IMPACT OF FAMILY HISTORY OF PREMATURE CORONARY ARTERY DISEASE ON CAROTID ULTRASOUND AND CORONARY CALCIUM FINDINGS

by

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ABSTRACT

First degree relatives (FDRs) of subjects with early onset of coronary heart disease (CHD) have higher risk of developing cardiovascular disease. We verified early CHD by angiography in the index patients and extensively phenotyped their FDRs to investigate the relationship of traditional and non-traditional cardiovascular risk factors to carotid ultrasound and coronary calcium scoring findings.

B-mode carotid ultrasound was used to assess the combined intima-media thickness and plaque burden in 111 FDRs. The biochemical and anthropometrical characteristics of the FDRs were compared with those of healthy controls matched for sex, age, ethnicity and BMI. Odds ratios indicate that FDRs are more likely to have positive carotid ultrasound findings compared to controls; 2.23 (95% CI 1.14 – 4.37) for intima-media thickness and 2.3 (95% CI 1.22 - 4.35) for average total thickness. In multivariate analysis positive carotid ultrasound findings were higher in FDRs independent of age, gender, total cholesterol over HDL-c ratio, systolic blood pressure and smoking but not homocysteine which had higher values in FDRs compared to controls. In conclusion FDRs of patients with angiographically confirmed CHD have higher burden of subclinical atherosclerosis even when considered in the context of traditional risk factors.

Coronary artery calcium scoring (CAC), assessed by 64-slice multi-detector computed tomography (MDCT), was used to assess burden of subclinical atherosclerosis in 57 FDRs compared to controls. FDRs have a two-fold increase in risk of having CAC

positive findings; odds ratios for the 75th percentile was 1.96 (95%CI 1.04 – 3.67, $p<0.05$) while for the 90th percentile odds ratio was 2.59 (95% 1.232 – 5.473, $p<0.05$). In summary, the risk of significant CAC findings, measured by 64-slice MDCT, is two-fold higher in FDRs than controls. These findings correlate highly with carotid ultrasound findings in the same cohort. Different thresholds for CAC may be appropriate when assessing male versus female FDRs.

Together increased carotid ultrasound findings and CAC scoring results in FDRs of patients with validated early onset of CHD suggest these imaging techniques as potentially useful tools in cardiovascular risk assessment that will go above and beyond the current diagnostic algorithms.

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ABBREVIATIOINS

CCA	Common Carotid Artery
ICA	Internal Carotid Artery
ECA	External Carotid Artery
HDL-c	High Density Lipoprotein Cholesterol
LDL-c	Low Density Lipoprotein Cholesterol
IDL-c	Intermediate Density Lipoprotein Cholesterol
VLDL-c	Very Low Density Lipoprotein Cholesterol
CHD	Coronary Heart Disease
NO	Nitric Oxide
PAI-1	Plasminogen Activator Inhibitor - type 1
FH	Familial Hypercholesterolemia
Apo-B100	Apolipoprotein B100
NEFA	Non-Esterified Fatty Acids
BMI	Body Mass Index
WC	Waist Circumference
CV	Cardiovascular
IL-6	Interleukin-6
CAD	Coronary Artery Disease
CRP	C-Reactive Protein
Lp(a)	Lipoprotein little (a)
FRS	Framingham Risk Score
PROCAM	PROspective CArdiovascular Munster

SCORE	Systematic COronary Risk Evaluation
IMT/CIMT	Carotid Intima-Media Thickness
MRI	Magnetic Resonance Imaging
MDCT	Multi-Detector Computed Tomography
CT	Computed Tomography
CAC/CACS	Coronary Artery Calcium Scoring
FDRs	First Degree Relatives
FACT	Family Atherosclerosis Counseling and Testing Study
M-CHAT	Multicultural Communities Health Assessment Trial
TPA	Total Plaque Area
TA	Total Area
AvgTT	Average Total Thickness
APT	Average Plaque Thickness
TC	Total Cholesterol
BP	Blood Pressure
OR	Odds Ratio
HR	Hazard Ratio
RR	Relative Risk
tHcy	Total Plasma Homocysteine

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DEDICATION

I dedicate this thesis

To my wife Cristina, that through her understanding and love helped me in following through with my education over the years. I would also have to apologize to my son for taking this time away from him with the hope that I'll be able to offer him a better life.

To the principle investigators and staff in the ASL lab and Healthy Heart Program, I have learned much from you during my training.

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CHAPTER I: INTRODUCTION

1.1 Prevalence of Cardiovascular Disease

Over 16 million people in the world died of cardiovascular disease in 2001 according to WHO with 7.2 million due only to Coronary Heart Disease (CHD) (1). In the US in 2004, CHD represented 43% of all cardiovascular and 16% of all-cause morbidity, and accounted for 869,724 deaths. This year more then 770,000 Americans will experience a new heart attack and 430,000 a recurrent coronary attack. The estimated cost for CHD alone in US is estimated to be $156.4 billion dollars in 2008 (2). The Heart and Stroke Foundation of Canada estimates that the cost for heart disease and stroke in Canada is approximately $18.5 billion dollars per year (in 2001), and that CHD is responsible of 36% of all-cause deaths (3).

Atherosclerosis is a progressive disease with heterogeneous manifestations in the arterial system, characterized by asymmetric focal thickening of the innermost layer of the medium/large arteries, also known as plaque. These pathological formations cause coronary thrombosis through volume increase or rupture, manifest as unstable angina, acute myocardial infarction or sudden cardiac death, or ischemic stroke. The major cause of Coronary Heart Disease (CHD) is atherosclerosis.

1.2 Pathology of Atherosclerosis

Atherosclerosis has a multifactorial etiology and an insidious slow progression, which represent a challenge, but also an opportunity to intervene in the natural course of the disease. For educational and research purposes, atherosclerotic lesions have been characterized based on morphology and temporal progression. Only the most frequent morphological features for each stage are described while it should be understood that progression from one type of lesion to another is not necessarily sequential. Contributors to the etiology of atherosclerosis include risk factors that can be classified based on which element, genetic or environmental, is predominant. Numerous family and twin studies have determined that the percent of atherosclerosis that can be explained by genetics is approximately 50% (4). The combined effects of, either genetic or environmental risk factors are not additive, making predictions of disease progression more difficult.

1.2.1 Endothelial Dysfunction

Plaque initiation, as it's currently known, is triggered by endothelial dysfunction in a topographically limited arterial inner-layer. One important factor is shear stress, forces that will ultimately change the morphology of the endothelial cells and thus disrupt their normal function (5). Genetics, smoking, hypercholesterolemia and cytokines released by inflammatory elements in response to oxidized lipids being entrapped in the matrix may all contribute to endothelial dysfunction (6, 7). Activation of endothelial

cells has multiple consequences including increased expression of adhesion molecules (P- and E-selectins) that can promote the recruitment of monocytes and lymphocytes into the arterial wall, vasoconstriction, increased permeability for low density lipoproteins (LDL), and increased expression of glycoproteins may generate a prothrombotic state. In all of these processes, one molecule, nitric oxide (NO) acts as key regulatory element; decreased NO production being responsible for the altered endothelial function (8).

1.2.2 Intima-media Thickening

Intima-media thickness corresponds to the histological media defined as the distance from the leading edge of the endothelium–intima interface (internal elastic lamina) to the leading edge of the media–adventitia interface (external elastic lamina). Initial lesions are characterized microscopically by clusters of macrophages with lipid inclusions. Increases in oxidized LDL particles trapped in the extracellular matrix are accompanied by eccentric deposition of proteoglycan, chondroitin sulfate and collagen. The integrity of the layers is maintained and changes are only noticeable in histological sections, though extracellular lipid droplets are not seen (9, 10). This type of lesion can be found as early as the first decade of life.

1.2.3 Fatty Streaks

Fatty streaks have been the first visible lesions described in autopsy studies as yellow spots or patches of fat. Also known as type II lesions, fatty streaks evolve at the point of an intima-media thickening and are characterized by the presence of extracellular

lipid deposits (Sudan staining), macrophage-derived foam cells, smooth muscle foam cells and also lymphocytes that infiltrate the sub-endothelial space. Not all type II lesions are visible at inspection of the inner artery, as lipids accumulate in the media. Lipid droplets (extracellular lipid accumulations) are formed by disruption of the foam cells and biochemical analysis indicates the presence of cholesteryl esters in addition to free cholesterol and phospholipids (11).

Only a small number of type II lesions will proceed to type III, these being characterized by migration of smooth muscle cells in between endothelial cells and internal elastic lamina (which shows discontinuation), higher accumulation of cellular elements and higher lipid content. The progression-prone type II lesions often coincide with regions of higher shear stress and their development is correlated with circulating lipid levels (12). This type of lesion can be found in younger individuals and are considered as still reversible since disorganization of the histological layers of the arterial wall are minimal.

1.2.4 Intermediate Lesions

Intermediate lesions, also known as type III lesions, are characterized by a visible thickening of the intima layer, which contains numerous smooth muscle cells and visible lipid droplets that disrupt histological layers; many smaller lipid pools may exist. As an intermediate phenotype, this entity consists of overlapping characteristics of the fatty streaks and atheroma lesions. Changes in lipid composition within droplets is demonstrated by their lower melting temperature when compared to type II lesions (13).

1.2.5 Atheroma Plaque

The type IV lesion, known as atheroma, is characterized by a large lipid core formed by a confluence of smaller lipid droplets described in type II lesions. The lipid accumulation continues as more foam cells are undergoing apoptosis/necrosis and is fed by circulating plasma lipids. Biochemical analysis of the plaque confirms that the predominant cholesteryl ester is cholesteryl linoleate, the same as in plasma low density lipoproteins (14). In this stage, small calcium particles are found free in the lipid core as well as in organelles of smooth muscle cells. The lesion cap is formed by smooth muscle cell and extracellular matrix mainly with few lymphocytes and mast cells, while internal elastic lamina has completely loss its integrity. A characteristic of type IV lesion is an increased thickening of the cap due to increase proteoglycan accumulation and an increase in collagen content (70% type I, 30% type III collagen fibers). The base of the lesion is enriched with inflammatory cellular elements, which tend to concentrate in the shoulder regions, as well as a substantial increase in connective tissue (15).

Type IV lesions are noticed in the same locations as fatty streaks, which are considered their predecessors. This type of lesion is considered irreversible, with well defined histo-pathological elements. Progression scenarios include plaque rupture, most likely in the shoulder region due to increased macrophage infiltrate and degrading extracellular matrix, or stabilization through fibrosis and calcification.

1.2.6 Advanced Atheroma Lesions

We talk about Type V or advanced lesions when considerable amounts of fibrous connective tissue have accumulated in the lesion. These lesions are protruding into the arterial lumen with various degrees of stenosis being present. The fibroatheroma has been described as a thick fibrous cap with a moderate lipid core or alternate fibrous tissue and lipid layers. Capillaries can be noticed in the vicinity of the lipid core, incorporated and organized thrombi, inflammatory cells as well as micro hematomas have been described. Another sub-type of advanced lesion is the calcified plaque, in which mineralization is the predominant process. This can affect the fibrous cap as well as the entire lipid core in some circumstances. Plaques where shoulder calcifying regions are adjacent to lipid pools are considered prone to rupture in contradiction to the general dogma that considered calcified plaques as stable (16). Yet another sub-type of advanced lesion is the fibrous plaque where the lipid core is replaced by fibrous tissue. This can happen through increased fibrosis in a fibroatheroma, thrombus incorporation and structuring or simply the regression of the lipid core.

Besides the increase in fibrous connective tissue, advanced plaques display neo-vascularization, disrupted or a rough endothelium and lymphocyte infiltration of the adventitia. Type V lesions are phenotypically diverse and evolve towards complicated lesions through thrombosis, hematomas or hemorrhage consequently obstructing the arterial lumen (15).

1.2.7 Complicated Lesions

Type VI or complicated lesions are the consequence of rupture, fissure or erosion of type IV/V plaques and manifest with hematomas, hemorrhage or thrombosis. Progression towards complicated plaque is not linear and varies with shear and tensile forces, high plasma fibrinogen levels, decreased fibrinolytic capacity (increase in plasminogen activator inhibitor type 1; PAI-1), platelet adhesion and aggregation properties. High levels of low density lipoproteins affects platelet function (17), while lipoprotein (a) due to its structural similarity to plasminogen may inhibit fibrinolysis (18). Acute events are caused by obstruction of the arterial lumen by thrombus formation or a cataclysmic expansion of the plaque due to hemorrhage in adventitia neo-capillaries. Atheroma plaque can also slowly grow to obstruct the arterial lumen through repeated incorporation of small thrombi and hematomas.

Not all fully obstructing thrombi will permanently block the blood flow. A small number of thrombi will become permeable and a false lumen will form to allow blood flow. Most advanced plaques have a rough surface and can shed thrombi which will become manifest in the periphery (cerebral ischemic attack). One other scenario in the evolution of the complicated plaque represents the atherosclerotic aneurysm. A consequence of matrix degradation and destruction of the external elastic lamina is the formation of an aneurysm (bulging of the arterial wall) that can preserve the arterial lumen while thrombi form eccentrically. Proteolytic enzyme activity probably in the presence of hyperlipidemia results in dilation and rupture of the arterial aneurysm (19).

1.2.8 Clinical Imaging of Atherosclerotic Lesions

Though important, histological classifications provide only limited information to the clinician using modern imaging methods to visualize plaques. Angiography the gold standard for evaluating arterial stenosis, is inefficient in detecting type I, II and III lesions and sometimes even type IV and V lesions containing a smooth surface, could be missed. Severity of stenosis is evaluated instead, with clinically significant blood flow reduction in > 50% stenotic lesions and severe obstruction in >70% stenosis (20). In two thirds of patients the acute events are triggered by fissure or rupture of lesions that do not affect the blood flow to that extent (21). Autopsy studies demonstrated that plaques prone to rupture have the following characteristics: thin fibrous cap, marked eccentricity, shoulders with high cellular content, rough or irregular edges, large lipid core and calcification isolated in the shoulder region (22).

New imaging methods can better describe the composition and characteristics of the plaque before an event and promise to better indicate the risk for future events. B-mode ultrasound can assess the thickness of the arterial wall, calcification and large lipid accumulations in addition to percent stenosis, though not applicable to coronary arteries. Intra-vascular ultrasound exploration can provide accurate structural information of the plaque with the shortfall of an invasive exploratory procedure that could destabilize lesions. Ultra-fast computed tomography can detect calcium depositions, indirectly correlated with number of plaques, but falls short to provide structural information beyond that unless contrast substance is injected. Nuclear magnetic resonance spectroscopy, though promising, needs further validation (22).

1.3 Risk Factors for Cardiovascular Disease

Large epidemiological studies have identified a series of risk factors for atherosclerosis and new risk factors are discovered in an effort to circumvent the natural evolution of the disease. For clinical purposes, these risk factors have been classified as modifiable (with a large environmental component) and non-modifiable (mainly inherited). Thus age, gender and family history are traits that can not be changed while the risk associated with the remaining risk factors can be altered through pharmaceutical or behavioral intervention.

1.3.1 Age and Gender

As any progressive / chronic disease, the burden of atherosclerosis increases with age thus the risk for cardiovascular events increases with time. With age, the composition of the atherosclerotic lesions changes towards an increased prevalence of fibrous tissue (stabilizes the plaque), less smooth muscle cells, inflammatory cellular elements and increases in proteolytic enzyme activity, some of which contribute to plaque rupture (23). The number of angiographically positive findings increases with age independent of other risk factors. Age, in the context of cardiovascular disease and atherosclerosis, should be understood as years passed in the presence of a detrimental risk factor, not to mention that the frequency of these risk factors increases with age as well (24). From this perspective, people age differently and by lowering all the other risk factors we can reduce the effects in time that we would otherwise observe.

Men are more likely to be affected by atherosclerosis then their women counterparts. Genders display noticeable differences in the frequency of risk factors like smoking, physical exercise and triglycerides, though these do not fully explain the differences in burden of disease noticed (25). The protective effects of estrogens in women may partially provide an explanation of these differences. Though risk increases in the postmenopausal women and differences in risk between men and women after the age of 60 disappear, estrogen replacement therapies did not prove to be beneficial (26). The effects of androgen hormones in the absence of estrogen activity could be a potential mechanism (27).

1.3.2 Smoking

Smoking shows a strong and graded relation with risk of myocardial infarction. Even for five cigarettes per day the risk is considerably higher reaching an odds ratio (OR) of 9.5 (95%CI 6.18 – 13.58) for those that smoke over forty cigarettes (28). No level of smoking is safe and the graded characteristic suggests that each cigarette less counts towards reducing risk. As with many risk factors, the relative risk is higher in younger subjects and decreases in older subjects, though the correlation to risk does not plateau (29, 30). Though higher mortality in men that smoke compared with women that smoke has been reported, this could be due to the difference in the frequency of smoking or associated risk factors between genders.

1.3.3 Dyslipidemia

Besides smoking, total cholesterol levels are the most important risk factor for atherosclerosis. A direct, graded relation between cholesterol levels and coronary heart disease mortality has been observed (31). This relation is independent of all other risk factors and doesn't plateau. Though the risk in women, prior to menopause, is lower then in men the effect of total cholesterol on risk of myocardial infarction parallels. A severe form of familial hypercholesterolemia (homozygous FH) has been associated with an increase risk of cardiovascular disease. The mechanism behind this extreme form of hypercholesterolemia has revolutionized the study of lipids and their relation to cardiovascular pathology (32).

Cholesterol in plasma is packed as low density lipoproteins (LDL-c), high density lipoproteins (HDL-c) and a small amount as very low density lipoproteins (VLDL). It is the LDL-c levels that are directly correlated with atherosclerotic build up and even more important are the number of such particles. Described mechanisms include abundance of small, cholesterol depleted LDL particles (assessed as Apo-B100; one per each LDL particle), which more easily enter the arterial wall become entrapped and are oxidized. Overwhelming evidence however comes from studies that show reduction in cardiovascular risk by lowering LDL-c levels through pharmaceutical or dietary (minor effect) intervention. This has been tested in both secondary (33-36) and primary prevention trials (37-39) and 1% reduction in LDL-c levels corresponds to a 1% reduction of risk.

HDL-c levels on the other hand are negatively correlated with risk for cardiovascular events. Total cholesterol to HDL-c ratio combines the predictive power of high LDL-c and low HDL-c for coronary events in one index. A wide variety of mechanisms have been probed to explain the protective effects of high HDL-c levels from its anti-inflammatory (40) and anti-oxidative (41) effects and the recirculation of cholesterol back to hepatic cells though a process known as reverse cholesterol transport (42).

The effects of triglyceride plasma levels in atherosclerosis are complex. In epidemiological studies, the triglyceride level is a strong predictor of coronary heart disease, though after adjustment for HDL-c, most of that power dissipates. Reducing triglyceride levels is demonstrated to be beneficial (43), but it remains unclear how much of that effect is due to increases in HDL-c or reduction in number of VLDL remnants.

1.3.4 High Blood Pressure

Hypertension is correlated with a significant increase in risk for development of atherosclerosis. Mechanisms involved include endothelial dysfunction, increased shear stress with consequent smooth cell proliferation and increased fibrosis. Also an increase in the rate of acute events due to plaque destabilization has been reported (44, 45). In medium risk patients, the blood pressure should be maintained under 140 / 90 mmHg while in high risk patients it should be under 135 / 80 mmHg. In secondary prevention, independent of blood pressure values, addition of B-blockers and angiotensin converting

enzyme inhibitors in addition to lowering lipid levels have been proven to increase survival (46).

1.3.5 Diabetes Mellitus and Glucose Intolerance

Presence of diabetes increases the risk for cardiovascular disease two to four times and it's now widely recognized that the presence of diabetes places the patient in the high risk category independent of other risk factors. Type 2 diabetes has been estimated to affect 226 million people by 2010, a 46% increase in almost 10 years affecting increasingly younger subjects (47). Possible mechanisms can be divided in glucose-related factors like hyperglycemia and insulin resistance and other factors like increased blood pressure, haemostatic abnormalities and dyslipidemia. Even in the presence of good glycaemic control, there is an increased VLDL production with consequent hypertriglyceridaemia. This maybe due to the inhibition of hormone-sensitive lipase and a subsequent increase in the release of non-esterified fatty acids (NEFA) from adipose tissue. Low HDL-c levels coexist with hypertriglyceridaemia and the LDL particle size decreases with obvious detrimental effects (48, 49). CARE, LIPID and 4S studies have conclusively showed that lipid lowering medication in addition to good glycemic control is of up most importance (33-35).

1.3.6 Abdominal Obesity

Obesity can be classified as either central/abdominal obesity or according to others as android /gynoid obesity. The Framingham Heart Study showed that an increase

in obesity is correlated with an increase in coronary heart disease events (50). When defined as an increased body mass index (BMI) the association with cardiovascular (CV) disease is weaker then when obesity is defined as an increase in waist-to-hip ratio. This indicates that visceral fat depositions are a better indicator of risk then subcutaneous fat (51). Potential mechanisms related to this increased risk are an increased release of nonesterified free fatty acids, increases in IL-6, tumor necrosis factor, leptin, resistin, adipokines and acute phase proteins. Gender differences in the distribution of fat could explain differences seen in strength of the correlation between BMI and cardiovascular risk. Decreasing cholesterol levels and weight loss associated with dieting can undoubtedly be beneficial through lowering insulin resistance, improving overall lipid profile and lowering risk for cardiovascular events.

1.3.7 Psychosocial factors

Psychosocial stress was demonstrated to correlate with the development of atherosclerosis and an increase in cardiovascular events (52). Stress has been correlated with a lack of physical exercise, unhealthy diet, smoking, lower economic status and an increase in inflammatory cytokines (53). From a mechanistic perspective, stress can increase epinephrine and norepinephrine hormones together with cortisol, proinflammatory cytokines and free fatty acids (54).

1.3.8 Family History of Premature CAD

Family history of premature CAD is defined as the presence of a cardiovascular event in any first degree relative (parent, sibling or children) under the age of 55 for men and 65 for women according to The National Cholesterol Education Program Third Adult Treatment Panel (NCEP-ATP III) (38). The risk attributable to family history of premature cardiovascular disease varies widely from 1.5- to a 7-fold increase (55-57). A family history of myocardial infarction explains 15% of the future coronary events in a first degree relative (58). In a different cohort, maternal family history had a higher impact then parental family history in predicting risk (59). While in a different study the sibling history was more strongly associated with subclinical coronary atherosclerosis than a parental history of premature CAD (60). Risk varies according to the age of the person being evaluated (risk seems to be lower in older subjects in which environmental components are predominant), the number of relatives affected and the age at which they have suffered the event, and the degree of genetic inheritance.

With many studies confirming the clustering of genetic factors in families with premature cardiovascular disease, genetic analyses could become a useful tool to assess risk. There are over 80 genes that participate in regulation of plasma lipid levels and thus impact the development of atherosclerosis (61). The polymorphism in the gene corresponding to the expression of angiotensin-converting enzyme was thought to be an independent contributor to cardiovascular risk (62). However, there are only a few cases in which a single gene mutation can be uniquely made responsible for the increase risk for atherosclerosis. Familial hypercholesterolemia, a defect in the LDL receptor, can explain 45% of events in men and 25% of events in women (63). Normally, complex

interactions between clusters of genetic factors contribute to increased atherosclerosis in families with a proven early onset of cardiovascular disease in one of their first degree relatives. Over 50% of cases can thus be explained by the genetics and this has been validated by comparing studies of adoptee with twin studies (64, 65).

Environmental components of a family history of cardiovascular disease are influential. Families share not only their predecessor's gene variants, but frequently the dietary, smoking, drinking habits, physical activity patterns, socio-economical status and sometimes education levels tend to be similar (66). Thus, the phenotype expressed by family members is the result of gene-environment interactions and are at the basis of the cumulative risk attributed to family history of premature cardiovascular disease.

1.3.9 Non-traditional / Emerging risk factors

1.3.9.1 Homocysteine

Total plasma concentrations of homocysteine, a non-essential sulfur-containing amino acid derived from methionine metabolism, are correlated in a graded, independent way with increased risk of CAD and stroke (67, 68). Higher plasma levels are determined by a congenital enzyme defects (the C677T polymorphism in the gene for methylene tetrahydropholate reductase) or a gross deficiency in folic acid and vitamin B12. In vitro cell culture studies have suggested that homocysteine may promote risk through injury to the endothelium (69), increased proliferation of smooth muscle cells (70), increased oxidation of LDL and interfering with the coagulation/fibrinolysis equilibrium (71).

Plasma concentrations of homocysteine can be lowered by food supplementation with folic acid and Vitamin B6, which have been suggested by some to be beneficial and may lower the risk for coronary heart disease (72). Food fortification with folic acid and vitamin B6 has been implemented in Canada since 1989. However, recent prospective epidemiological studies like VISP and HOPE-2 have definitely proven that lowering total plasma homocysteine by administering folic acid and Vitamin B6, B12 supplements does not decrease the risk for CAD (73, 74).

1.3.9.2 C-Reactive Protein and inflammation

C-reactive protein (CRP) is an acute-phase protein that is increased in systemic inflammation, tissue injury and infections all of which correlate with atherosclerosis progression. A high sensitivity test (detects levels as low as 0.004mmol/L or 0.175mg/L) is currently used to measure this liver-synthesized marker of inflammation. Small increases in hs-CRP levels are predictive of increased cardiovascular risk in apparently healthy individuals (75). An approximately 1.5-fold increase in risk for cardiovascular death and myocardial infarction has been described in people in the higher quartile versus the low quartile for CRP (76). Potential mechanisms related to a CRP-mediated increase in risk include an inhibitory effect on endothelial progenitor cells, the inhibition of NO production, the induction of apoptosis of smooth muscle cells and the stimulation of the production of reactive oxygen species (77, 78). CRP may improve prediction above the Framingham Risk Score (FRS); CRP over 3mg/L indicated high risk in up to 32% of people previously classified as medium risk according to FRS (79). However the

measurement of CRP remains a relatively non-specific marker of inflammation and it is not entirely clear if elevated CRP represents a direct mechanistic link to atherosclerosis or if CRP is merely a marker of increased inflammatory status.

1.3.9.3 Lipoprotein (a)

Structurally, Lipoprotein (a) is similar to the LDL particle with the addition of apoprotein (a) which is covalently bonded to apoprotein B100. Apoprotein little (a) is structurally homologous to plasminogen and suggests a possible involvement of Lp(a) in thrombosis and coagulation. The exact biological role of Lp(a) is unknown while plasma concentrations seem to be mainly genetically predetermined. Concentrations of Lp(a) above 30mg/dL confer a two fold increase in risk for CAD particularly when associated with high LDL-c levels (80). High levels of Lp(a) in the presence of other risk factors has been associated with angiographic progression of atherosclerotic lesions, however, to date there are no prospective clinical trials to confirm the observed associations to risk nor if assessment of Lp(a) provides additional information to the usual FRS estimation of risk.

1.4 Risk Assessment

The risk for developing atherosclerotic disease can be assessed by fully appraising the multivariate risk factors as they seldom manifest in isolation where clustering confers more than an additive effect. Key concepts include absolute risk which is the probability of a subject to have an event in 10 years or in their remaining lifetime (global risk) and the relative risk which is more useful in determining causality as it compares events in people with and without a certain risk factor. The clinician can decide whether to assess the absolute risk according to one of the validated methods (PROCAM, Framingham Risk Score) and treat according to risk estimates or assess the risk using these tools and then adjust the calculated risk based of the presence or absence of other factors not considered: family history of CAD for FRS, hs-CRP, Lp(a), total homocysteine, arrhythmias, results of the stress test, CAC (coronary artery calcium), or CUS (carotid ultrasound).

1.4.1 Framingham Risk Score

The Framingham Risk Score (FRS) is based on data derived from 5300 subjects recruited in the Framingham Heart Study, age 30 to 74 at the time of recruitment which were followed for a period of 12 years. The NCEP-ATP III (38) recommendation to assess risk are based on the FRS algorithm which indicates the 10 year risk for a coronary hard event. Risk factors included in the score are age, gender, total cholesterol to HDL-c ratio, systolic blood pressure (on treatment or not), and smoking. Presence of Diabetes

Mellitus is considered equivalent with the presence of CHD and thus high risk; the same applies for aortic aneurysm and peripheral vascular disease. Categories defined: <10%FRS low risk, between 10% and 20% medium risk and >20% high risk category.

The ease of use of the FRS makes it an attractive clinical tool. However, some disadvantages include: limited applicability to populations of different ethnicities, variables are categorical and not continuous thus false thresholds are created, does not account for a family history of cardiovascular disease or other new risk factors or factors in the results of exploratory tests (stress test, B-ultrasound, chest x-Ray). Also, it does not differentiate between on and off treatment for lipids and doesn't consider the possibility that clustering of risk factors could increase exponentially the risk. Furthermore, the risk assessed is an absolute 10 years risk, which is significantly less then the corresponding life time risk (81).

1.4.2 PROCAM Score

The PROspective CArdiovascular Munster (PROCAM) study has derived its data from 5389 men age 35 to 65 from Munster, Germany after they've been followed for 10 years. Risk factors included are smoking, systolic blood pressure, LDL-c, HDL-c, triglycerides, family history of myocardial infarction, Diabetes Mellitus and age. One disadvantage is the lack of a table estimated for women (it's calculated as a fourth of the risk of a man). It has coefficients of adjustments for various European populations in which FRS would overestimate the risk. PROCAM used only hard endpoints of fatal and non-fatal MI and sudden coronary death.

1.4.3 SCORE algorithm

SCORE comes from the Systematic COronary Risk Evaluation and it is an attempt to adjust risk estimated form various parts of Europe in which mortality data varies. This algorithm focuses on mortality form cardiovascular disease and not coronary events fatal or non-fatal like its predecessors (82). Major shortcomings are drawn form ignoring important risk factors like: family history of CAD, impaired glucose intolerance, hypertriglyceridaemia thus this algorithm likely underestimates risk.

1.5 Non-invasive Risk Assessment in Asymptomatic Subjects

Besides the assessment of risk using validated risk prediction algorithms the clinician has access to a variety of non-invasive assessment tools to further explore the issue. Most methods in use today are the stress test, cardiac perfusion test, brachial artery index, non-invasive coronary angiography, carotid intima-media thickness (CIMT) and coronary artery calcium scoring (CAC).

The stress test is a useful non-invasive method of assessing risk in subjects with or suspected CAD. In selected asymptomatic subjects at medium risk, the stress test provides useful information to increase or decrease the attributed risk (83). The diagnostic accuracy of the test however depends on the pretest likelihood of CAD which in the case of asymptomatic subjects is low, especially in young or female patients. This is why in young or low risk asymptomatic patients this test will generate an unnecessary high number of false positive results and it is usually not recommended.

The cardiac perfusion imaging test is a valuable tool to categorize patients based on their risk of future events especially in its ability to detect subjects at very low risk which consequently impacted the referral rate for revascularization (84, 85). Thallium-201 or technetium-99 radioisotopes are injected to detect a signal. The event rate is less then 1% per year in subjects with normal perfusion studies. Different predictive power is described in pharmacologic versus stress perfusion imaging tests but both allow

evaluation of myocardial perfusion, ventricular size and systolic function. In asymptomatic subjects, the usefulness of this investigation is limited as only severely obstructive atherosclerotic lesions would produce perfusion defects (86).

Endothelial function impairment is characteristic for pro-atherosclerotic status and directly correlated with progression of disease (87). The degree of endothelial dysfunction can be assessed by ultrasound assessment of brachial artery flow-mediated dilation and has been proposed as an independent predictor of long-term cardiovascular events (88). In one study, preserved endothelial function reduces the risk for future events in people with high burden of plaque assessed by carotid ultrasound (89). Further assessment of this method and its predictive value in asymptomatic subjects is needed.

Non-invasive coronary angiography includes both magnetic resonance imaging (MRI) and CT- angiography techniques. To date the usefulness of MRI in assessment of risk for CAD is hypothetical as no large studies to evaluate outcomes have been conducted. Myocardial transmural perfusion defects assessed by MRI after injecting a contrast substance has a sensitivity of 84% and a specificity of 85% (90). Limited information on the suitability of CT-angiography in risk assessment is due to the relatively new introduction of the 64-slice MDCT which is approaching 99% sensitivity and 95% specificity for atherosclerotic lesions. However, it is unlikely that either of these methods is suitable to be applied as first line of screening techniques to assess risk in asymptomatic patients.

1.5.1 Ultrasound Exploration of Carotid Arteries

High-resolution B-mode ultrasound of the carotid arterial segment is a non-invasive method to assess intima-media thickness and /or focal thickening (plaque) of the arterial wall. Intima-media layer is defined between two hyperechogenic lines: one at the interface between luminal flow and endothelium and the other between external elastic lamina and adventitia. Increase in thickness of the intima-media layer can be due to hypertrophy and hyperplasia as well as the pathological changes related to atherosclerosis like smooth muscle cell infiltration and subsequent lipid loading. Focal thickening observed as increase thickness relative to the adjacent region are more clearly attributable to atheroma build up. However both intima-media thickness and plaques assessed by B-mode ultrasound have been correlated with morbidity and mortality data and are considered accurate predictors of future cardiovascular events (91, 92). More important carotid IMT progression, lack of progression or regression is used today as a validated surrogate marker in interventional clinical trials (93, 94).

1.5.2 Calcium Scoring

Normal arteries do not contain calcified crystals unless there is a major structural disruptive process like the accumulation of atheroma material. Plaques have been shown to contain osteocalcin (nonhepatic Gla-containing proteins involved in Ca trafficking), osteopontin (bone mineralization) and bone morphogenic protein-2a (osteoblastic differentiation), suggesting a dynamic possible reversible process (95). Coronary artery calcium (CAC) scoring, assessed by electron beam computed tomography or multi-detector computed tomography, correlates well with the extent of atherosclerosis burden (96, 97). CAC scoring is demonstrated to be a good predictor of future cardiovascular

events (98-100). CAC findings are a reflection of total atherosclerotic burden as it seems that a ratio between calcified and non-calcified plaques exists. Though CAC findings suggest presence of a larger lesion it does not correlate with the degree of stenosis. No one-to-one relation between calcium deposition and obstructive plaque has been observed. Zero calcium scan does not exclude the presence of soft plaque though very low rates of events have been demonstrated (101). Though previous studies suggested that a slow progression of CAC related to a lower rate of events (102), recent evidence from randomized double-blind trials failed to demonstrate slower progression with lipid lowering treatment and reduction in LDL levels (103, 104).

1.6 Rationale

With death rate reaching 36% of all cause mortality, CAD is a major health concern in Canada. Secondary and now primary prevention and treatment of this disease should represent a priority in national health practices.

Primary prevention is the most effective strategy to fight cardiovascular disease. To implement primary prevention, early identification of medium to high-risk individuals and/or populations is needed. Traditional risk assessment, such as Framingham score, predicts only 60% to 65% of hard cardiac events. Even though positive family history of early cardiovascular disease improves this prediction algorithm (as a two-fold increase is considered), the exact amount of risk in asymptomatic first degree relative eludes classical risk assessment.

My thesis compares traditional and non-traditional risk factors with the number and frequency of findings at carotid ultrasound and coronary artery calcium scoring scans. This will give us a better understanding of the effectiveness of various approaches to risk assessment in individuals with a family history of early cardiovascular disease.

The knowledge gained from this research will provide insight into the appropriate risk assessment tools to be used in first degree relatives of patients with early onset of CAD, a population known to be at high risk, but often neglected. This will provide a basis for the education of health professionals into better detection strategies for early atherosclerotic lesions and management of cardiovascular health risks in this specific population.

1.7 Hypothesis

- In first degree relatives (FDRs) of patients with early onset of coronary artery disease (CAD) (males < 50, females < 60) there will be an increased frequency and severity of carotid ultrasound findings when compared with controls.

- There will be significant correlations between both the traditional (lipids, apoproteins, hypertension, diabetes, smoking) and non-traditional (Lp(a), CRP, homocysteine) risk factors with carotid ultrasound traits.

- In asymptomatic FDRs of patients with early onset of CAD (males < 50, females < 60) there will be an increased frequency and severity of positive findings in coronary artery calcium scoring compared with controls.

1.8 Experimental Design

The study design is illustrated in Figure1-1.

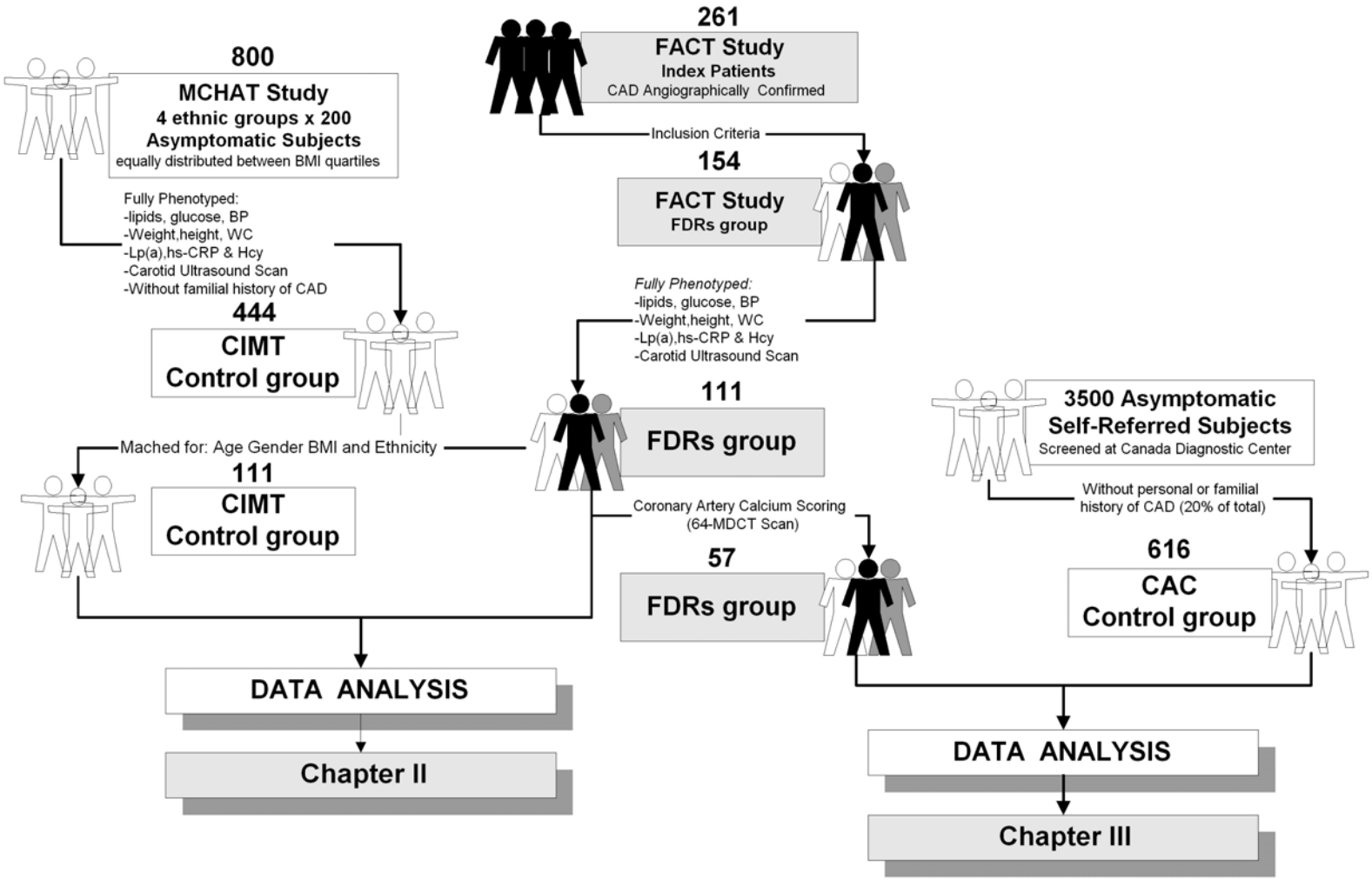

Figure 1-1. Experimental design *(flow chart):* First degree relatives of patients with, angiographically confirmed, premature onset of coronary artery disease will have higher frequency of findings at carotid ultrasound scan (Chapter II) and at coronary artery calcium scoring (Chapter III); these findings will correlate with the traditioinal and non-traditional risk factors evaluated.

1.9 Structure of the Research Project

Chapter 2. This chapter evaluates the potential of carotid ultrasound indexes with respect to their ability to detect differences in cardiovascular risk between subjects with and without family history of premature coronary artery disease. We also evaluated the correlation between traditional and non-traditional risk factors and the carotid ultrasound findings.

Chapter 3. This chapter compares the frequency of positive coronary artery calcium scoring findings, assessed by multi-detector computed tomography, in asymptomatic individuals with and without family history of early coronary artery disease.

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CHAPTER II: CAROTID ULTRASOUNFD FINDINGS IN ASYMPTOMATIC FIRST DEGREE RELATIVES OF PATIENTS WITH EARLY ONSET OF CORONARY ARTERY DISEASE[1]

2.1 Introduction and Rationale

Family history of coronary heart disease (CHD) is a risk factor for cardiovascular disease (1-5). Based on CHD onset and the relation to the first degree relative (FDR), the reported risk increase ranges from 1.5 to 7-fold (6-9) and is likely due to variable penetrance of genetic contributors and different environmental factors affecting the various age categories studied (10, 11). A more conservative two-fold increase in risk for cardiovascular disease with positive history of premature CHD is currently used by most experts (12, 13) although the Framingham score (14, 15) does not include family history to adjust the ten year estimate for an acute event. New methods for predicting risk in FDRs that will go beyond the traditional risk assessment algorithms are needed.

Carotid ultrasound techniques, such as measurement of the intima–media thickness (IMT), have been used in epidemiological studies as well as in clinical trials to predict the risk of future events (16-18). Ultrasound screening in people with family

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history of premature CHD thus may be an appropriate method to detect individuals at high risk who would benefit the most from primary prevention (19). Early atherosclerosis may manifest as either diffuse intimal thickening or as focal plaque development. Assessment of both these forms of early atherosclerosis may be important in fully characterize the vascular comprise in such patients. Composite measurements such as plaque area, average total thickness or combining the IMT data with those of plaque findings could be used to assess subclinical atherosclerosis in younger subjects (20).

Our prospective case – control study, was conducted to evaluate a number of carotid total ultrasound indexes with respect to their ability to detect differences in cardiovascular risk between subjects with and without family history of premature coronary artery disease. We related these findings to a number of cardiovascular risk factors in an effort to find out which of these best characterizes the differences in carotid ultrasound findings between FDRs and controls.

Unlike other studies our cohort consisted of FDRs of patients with angiographically proven CHD and the phenotyping included both traditional and non-traditional cardiovascular risk factors.

2.2 Materials and methods

2.2.1 Study Populations

2.2.1.1 Cases – First Degree Relatives

First degree relatives (n=111, children n=44, parents n=4 or siblings n=63) of patients with early onset of CHD have been thoroughly phenotyped for traditional and non-traditional cardiovascular risk factors. Patients with early onset of CHD, (men <50 years, women <60 years) proven by angiography consequent to myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty or angina with ECG changes, were asked for permission to contact their asymptomatic FDRs within the Greater Vancouver area. Between May 2002 and September 2005, 261 patients (37% female, 63% male; 54.2 $\pm$ 11.4 years) with premature CHD have been identified in the St. Paul Hospital's interventional catheterization laboratory, cardiac inpatient wards, heart transplant and heart function clinics, as well in the Healthy Heart's lipid clinic and cardiovascular rehabilitation clinic. FDRs of 82 index patients (31% female, 68% male; 46.3 $\pm$ 6.4 years; **Table 2-1.**), 16 years of age or older, were invited to the clinic for a 20 minutes visit; starting with an interview to record their personal and family medical history and ending with a brief clinical examination while anthropometrical measurements, blood pressure and 12h fasting blood samples were taken. The lipid profile (triglycerides, total cholesterol, high density lipoprotein cholesterol), apolipoprotein AI, apolipoprotein B100, lipoprotein(a), high sensitivity C-reactive protein and 12h fasting glucose levels were measured at the St. Paul's Hospital laboratory

Table 2-1. Index Patients - characteristics of the event*:

Index patients (n=73)	**Women** (n=23)	**Men** (n=50)
Age at time of event:	50 ± 6.3 yrs	44.8 ± 5.8 yrs
Type of Event:		
Myocardial Infarction	56% (13/23)	50% (29/59)
PTCA / CABG	21% (5/23)	26% (15/59)
Stable Angina	18% (4/23)	16% (9/59)
Unstable Angina	5% (1/23)	8% (5/59)
Angiography findings:		
Single Vessel Disease	56% (13/23)	58% (34/59)
Two Vessel Disease	13% (3/23)	12% (7/59)
Three Vessel Disease	30% (7/23)	18% (18/59)

Note: This table summarizes the angiography data of 73 patients out of 82 index patients included in the study (9 reports missing from the charts); * frequency data is presented as % and ratio (n/total) except for age of the patient at the time of event which is expressed as mean ± SD.

by routine laboratory methods. Low density lipoprotein cholesterol was calculated using Friedewald formula and the atherogenic index was expressed as logarithm of the ratio of molar concentration of triglycerides to HDL-cholesterol(21). Total plasma homocysteine was analyzed at Vancouver General Hospital laboratory by direct chemiluminescent immunoassay (22). The 10-year cardiovascular risk of future events was calculated using Framingham Risk Score tables(14, 15). Subject's calorie and vitamin daily intake was assessed using the food frequency questionnaire developed by Harvard School of Public Health (23).

All subjects underwent carotid ultrasound scan within a year from the baseline assessment. Out of the 162 FDRs recruited for the study, 111 (65 female, 44.7 $\pm$ 13 years; 46 male, 44.4 $\pm$ 11 years) from 82 families completed the assessment and are included in this analysis.

2.2.1.2 Contemporary Controls

Matched control subjects were selected from the Multicultural Community Health Assessment Trial (M-CHAT), a prospective observational study designed to investigate the differences in adipose tissue distribution (visceral versus total body fat) in men versus women of four ethnic origins: Aboriginal, Chinese, European and South-Asian. Selection criteria for M-CHAT study have been previously described (24). In brief, equal number of apparently healthy men and women were recruited for each of the four ethnic groups. The recruitment was equally distributed between three categories of the body mass index (BMI): 18.5 to 24.9, 25 to 29.9 and > 30. Out of the 802 M-CHAT subjects fully

characterized, 444 without familial history of cardiovascular disease have been matched to our FDRs by gender, age (± 5 years), ethnicity (90% successfully), and BMI (± 2). When two or more subjects fitted the matching criteria the first available subject from the database was chosen. Matching the FDRs, by gender, age, ethnicity and BMI, was successful and verified using Pearson correlations for the studied variables. FDRs cohort was slightly younger (44.6 ± 12.7 years of age) with a higher incidence of current smokers (16 out of 111) compared to M-CHAT cohort (46.7 ± 10.2 years old) with only 9 smokers out of the 111 matched participants. A predominantly European FDRs cohort and the limited availability of European females in the control population necessitated to match two of them with controls of South-Asian and four with controls of Chinese ethnic background, matching for the other criteria.

Historical controls were defined based on the 75th percentile, age and sex adjusted carotid ultrasound values, published in a number of large studies (for references see (25)).

2.2.2 Ethics and Data Collection

Both studies have been conducted with the approval of institutional ethical review boards of St. Paul Hospital and University of British Columbia. For publication purposes the study has been registered with ClinicalTrials.Gov. **(Appendix 1, 2)**

Hardcopies of medical records for all study participants have been stored onsite (Healthy Heart Research office) with access restricted to FACT study research team. An MS Access database has been created and independent double data entry performed. The

two datasets have been reconciled against hardcopy data. Access to the electronic data has been restricted to FACT study research team; a combination of network/user restrictions at database level has been applied to maintain the integrity and privacy of participant's records. (**Appendix 3)**

2.2.3 Ultrasonography Carotid Measurements

Both FDRs and control cohorts had their carotid ultrasound scans done at Healthy Heart Program, St. Paul Hospital by one of two staff technicians. The examination was performed bilaterally, with subjects in supine position, neck extended, using a 7.5 - 10 MHz, L1038 linear-array transducer (HPM2410B Image Point HX, Hewlett Packard ™, Andover, MA). Scanning begins with a sagital image of distal CCA (both near and far wall clearly visible), approximate 30 sec recording, followed by a sagital scan of the bifurcation identifying ECA and ICA flow divider (good visualization of the ICA far wall is important). Transverse scan is performed from the 2-3 cm of CCA, past the bulb, and followed by proximal 3-4 cm of ICA and ECA to show layered plaques and help the analyst locate the plaques to be measured in sagital still-shoots. The scans were sent to the Cardiovascular Imaging Research Core laboratory, Vancouver General Hospital, were they were digitized for downstream analysis (using edge detection software - Vascular Imager 5.0, Medical Imaging Applications, LLC, Coralville, IA). **(Appendix 4, 5)**

The methods for measuring bilateral average IMT, calculating carotid ultrasound indexes as well as intra- and inter-observer variability were fully described before (20, 26). Briefly, individual IMT measurements are taken as the average thickness over 10

mm segment of the far wall of the right and left CCA free of plaque, within 2 cm proximity from the carotid bulb. Presence of plaque (focal increase of the intima-media thickness) in any carotid segment is identified by consensus of two observers while still images are recorded for analysis. Carotid ultrasound indexes reported herein include total plaque area (TPA) which is the product of thicknesses and lengths of lesions, total area (TA; which incorporates the TPA with the area of the IMT), a sum of the area of carotid wall and plaques measured and average total thickness (AvgTT; which is the TA divided by the total length of plaque and wall measured; **Figure 2-1.)** Total plaque number per subject was recorded independent of location or size of the lesion observed.

2.2.4 Statistical Analysis

Characteristics of the study cohorts were expressed as mean and standard deviation for all variables except homocysteine, Lp(a), high sensitivity C-reactive protein and the carotid ultrasound measurements for which median and interquartile range were used instead. Since no difference was observed between the actual values and logarithmically normalized ultrasound indexes, only the former are reported herein. For means, paired student t-test was used whenever Gaussian distribution could be assumed while Wilcoxon ranking test was used for others; McNemar's test was used for categorical variables. The results were the same when Mann-Whitney ranking test, which assumes independence of the two groups compared, was used. Two tail and 95% confidence intervals were used in all analyses. The degree of association between carotid

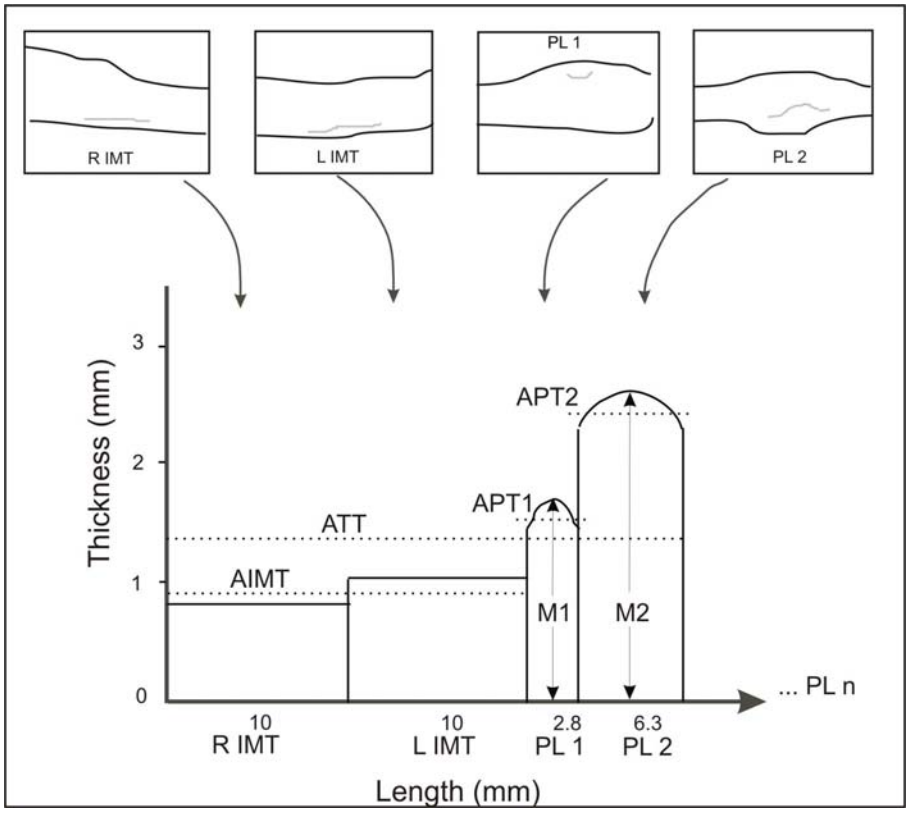

Figure 2-1. Quantitative calculations undertaken for each scan:

AIMT is the average for IMT derives form the right and left measurements; APT1 & APT2 designates the average thickness of that plaque; ATT is the average total thickness and is given by the total area divided by the total length shown on the Y axis.

Note: This image has been reproduced from: Mancini GB et. al., Validation of a new ultrasound method for the measurement of carotid artery intima medial thickness and plaque dimensions. Reproduced with permission, *Can J Cardiol* 2004; 20(13):1355-9. **(Appendix 6)**

ultrasound indexes and other known risk factors was assessed by univariate analysis and Spearman's correlation test.

Multivariate linear regression analysis was employed to answer the question whether family history of CHD is predictive of carotid ultrasound indexes after adjustment for confounders. For this purpose we removed from the FDRs cohort 14 subjects that declared that they've experienced thoracic pain or those with diabetes. Variables were entered in the model one at a time and collectively to assess their influence. Higher impact variables like age, TC/HDL-C ratio, systolic BP, waist circumference and smoking were considered covariates in our base model. We chose waist circumference and not BMI to adjust for abdominal obesity in our model as it correlated better with carotid ultrasound indexes. Since homocysteine was significantly higher in FDRs we excluded this variable from the base model and created an extended model to include it, thus isolating the effect this difference may have on carotid ultrasound measurements.

Odds ratios with 95% confidence intervals were calculated (using loglinear general model) to determine the odds of increased likelihood of positive findings in FDRs compared to controls. We defined positive findings as values over the 75^{th} percentile (age and gender matched) for the historical control(25). A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA) software package.

2.3 Results

2.3.1 Clinical and Biochemical Characteristics

Table 2-2. shows the anthropometric and biochemical data of the FDRs and the control group. The only significant differences were in total plasma homocysteine ($p<0.001$) and HDL cholesterol ($p<0.05$) levels, both of which were higher in the FDRs. Dividing the subjects into low, medium and high risk categories based on Framingham risk score showed no significant differences between the cohorts. FDRs had seven medium risk and two high risk subjects as opposed to two medium and one high risk subject in the control group. Surprisingly, no differences were found in lipid profiles, 12-hours fasting glucose, apolipoprotein levels or non-traditional risk factors such as high sensitivity C-reactive protein or lipoprotein (a). Calculated atherogenic index reflecting lipoprotein particle size distribution was also similar between FDRs and controls.

2.3.2 Carotid B-mode ultrasound findings

Wilcoxon nonparametric ranking test was used to test if the B-mode ultrasound measurements, as continuous variables, were different between studied cohorts **(Figure 2-2.)**. Ultrasound measurements of average IMT did not reach statistically significant differences when FDRs were compared to either M-CHAT or historical controls [0.66 (0.60 – 0.74) vs. 0.65 (0.58 – 0.72) or 0.67 (0.58 – 0.72) mm]. However, all the other B-mode ultrasound indexes that indicated the number and/or size of the plaques were

Table 2-2. Characteristics of FDRs and their matched controls (M-CHAT Study): #

Characteristic	FDRs (n=111)	MCHAT (n=111)
BMI	26.8 ± 5.2	27.3 ± 5.1
Waist circumference (cm)	86.8 ± 19.4	87.4 ± 12.9
Systolic BP (mmHg)	119 ± 18.17	116 ± 17.13
Diastolic BP (mmHg)	75.00 ± 12.58	75.32 ± 11.70
Fasting Glucose (mmol/L)	5.00 ± 1.10	5.14 ± 0.63
Total Cholesterol (mmol/L)	5.37 ± 1.23	5.24 ± 1.08
Triglycerides (mmol/L)	1.43 ± 0.89	1.39 ± 0.93
LDL-C (mmol/L)	3.26 ± 1.14	3.28 ± 0.99
HDL-C (mmol/L)	1.42 ± 0.46 *	1.32 ± 0.35
TC/HDL-C	4.09 ± 1.39	4.27 ± 1.49
Log [TG/HDL-C]	- 0.11 ± 0.81	- 0.07 ± 0.78
Lp(a) (mg/L)	170 (45 - 466)	174 (95 - 319)
Total homocysteine (mg/L)	9.6 (8.02 - 11.1)**	7.5 (6.4 - 8.7)
hs-CRP (mg/L)	1.0 (0.6 – 2.6)	1.0 (0.7 – 3.1)
Apo-AI (g/L)	1.52 ± 0.30	-
Apo-B100 (g/L)	1.03 ± 0.28	0.99 ± 0.28
Smoking (n)	16	9

Note: * $p<0.05$;** Sig. $p<0.001$; # data is presented as mean ± SD, except for homocysteine, Lp(a) and hs-CRP where median and interquartile range are shown.

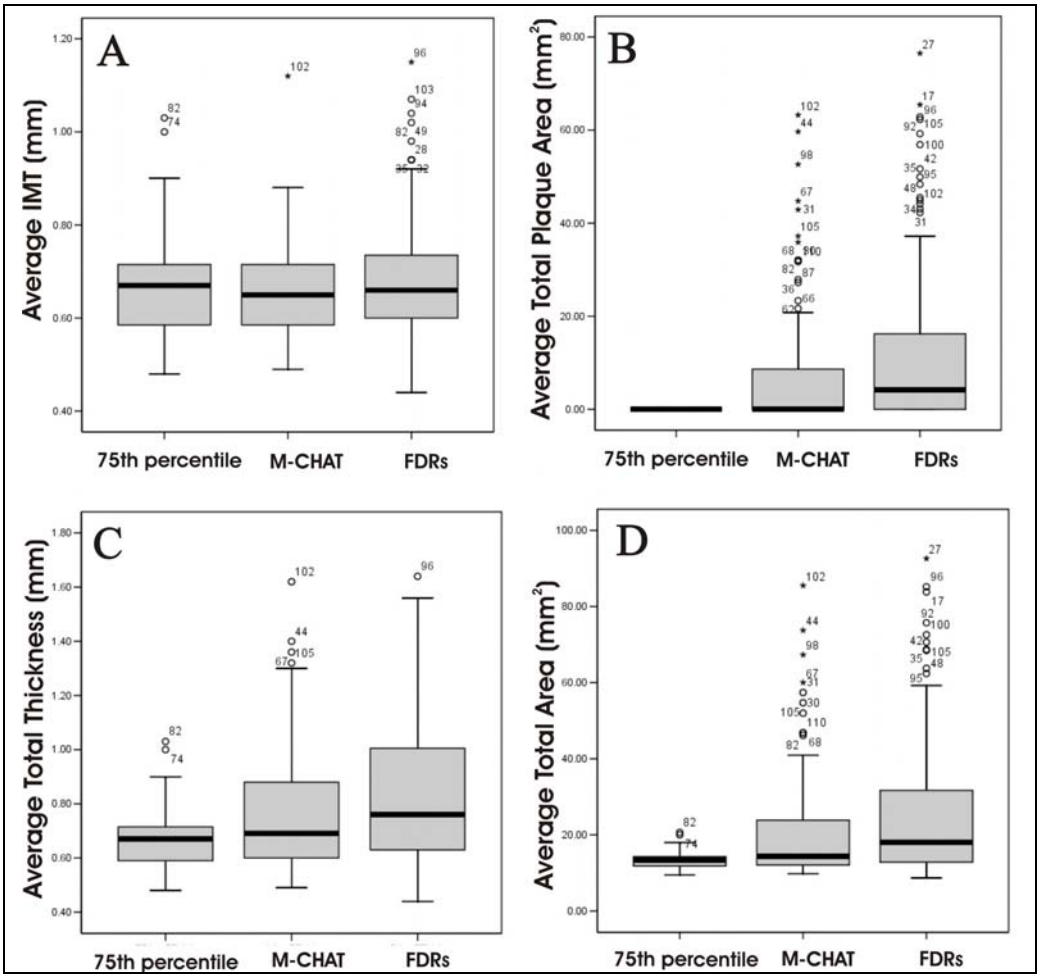

Figure 2-2. β-mode carotid ultrasound measurements:

A: Average intima-media thickness (mm); **B:** Average total plaque area (mm^2); **C:** Average total thickness (mm); **D:** Average total area (mm^2); Box plots (median and 25^{th} to 75^{th} interquartile range) for FDRs compared to prospective (M-CHAT) and historical controls (75^{th} percentile cut-off point for the normal population – age and gender adjusted values);

significantly different between FDRs and M-CHAT controls. These included the total plaque area [4.17 (0 – 16.71) vs. 0 (0 – 8.67) mm^2, $p < 0.05$], average total thickness [0.76 (0.63 – 1.01) vs. 0.69 (0.60 – 0.88) mm, $p < 0.001$] and total area [18.00 (12.8 – 32) vs. 14.42 (12.04 – 24.72) mm^2, $p < 0.05$]. These differences were even greater when FDRs data were compared with the historical controls: total plaque area [4.17 (0 – 16.71) vs. 0 mm^2, $p < 0.001$], average total thickness [0.76 (0.63 – 1.01) vs. 0.67 (0.59 – 0.72) mm, $p < 0.001$], total area [18 (12.8 – 32) vs. 13.3 (11.78 – 14.36) mm^2, $p < 0.001$]. **(Table 2-3.)**

The loglinear general model was used to calculate the odds ratio of increased total area, increased average total thickness or increased IMT findings between FDRs and controls. The odds of positive findings in total area, average IMT (OR 2.235, 95%CI 1.146 – 4.373; $p<0.02$) and average total thickness (OR 2.313, 95%CI 1.227 – 4.359; $p<0.02$) were significantly higher in FDRs compared to controls.

2.3.3 Correlations Strength and Predictive Values

Table 2-4. shows the strength of the correlations (assessed using Spearman rank correlations test) between the carotid ultrasound measurements (those that were significantly different between the subjects with and without family history of premature CAD) and the traditional and non-traditional risk factors for CAD.

There was a statistically significant ($p<0.05$ to 0.005) negative correlation (rho ranging between -0.228 and -0.292) between the age of the index patient at the time of

Table 2-3. Summary of carotid ultrasound findings in FRDs, M-CHAT and historical controls#:

	FDRs	M-CHAT	Historical
n	111	111	n/a
Average IMT (mm)	0.66 (0.60 - 0.74)	0.65 (0.58 - 0.72)	0.67 (0.58 - 0.72)
Total plaque area (mm^2)	4.17 (0 - 16.71) **	0 (0 - 8.67)*	0
Average total thickness (mm)	0.76 (0.63 - 1.01) **	0.69 (0.60 - 0.88) **	0.67 (0.59 - 0.72)
Total area (mm^2)	18 (12.8 - 32) **	14.42 (12.04 - 24.72)*	13.3 (11.78 0 14.36)
Plaque No	1 (0 - 3) **	0 (0 - 1) **	0

Note: * $p<0.05$; ** $p<0.001$; # Data is expressed as median and interquartile range. Wilcoxcon nonparametric ranking test is used to assess the differences between FDRs and matched controls (M-CHAT) or between FDRs and historical controls (the 75th percentile, age and gender matched, customarily used as a threshold to define positive findings);

Table 2-4. Spearman's rho* correlations (carotid B-ultrasound vs. other markers)

Variables	AvgIMT	TPArea	AvgTThick	TArea	PlaqueNo
Age	0.575	0.363	0.561	0.519	0.328
Systolic BP	0.358	0.253	0.378	0.357	0.226
Waist circumference	0.310	0.279	0.319	0.320	0.245
f-glucose	0.326	0.179	0.284	0.267	0.173
Total Cholesterol	0.259	0.291	0.352	0.340	0.282
Apo-B100	0.301	0.295	0.359	0.340	0.287
LDL-c	0.277	0.287	0.360	0.345	0.271
TC/HDL-c	0.283	0.224	0.299	0.271	0.205
Homocysteine	0.251	0.235	0.281	0.282	0.244

Note: Bivariant Spearman's test was employed to assess the strength of correlations between carotid ultrasound indexes (in the heading) with other risk factors (listed in the first column) in the FDRs cohort; * rho - correlation coefficient (strength of correlation ranges form 0 to 1; 0 = no correlation); all values in the table were statistically significant $p<0.001$ (2-tailed); AvgIMT = average intima-media thickness; TPArea = total plaque area; AvgTThick = average total thickness; TArea = total plaque area; PlaqueNo = number of plaques;

the first cardiovascular event and FDRs carotid findings namely plaque number, total plaque area, total area and average total thickness. In addition, carotid ultrasound indexes correlated with kinship, the relation being stronger for siblings (rho ranging between 0.239 and 0.323, $p<0.01$) than parents or children.

Homocysteine levels correlated with age ($r = 0.175$, $p<0.01$) systolic blood pressure ($r = 0.176$, $p < 0.01$) and waist circumference ($r = 0.214$, $p < 0.05$). The strength of the correlation between homocysteine and carotid ultrasound indexes (TA and TPA) was higher in the FDRs (R-squared = 0.16, $p<0.001$) then in controls (R-squared = 0.07, $p<0.05$).

Multivariate linear regression modeling assessed the predictive value of family history of premature CHD on carotid ultrasound findings. In the base model (model 2), adjusting for age ($B = 0.441$, $p = 0.001$), TC / HDL-cholesterol ratio ($B = 0.259$, $p = 0.001$), systolic blood pressure ($B = 0.100$, $p = 0.105$), waist circumference ($B = 0.092$, $p = 0.139$) and smoking ($B = 0.055$, $p = 0.343$), did not affect the statistical significance for all the carotid ultrasound indexes except for the average IMT. **(Table 2-5.)** Adjusting for total plasma homocysteine levels in the extended model (model 3), at any point of constructing this model, made group assignment (first degree relatives vs. controls) loose its strength to predict positive carotid ultrasound findings. However, group assignment has maintained its predictive value for high homocysteine levels after adjusting for all of the other risk factors including the carotid ultrasound findings. In the gender subgroup analysis the predictive value of family history for carotid ultrasound indexes was stronger for men then for women. **(Table 2-6.)**

Table 2-5. Impact of family history on carotid ultrasound traits - adjusting for confounders:

	AvgTThickness		AvgTPArea		AvgTArea		Plaque No	
	Beta	Sig.	Beta	Sig.	Beta	Sig.	Beta	Sig.
Adjusted*								
Model 1#	0.139	0.027	0.140	0.039	0.144	0.030	0.158	0.019
Model 2&	0.143	0.017	0.144	0.030	0.148	0.021	0.157	0.017
Model 3§	0.055	0.343	0.028	0.663	0.031	0.616	0.034	0.628

Note: Table summarizes the multivariate linear regression analysis used to assess the predictive value of family history for carotid ultrasound findings after adjusting for confounders as per the following models: # Adjusted for age only; & Adjusted for age, TC/HDL-c ratio, systolic BP, waist circumference and smoking; § Adjusted for model 2 covariates plus homocysteine; * Standardized β length and significance values for the group assignment (FDRs vs. M-CHAT).

Table 2-6. Gender subgroup analysis - Impact of family history on carotid traits after covariate adjustment:

	AvgTThickness		AvgTPArea		AvgTArea		Plaque No	
	Beta*	Sig.	Beta	Sig.	Beta	Sig.	Beta	Sig.
Adjusted#								
Male	0.210	0.033	0.195	0.072	0.200	0.059	0.212	0.045
Female	0.145	0.062	0.134	0.130	0.143	0.094	0.142	0.110

Note: The table summarizes the predictive value of family history for carotid ultrasound findings after adjusting# for age, TC/HDL-c ratio, systolic BP, waist circumference and smoking male versus female subjects; * Standardized β length and significance values for the group assignment (FDRs vs. M-CHAT).

2.4 Discussions

Our study indicates that family history of early onset of CHD doubles the odds of subclinical atherosclerosis in asymptomatic subjects **(Table 2-7.)**. We have demonstrated that family history is a strong predictor of carotid ultrasound findings independent of age, serum lipids, systolic blood pressure, waist circumference and smoking status. In FDRs, using composite indexes of IMT and plaque burden such as total area, average total thickness or plaque measurements such as total plaque area and plaque number are superior to using IMT measurement alone. Carotid ultrasound findings were strongly correlated with all traditional cardiovascular risk factors. We also observed that carotid ultrasound findings were strongly correlated with all cardiovascular risk factors measured and that total plasma homocysteine, the only risk factor that was statistically different between the two cohorts, shows the lowest correlation strength among them. The predictive value of family history for carotid abnormalities however was diminished by adjusting for homocysteine in a multivariate linear regression analysis. This suggests that in this cohort plasma homocysteine level may be one of the factors responsible for the noted differences in the carotid findings.

In the largest prospective study to date, 12,802 asymptomatic subjects from the Atherosclerosis Risk in Communities Study (27) were investigated using carotid ultrasound while their family history (assessed by recall) was quantified as the 'family risk score'. There was a high correlation between mean IMT and the family risk score which could not be fully explained by the assessed risk factors in whites, as it could in

Table 2-7. Odds Ratios (95% CI) of positive findings at carotid ultrasound in FDRs versus matched controls*:

&	OR	95% CI	Sig.
Average IMT	2.235	(1.146 - 4.373)	0.019
Average Total Thickness	2.313	(1.227 - 4.359)	0.010
Average Total Plaque Area	1.318	(0.763 - 2.276)	0.322
Total Area	2.235	(1.143 - 4.373)	0.019
Plaque No	1.417	(0.820 - 2.449)	0.212

Note: Logistics regression was used to calculate odds ratio of positive findings at carotid ultrasound scan in FDRs versus controls. & Data was expressed as case-control odds ratio, 95% confidence interval and significance.

* Positive findings for coronary indexes were defined as > 75th percentile of normal, age and gender adjusted;

African-Americans. In the first prospective assessment of family history of CHD in 1,662 offspring of participants from Framingham heart study, Wang et al. (28) found that family history of premature CHD correlated independently with internal carotid artery (ICA) IMT, while family history of late onset of CHD can be fully explained by the existing risk factors. Some studies (17, 29) indicate that ICA-IMT is a better indicator of risk than common carotid artery (CCA) IMT, while others (29) reported that parental history of MI was correlated with CCA-IMT but not with ICA-IMT. In much younger subjects (27-30 years old), Oren et al. (30) noticed no contribution of family history to IMT while Juonala et al.(31) reported small but significant differences in CCA-IMT with higher correlations between IMT and other risk factors in subjects with family history of CHD.

Our findings show, similar to previous studies, that asymptomatic subjects with positive family history of CHD are more likely to have subclinical manifestations of atherosclerosis than controls. However, unlike others we have not observed a difference in average CCA-IMT between the groups. This may be due to the smaller size of our cohort or to methodological differences. We measured IMT of the far-wall in a segment of CCA free of plaques, while plaques anywhere in CCA or ICA were considered. This allowed making the distinction between early atherosclerotic lesions (plaques) and other causes of increased IMT like hyperplasia and hypertrophy which could also have a genetic component but may involve different pathological pathways. As plaques progress 2.4 times faster then IMT thickening, measuring plaque area or volume in early stages of the disease could increase the sensitivity of the method(32). Minute increases in IMT with years of risk factors exposure make IMT a less useful measurement than plaque assessment in younger individuals where heredity rather than environmental risk factors

are the strongest contributors to atherosclerosis(33). The presence of plaque in carotid arteries of asymptomatic individuals with family history may signal genetic causes of the disease; this may be more useful in clinical decisions.

Spence et al. (34) found high correlation between tHcy levels and carotid plaque area. In the largest study (1467 subjects) to assess the impact of homocysteine levels on carotid IMT, Tsai et al. (35) also indicated high, independent correlation between total plasma homocysteine (tHcy) and IMT. Smaller case-control studies (34) indicated that while tHcy correlates with carotid IMT, no such relation exists with the C677T mutation (35). No relation of tHcy and family history of early CHD on carotid ultrasound findings have been reported in previous studies (28-30). In patients with cerebrovascular disease, Linnebank et al. (36) found no correlation between tHcy or its gene polymorphism and IMT. In view of the above data, it is likely that increased levels of tHcy in the FDRs partially explain the differences seen in the carotid ultrasound findings particularly the increase in plaque formation.

We found that homocysteine levels were correlated with family history of CHD independent of the traditional risk factors and carotid ultrasound findings. With evidence from VISP (37) and HOPE-2 (38) trials, now completed, it is likely that tHcy levels are merely an indicator of higher risk in this population rather than a cause of the findings (without excluding its confounding effect on carotid ultrasound assessment).

These findings may be due to the recruitment bias: only relatives from 82 families participated in the study. Though unlikely, it is possible that a cluster of genes or an environmental component may have resulted in the increased levels of total

homocysteine. Previous studies indicate that adjusting for glomerular filtration rate in patients with chronic kidney disease cancels the predictive value of total plasma homocysteine for coronary events (39). Though unlikely, since we did not assessed kidney function in FDRs we cannot exclude that a degree of kidney function impairment in these subject has skewed our results. Plasma homocysteine levels are increased by folic acid and vitamin B12 deficiency, thus we cannot exclude diet as a factor. This is unlikely as well, since fortification of wheat flour, pasta and cornmeal with folic acid became mandatory in Canada effective November 1998. When we assessed the levels of vitamins consumption using the food frequency questionnaire developed by Harvard School of Public Health (23), no correlations were found between vitamin B12 consumption and total plasma homocysteine levels in the studied cohort. These findings suggest that homocysteine is an indicator of increased risk in the FDRs.

Fasting glucose, lost its predictive value for group assignment and thus was eliminated, as a covariant, from our base model when we've excluded those 14 subjects that declared diabetes, glucose intolerance or thoracic pain. Eliminating these subjects from the analyses comparing the two groups may have weakened our results but was necessary to validate our hypothesis.

Correlations between carotid ultrasound indexes with kinship and age of the index patient at the time of the cardiovascular event suggest these factors are important, but this study was not powered to address these questions. Carotid ultrasound scan was performed one year after the baseline visit. This may have increased the participant's awareness of risk and slightly modify the findings.

Technical variations in the way carotid ultrasound abnormalities are explored make the direct comparison of our results with those from similar studies difficult. Our FDRs cohort was predominantly European and thus the results cannot be generalized to other ethnic groups.

Our study is unique in the angiographic confirmation of CHD in index patients which eliminated possible recall bias, in thorough phenotyping of the participants and the inclusion of composite plaque-IMT measurements to assess subclinical carotid atherosclerosis in young asymptomatic patients.

2.5 Conclusion

Simultaneous assessment of carotid plaques and intima-media thickness is likely to improve the prediction of CV risk in subjects with family history of premature CHD. While the observed differences in plasma homocysteine levels partially explain the increased frequency of positive carotid ultrasound findings in FDRs other genetic or environmental factors could be responsible for these observations. Measuring total homocysteine levels as well as assessing carotid arteries for plaques or abnormal thickening may provide additional information about the cardiovascular risk in FDRs.

2.6 Acknowledgements

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CHAPTER III: CORONARY ARTERY CALCIFICATION ASSESSED BY 64-SLICE MDCT IN ASYMPTOMATIC SUBJECTS WITH FAMILY HISTORY OF PREMATURE CORONARY ARTERY DISEASE [1]

3.1 Introduction and Rationale

Validated family history of premature coronary artery disease (CAD), men< 55 and women <65 years of age, is an independent risk factor for cardiovascular disease (CVD) (1-5). Although Framingham risk score (6, 7) does not include family history, European SCORE risk tools (8) and current guidelines for management of dyslipidemia (9, 10) suggest a two-fold increase in risk of developing CVD in the presence of positive family history of CAD.

Calcium deposits in the coronary arteries are considered an accurate marker of atherosclerotic burden (11). Sangiorgi et al. demonstrated a very high correlation (r = 0.8 - 0.9) between the amount of calcium quantified by electron beam computed tomography (EBCT) and calcified area in histological sections (12). CAC assessed by computed tomography (CT) is a strong predictor of cardiovascular (Relative Risk [RR]=9.6) and coronary events (RR=11.1), non-fatal myocardial infarction (RR=9.2) and an independent predictor of all-cause mortality (13-15). Today, the coronary artery

[1] A version of this chapter is under review for publication. Taraboanta C. et. al., Coronary artery calcification assessed by 64-slice MDCT in asymptomatic subjects with family history of premature coronary heart disease;

calcification (CAC) score is recognized as a highly sensitive but less specific method to measure subclinical atherosclerosis in asymptomatic patients (13, 14, 16-18). CAC scans provide incremental, independent prognostic value above the classic Framingham risk factors in young asymptomatic men (18). The benefits of CAC scoring to assess risk in asymptomatic individuals known to belong to a high risk population remain controversial.

Our study, using a prospective case – control design, compared the frequency of positive CAC findings, assessed by multi-detector computed tomography (MDCT), in asymptomatic individuals with and without family history of early CAD. Multiple thresholds defining positive findings at CAC scan were tested. In addition we compared CAC findings with carotid ultrasound indexes of early atherosclerosis (combined measurements of carotid intima-media thickness and plaque), as well as other traditional and non-traditional risk factors assessed in the first degree relatives (FDRs) cohort.

3.2 Materials and methods

The study protocol was approved by both institutional ethical review boards of St. Paul Hospital and University of British Columbia; all participants gave informed consent.

3.2.1 Study Populations

3.2.1.1 Cases – First Degree Relatives

First degree relatives (FDRs) of patients with angiographically confirmed premature CAD (men <50 years, women <60 years), had previously been phenotyped for their risk factors profile and carotid ultrasound findings (10.106/j.ahj.2007.11.046). In brief, classical (age, gender, smoking status, blood pressure, lipid profile, apolipoprotein and fasting glucose) as well as newly recognized risk factors (lipoprotein (a), high sensitivity C-reactive protein and total homocysteine) had been measured. Carotid B-mode ultrasound was carried out in 111 FDRs with carotid intima-media thickness (CIMT), plaque size and number recorded. A combination of IMT and plaque measurements was used to assess burden of atherosclerosis in these asymptomatic subjects using previously validated methods (19, 20). In short, total plaque area (TPA) the product of length and thickness of the lesion(s), total area (TA) the sum of all areas of carotid wall measured and average total thickness which is TA divided by the total length of carotid wall measured were recorded.

Subjects who had a chest X-ray or CT in the previous 12 months, pregnant women or women who might have been pregnant were excluded from the study. A total

of 74 consenting FDRs were referred to undergo CAC scoring of whom 57 (2 parents, 18 children and 37 siblings), 25 years of age and older, without diabetes or chest pain completed the assessment and are included in this analysis. Participants underwent the MDCT scan within one year of the carotid ultrasound and within a year and a half of first contact. A copy of the results has been mailed to the study subjects and their physician, if the participant so requested.

3.2.1.2 Contemporary Controls

Controls were drawn from an initial cohort of 3500 asymptomatic subjects who underwent CAC scoring between January 2002 and May 2007 at a private imaging center. Exclusion criteria included presence of CAD, prior assessment of CAC (no follow-up examinations were included in the control group) or age under 40. These subjects were either self-referred, referred to the clinic by a physician or were executives having the scan done as part of their health plan. To match the criteria applied to the study subjects we excluded control subjects that declared on the pre-scan questionnaire that they had chest pain or diabetes.

A total of 616 subjects (57.2 + 10 years old; 76% men, 24% women) served as the control group. This represents 20% of the total number of subjects scanned, who stated, on the pre-scan questionnaire, no family history of cardiovascular disease. Smoking status was confirmed in 416 subjects. Therefore at the time of the scan 43 of them were smokers (defined as currently smoking or gave up in the last month); extrapolating the results, smokers represent 10% of the control group.

3.2.2 Ethics and Data Collection

The study has been conducted with the approval of institutional ethical review boards of St. Paul Hospital and University of British Columbia. For publication purposes the study has been registered with ClinicalTrials.Gov. **(Appendix 1, 2)**

Hardcopies of medical records for FDRs have been stored onsite (Healthy Heart Research office) with access restricted to FACT study research team. An MS Access database has been created and independent double data entry performed. The two datasets have been reconciled against hardcopy data. Access to the electronic data has been restricted to FACT study research team; a combination of network/user restrictions at database level has been applied to maintain the integrity and privacy of participant's records. **(Appendix 7)**

MDCT scan results for CAC scoring have been provided by the Canada Diagnostic Center staff when study has been completed. Pre-scan questionnaires, and scan results for the controls were stored at Canada Diagnostic Center with access restricted to their staff; guided by internal policies. Canada Diagnostic Center provided the scan results for the controls in a separate datasheet after personal identifiable information has been deleted. **(Appendix 8)**

3.2.3 Calcium Score Assessment - (64-slice MDCT)

All FDRs were scanned with a 64-slice MDCT (Aquilion 64, Toshiba America Medical Systems, Tustin, California) while subjects from the control group were scanned

with either 64 or 8-slice MDCT (Lightspeed Ultra, GE, Milwaukee, Wisconsin). The participants were told to abstain from caffeine the morning of the day of the scan. No pharmacological intervention or oxygen was administered prior to the scan to reduce the heart rate. The scan extended from the ascending aorta 12 cm inferiorly towards the cardiac apex with a slice collimation of 64 x 0.5 mm (100mA, 120KVp, average effective dose 0.9-1.1mSv) for the Aquilion and 8 x 2.5 mm (230mA, 120KVp, average effective dose 1mSv) for Lightspeed Ultra. Breath-hold image acquisition used prospective ECG gating (triggered at 50% of cardiac cycle) to minimize radiation dose (21).

CAC scoring was performed by two physicians, by consensus, on an offline computer station using the VScoreTM with AutoGateTM (Vitrea, Vital Image Software package; version 3.9). The radiologists were not blinded when reading FDRs scans. Under radiologist control each coronary artery (left main, left anterior descending, left circumflex, right and posterior descending artery) was selected; Agatston and Volume scores calculated by the software package. In calculating the Agatston score (22) plaques with greater attenuation were weighted higher (pixels 0.26-0.35 mm2; >130 Hounsfield units), while volumetric score using isometric interpolation eliminates partial volume effect (23). The total Agatston and total Volume scores reported herein are the sum of partial scores obtained for the five coronary artery readings. **(Appendix 9)**

3.2.4 Statistical Analysis

Descriptive statistics for the study cohorts were presented as mean and standard deviation for all variables except total homocysteine, Lp(a), high sensitivity C-reactive

protein, carotid ultrasound measurements and CAC scores for which median and interquartile range were used. To test for the difference between means, Mann-Whitney rank test, which assumes independence of the two groups, was used. Fisher's test and/or Chi-square were used to test for differences in dichotomous variables. Two tail tests and 95% confidence intervals were used for all analyses. The strength of correlation between the CAC (total Agatston and total Volume) score, other known risk factors and carotid ultrasound indexes in the FDRs was assessed by univariate analysis and Spearman's rank correlation test. To determine whether family history of premature CAD is predictive of higher CAC scores after adjusting for age and gender we used a multivariate linear regression analysis. To determine if family history of early CAD increases the likelihood of a positive findings at CAC scan we employed Chi-square analysis. We defined positive findings as values over 50th, 75th or 90th percentile of age and gender adjusted values (24, 25).

Odds ratios with 95% confidence intervals were calculated using a loglinear general model to determine the odds of positive CAC findings in FDRs compared to controls. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA) software package.

3.3 Results

3.3.1 Clinical and Biochemical Characteristics of the FDRs

The FDRs were predominantly European (48 out of 54) with only 6 South-Asian, 2 Chinese and one person Aboriginal. There was a significant difference in gender distribution between the study group in which there were slightly more women (56% women, 44% men) and the control group (24% women, 76% men) in which men were predominant. FDRs group (47.3 $\pm$ 8.9 years old) was 10 years older then the control group (57.2 $\pm$ 10 years, $p<0.001$). The slightly higher incidence of smoking in FDRs (14%) compared to controls (10%) was not statistically significant. Unadjusted values for CAC findings were not significantly different between FDRs (total Agatston 0 [0 – 41.5] and total volume 0 [0 – 50.5]) compared with controls (total Agatston 0 [0 – 83] and total volume 0 [0 – 69.7]). **Table 3-1.** lists the biochemical and anthropometrical characteristics of the FDRs cohort which were not available in controls. This does not allow us a direct comparison, though we can point out a rather unremarkable biochemical profile for the FDRs with most values within normal range.

3.3.2 Correlations strength and likelihood of findings

The relationship between assessed risk factors, carotid ultrasound findings and the extent of coronary calcification are summarized in **Table 3-2.** Higher correlations were attained by carotid ultrasound indexes measuring plaque and intima-media thickness than CIMT alone. All carotid ultrasound measurements had a higher correlation with CAC scores than with any other risk factor including Framingham risk score. After adjusting

Table 3-1. Characteristics of FDRs:

Characteristic	FDRs
n	57
Age	47.3 ± 8.9
BMI	27.5 ± 4.5
Waist circumference	90.3 ± 15.2
Systolic BP	117.5 ± 17.4
Diastolic BP	74.9 ± 14.0
Fasting Glucose	4.92 ± 0.8
Total Cholesterol	5.43 ± 1.1
Triglycerides	1.33 ± 0.8
LDL-c	3.40 ± 1.0
HDL-c	1.42 ± 0.43
TC/HDL-c	4.15 ± 1.48
Lp(a)	153 (43 - 467)
Total homocysteine	9.6 (8.8 - 11.0)
hs-CRP	0.9 (0.7 - 3.25)
Apo-AI	1.54 ± 0.31
Apo-B100	1.04 ± 0.29
Smoking (n,%)	8 (14%)
MDCT scores	
Total Agatston score	0 (0 - 41.5)
Total Volumetric score	0 (0 - 50.5)
Carotid Ultrasound Indexes	
Average IMT	0.67 (0.62 - 0.73)
Total plaque area	5.59 (0 - 21.34)
Average total thickness	0.77 (0.67 - 1.00)
Total area	19.0 (13.45 - 35.44)
Plaque No	1 (0 - 3)

Note: data is presented as mean ± SD, except for homocysteine, hs-CRP, Lp(a) or the imagistic indexes were median and interquartile range was used.

Table 3-2. Correlations of total Agatston and total volume scores with risk factors and carotid ultrasound findings in FDRs:

	Total Agatston		Total Volume	
	rho*	p	rho*	p
n = 57				
Age	0.425	0.001	0.421	0.001
Diastolic BP	0.267	0.045	0.267	0.045
Framingham risk score	0.279	0.036	0.280	0.035
fasting Glucose	0.298	0.017	0.283	0.024
Total Cholesterol / HDLc	0.256	0.041	0.259	0.039
Ultrasound indexes				
AvgIMT	0.398	0.002	0.402	0.002
TotalParea	0.458	0.000	0.460	0.000
AvgTThick	0.520	0.000	0.525	0.000
TArea	0.495	0.000	0.498	0.000
Plaque No	0.480	0.000	0.478	0.000

* rho - correlation coefficient (strength of correlation ranges form 0 to 1; 0 = no correlation) ;statistically significant $p<0.05$; highly significant $p<0.005$ (2-tailed).

Note: Spearman's test measures the strength of correlations between calcium scores (in the heading) and other risk factors (listed in the first column) in the FDRs cohort; Carotid Ultrasound Indexes: AvgIMT = average intima-media thickness; TPArea = total plaque area; AvgTThick = average total thickness; TArea = total plaque area; PlaqueNo = number of plaques;

for age and gender in a multivariate linear regression model, family history of CAD was highly predictive of coronary calcium findings. When adjusting only for age **(Table 3-3.)**, in sub-gender analysis, this relationship holds true for women but not for men.

Highly skewed distribution of Agatston and Volume scores required their transformation in dichotomous variables, age and gender adjusted cut-off points, to analyze for differences between cohorts. Chi-square analysis was employed to test for differences in likelihood of positive findings at CAC scan between FDRs and controls. **Table 3-4.** indicates FDRs as more likely to have positive CAC findings compared with controls at any of the previously used thresholds; this indicates however that the 90th percentile best distinguishes between people with and without family history of early CAD.

The odds of having positive CAC findings in FDRs versus controls were higher for the 90th percentile (OR 2.597, 95%CI 1.232 – 5.473; $p<0.05$) then the 75th percentile (OR 1.959, 95%CI 1.044 - 3.673; $p<0.05$) though the confidence interval was much wider. **Table 3-5.** displays the gender analysis indicating that 75th percentile better differentiates men with family history from those without, while the 90th percentile better differentiates women with from those without family history of premature CHD.

Table 3-3. The predictive value of CAC scoring assessd by 64-slice MDCT for presence of family history of premature CAD

	Total Agatston		Total Volume	
Family history	Beta	Sig.	Beta	Sig.
Total †	0.102*	0.009	0.103*	0.011
Women	0.185*	0.022	0.185*	0.022
Men	0.053	0.227	0.051	0.267

* significant $p<0.05$;† Multivariate linear regresion model, age and gender adjusted;

Table 3-4. Chi-square differences in the likelihood of having positive CAC findings in FDRs vs. controls:

	$\chi^{2\,\dagger}$	P
χ^2 (1, 673) =		
>50th percentile	6.33	0.012
>75th percentile	12.78	0.000
>90th percentile	32.08	0.000

*Note: the 50th, 75th and 90th percentile have been used as cut-off points defining positive CAC findings; age and gender adjusted values; † weighted by group

Table 3-5. Association of family history of premature CHD with coronary arteries calcification as assessed by 64-slice MDCT.

	OR*	95% CI	P
Family history†			
(Age and gender adjusted)			
75th percentile	1.959	1.044 - 3.673	0.036
Male	2.460	1.024 - 5.905	0.044
Female	1.806	0.691 - 4.723	0.228
90th percentile	2.597	1.232 - 5.473	0.012
Male	2.192	0.718 - 6.692	0.168
Female	3.525	1.139 - 10.906	0.029

† Angiographically confirmed family history of CHD vs. no reported presence of any FHx of cardiovascular disease;

* Mantel-Haenszel common odds ratio estimate.

3.4 Discussions

Our study demonstrates that in asymptomatic subjects with family history of early CAD the odds of having CAC above the 75th or the 90th percentile, age and gender adjusted, is twice as high as in controls. The likelihood of severe CAC findings (>90th percentile) in FDRs compared to controls is higher than the likelihood of having mild CAC (> 50th percentile). Cut-off values, indicating increased risk, for CAC scoring appear to differ between men and women with positive family history of CAD even after adjusting for age and gender differences. Though not powered to address this question, these results indicate a higher frequency of findings in men and a higher severity of findings in women. We also had the opportunity to demonstrate high correlations between CAC scan results and carotid ultrasound indexes of early atherosclerosis, particularly those indexes that combine plaque burden and intima-media thickness measurements. Our study is supportive of the possible use of CAC scoring to improve risk assessment in asymptomatic FDRs in addition to Framingham risk score and carotid ultrasound indexes.

Terry et. al. showed CAC (AUC 0.91) to be superior to IMT (AUC 0.73) as predictors of angiographic CAD. This was also suggested by Sesse et al. a decade earlier (26, 27). Similarly, in our cohort, CAC had higher odds ratio (OR) then IMT in predicting the presence of family history (10.106/j.ahj.2007.11. 046). When using the 90th percentile, the CAC odds ratios were higher for women then for men, an observation

consistent with previous studies suggesting that CAC is highly indicative of CAD in women (27, 28). In contrast, we demonstrated that if the 75th percentile is utilized, the CAC odds ratios are higher for men then for women. It s unclear form this investigation whether or not this finding pertains solely to first degree relatives.

In the largest study to date that evaluated the impact of various risk factors on CAC findings, Budoff et al. showed that CAC can predict all cause mortality, independent of family history of premature CAD (15). In the Framingham study cohort, a validated family history of CHD correlated with a two fold increase in CAC findings, when using the 90th percentile cut-of point in a younger cohort (29). Independent of Framingham Risk Score (FRS), a CAC scan can improve assessment of risk of coronary events in subjects with FRS <10% but not for those with FRS > 10% (30). In the Dallas Heart study, significant association between family history of MI and CAC was shown in younger (men <45, women <55) but not in older subjects (31). Similar to previous studies our results indicate that in middle-aged (39-55 years old) asymptomatic FDRs the incidence of positive CAC findings double that of controls. Our study uniquely suggests that that this is true at any CAC defined cut-off point and that the differential threshold might need to be gender specific to optimize sensitivity.

Taylor et al., in a prospective 4-year follow up study, demonstrated a significant and independent relation between carotid non-calcified atherosclerosis and progression of CAC. In the same study (Prospective Army Coronary Calcium Rescan Project) CAC was associated with a 11.8-fold increase in risk of CHD events after controlling for traditional risk factors (18, 32). In accordance with these findings, our study demonstrated high correlations between carotid ultrasound indexes and CAC score.

Qualitative analysis of the data assessed the relation between anatomical changes in the carotid arteries and calcification of the coronaries. Of the 57 asymptomatic FDRs included in this study 10 had no plaques and no increase in IMT, 22 had plaques present but no increase in IMT and only 15 had both plaques and increase in IMT. One subject with no plaque and no increase in IMT had calcium score falling in the second quartile. Having plaques detected by carotid ultrasound increased the risk of having a positive coronary artery calcium scan, defined as above the 75th percentile (age and gender adjusted), by 2.3 times. In practice, an Agatston score above 100 is considered to be highly predictive for CAD (33). All 10 FDRs with an Agatston score over 100 had plaques detected at carotid ultrasound scan. However, not all subjects with plaques alone or plaques and increased IMT, had positive CAC findings. Out of 38 FDRs with zero Agatston score 22 had detectable carotid plaques. Thirteen of these individuals had at least one or more cardiovascular risk factors (high blood pressure, dyslipidemia or smoking). Very low event rates for subjects with zero Agatston score are reported in a multitude of studies (14, 15, 27, 34). Both CAC scoring and carotid ultrasound methods are used to evaluate burden of subclinical atherosclerosis and thus a degree of overlap in findings is to be expected. The qualitative analysis of these results suggests that using carotid ultrasound investigation in addition to the risk factor assessment in FDRs can identify all subjects in which subclinical atherosclerosis is present. Nevertheless, CAC findings, which may require longer progression of disease, could change the risk perception, when present, and result in more aggressive intervention. Thus, CAC scan may further improve risk stratification of asymptomatic FDRs, in whom traditional risk factors assessment indicates a low risk of CVD.

Our study provides a unique focus on FDRs of patients with early onset of angiographically proven CAD, and compares CAC findings to ultrasound indexes that assess both IMT and plaque burden. There are, however, limitations. The study cohort is of predominantly European ethnic background and thus the results may not be applicable to non-Caucasian populations. A mixture of 8 and 64-slice MDCT scans were used in the control group. Even so, The Multi-Ethnic Study of Atherosclerosis (MESA) study showed only negligible (less then 4%) differences exist between these two CT detector platforms (35). Due to the unavailability of biochemical and carotid ultrasound data in the controls we could not adjust for confounding risk factors other than age and gender. The study was not powered to compare directly the frequency of CAC to CIMT findings in the FDRs compared to controls and to assess how gender may influence these findings.

3.5 Conclusion

Asymptomatic FDRs have a two-fold increase in the likelihood of positive findings at CAC scoring compared to controls. CAC scores correlated highly with carotid ultrasound indexes such as total plaque area, average total thickness, total area, plaque number or average intima-media thickness. Gender-specific thresholds for CAC scoring may be appropriate to increase sensitivity in defining high risk in men and women with positive family history of premature CAD.

CAC scoring, in addition to the carotid ultrasound and traditional risk factors evaluation, may improve assessment of asymptomatic family members of patients with early onset of CAD, who are otherwise clinically unremarkable.

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CHAPTER IV: GENERAL DISCUSSION AND CONCLUSION

4.1 Summary of the main findings

Confirmed, family history of premature coronary heart disease (CHD) (men<60, women<50) in a first degree relative doubles the risk for future cardiovascular events, independent of other risk factors (1-4). More importantly, in younger adult subjects, this risk should be carefully weighted in assessing risk as traditional risk factors are not overtly expressed. In middle-age men and women with a family history of early CHD, measuring the subclinical burden of atherosclerosis may be an important clinical tool. In the present thesis, I have described increased plaque burden in carotid and coronary arteries, assessed by either carotid B-ultrasound or 64-slice MDCT respectively, which corresponds to an estimated two-fold increase in risk previously described in this selected population.

Previous studies looking at the relationship between family history of CHD and carotid intima-media thickness (IMT) showed increased IMT findings in first degree relatives (FDRs) compared to controls that are unaccounted after adjusting for the traditional risk factors (5-7). In agreement with previous studies, I have demonstrated herein that family history of early CHD is a strong predictor of positive carotid ultrasound findings, independent of age, total cholesterol to HDL-c ratio, systolic blood pressure, waist circumference and smoking, but not total homocysteine. If we

meticulously exclude plaques (focal thickening of the intima-media layer) from the IMT measurement, my data demonstrate that measuring plaques, as number and size, is important to assess risk in FDRs, while IMT alone is not. Though not powered to answer these additional findings, I noticed stronger correlations of the carotid ultrasound findings with male gender, sibling history and with a lower age of onset for the coronary event in the index patient. As the predictive value of family history for carotid ultrasound findings is diminished when adjusting for total plasma homocysteine, I have to suggest the potential use of total homocysteine values as a predictor of increased risk in FDRs.

Similar to previous studies, I show that a family history of early onset of CHD doubles the odds of having positive coronary calcium artery scans, defined as above the 90^{th} percentile, age and gender adjusted when compared to controls (8-10). However, my analysis demonstrates that the 75^{th} percentile, age and gender adjusted values, for CAC scoring is a better indicator of increased risk in male FDRs, while the 90^{th} percentile is a better indicator of risk in female FDRs. Corroborating the current literature which indicates CAC findings as highly predictive of CAD in women (11, 12), I suggest the use of different threshold levels, to define positive CAC findings, in men versus women FDRs. CAC findings correlate with carotid ultrasound measurements at higher levels then any other risk factor assessed, including the Framingham Risk Score. While the 10 FDRs with CAC score above 100, generally recognized by practitioners as equivalent of CAD, had plaques and /or increased IMT, 22 of the 38 subjects with 0 CAC score had plaques at their carotid ultrasound scan. This suggests that CAC scoring may be useful to upgrade or downgrade risk in FDRs, with positive carotid ultrasound findings, which by FRS would be classified as low or medium risk.

In conclusion, my thesis provides additional evidence that justifies the use of carotid ultrasound assessment of plaque burden in FDRs of patients with confirmed early onset of CHD. Measurement of total plasma homocysteine, as a marker of increased risk, in addition to the traditional risk factors may be appropriate in FDRs. CAC scoring in FDRs may further improve risk assessment above and beyond classical risk factor assessment and carotid arteries ultrasound exploration. Based on today's understanding and evidence provided herein the clinician has the choice of using either of these imaging techniques to assess risk in asymptomatic FDRs in addition to the classical risk assessment.

4.2 Strengths and limitations

The early onset of CHD in the index patients was confirmed by catheter angiography, eliminating the recall bias. Extensive characterization of the FDRs subjects including non-traditional risk factors like total plasma homocysteine, high sensitivity C-reactive protein and lipoprotein (a), has been performed. Carotid ultrasound measurements have been performed in a well validated cardiovascular imaging center, which allowed us to make the distinction between plaque burden and actual intima-media thickening, a process that may include pathology that is distinct from atherosclerosis. Coronary artery calcium score has been performed on state-of-the-art 64-slice Multi Detector Computed Tomography, assuring the highest level of accuracy attainable to date. Recruitment bias: the FDRs cohort is formed mainly form Caucasian subjects and derived from the original pool of 82 index patients. Thus, potential clustering of genetic or environmental factors and limited applicability of this data to other ethnic populations have to be acknowledged. The CAC control group was less perfect as missing biochemical and anthropometrical data hindered our ability to adjust for the confounders in multivariate analysis. The relative smaller size of the cohort in the CAC analysis did not allow us to see statistically significant differences in some of the trends reported herein.

4.3 Future directions

- The role of CAC scoring remains controversial as a method of detecting increased risk in asymptomatic individuals, thus further larger studies should be conducted to evaluate this technique in special population known to be a high risk: first degree relatives, diabetics and metabolic syndrome.

- A cost-efficiency study shall compare and contrast carotid ultrasound with CAC scoring as risk assessment tools and the suitability for screening asymptomatic individuals.

- A meta-analysis could be an alternative prior to morbidity and mortality data becomes available. This could also serve as a building point for clinical guidance on the appropriate assessment tools that should be used in FDRs.

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10. Parikh NI, Hwang SJ, Larson MG, et al. Parental occurrence of premature cardiovascular disease predicts increased coronary artery and abdominal aortic calcification in the Framingham Offspring and Third Generation cohorts. Circulation. 2007;116(13):1473-81. Epub 2007 Sep 4.

11. Wexler L, Brundage B, Crouse J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation 1996;94(5):1175-1192.

12. Terry JG, Carr JJ, Tang R, et al. Coronary artery calcium outperforms carotid artery intima-media thickness as a noninvasive index of prevalent coronary artery stenosis. Arterioscler Thromb Vasc Biol 2005;25(8):1723-8. Epub 2005 Jun 9.

Appendix 1. Approval of the Instititional review board for St. paul Hospital and University of British Columbia granting permition to conduct clinical research involving human subjects.

UBC/PROVIDENCE HEALTH CARE
OFFICE OF RESEARCH SERVICES
Research Ethics Board

Certificate of Research Ethics Board Approval*

*** PLEASE NOTE: This does not mean final project approval has been granted. Final project approval will not be granted until the following approvals have been received:**

Cardiac/ECG	☐	Laboratory	☐	Respiratory	☐
Radiology/Cath Lab	☐	Pharmacy	☐	Nursing	☐
Contract	☑	Other	☐		

Principal Investigator:	Department:	Reference Number:
Dr. J Frohlich	Healthy Heart Program	P02-0103
Co-investigators:		
Sponsoring Agencies:		Term (Years):
Pfizer Canada		1
Project Title:		
Family Atherosclerosis Counseling and Testing (FACT)		
Date Submitted:	Date Approved:	Amendment Approved:
May 17, 2002	OCT 2 3 2002	

Comments/Amendments:

This certificate approves of the Research Application form, Request for Ethical Review form, revised Informed Consent form for Index Patients (99-10-10), revised Informed Consent form for First Degree Relative (99-10-10), Baseline Questionnaire and Protocol

The protocol and consent form for the above-mentioned project have been reviewed by the Board and the experimental procedures were found to be acceptable on ethical grounds for research involving human subjects.

Name:
Dr. J. Kennedy, Chair
Ms. K. Dunstan
Dr. D. MacDonald
Ms. M. Mackay
Mr. J. Saunders
Mr. K. Murphy
Dr. S. Shalansky
Dr. I. Fedoroff
Dr. N. Press
Mr. G. Hillson

Dr. J. Kennedy, Chair
UBC/Providence Health Care
Research Ethics Board
Date: OCT 2 3 2002

This Certificate of Approval is valid for the above term from the original date of approval. Any changes to the protocol must be submitted to the Research Ethics Board for the continuation of approval.

UBC/PROVIDENCE HEALTH CARE
OFFICE OF RESEARCH SERVICES

Certificate of Final Approval

Principal Investigator:	Department:	Reference Number:
Dr. J Frohlich	Healthy Heart Program	P02-0103
Co-investigators:		
Sponsoring Agencies: Pfizer Canada		Term (Years): 1
Project Title: Family Atherosclerosis Counseling and Testing (FACT)		
Date Submitted: May 17, 2002	Date Ethical Approval: October 23, 2002	Date Final Approval: April 16, 2003

The above-mentioned study has recently been approved by the UBC/PHC Research Ethics Board. All other necessary departmental approvals (*Laboratory, Nursing and Contract with Pfizer Canada*) are now in place and I am pleased to inform you that you have the permission of the hospital to begin your study.

Dr. M.V. O'Shaughnessy
Vice President, Research
Providence Health Care

Date: April 16, 2003

UBC/PROVIDENCE HEALTH CARE
OFFICE OF RESEARCH SERVICES
Research Ethics Board

Certificate of Research Ethics Board Approval - Amendment

Principal Investigator:	Department:	Reference Number:
Dr. J Frohlich	Healthy Heart Program	P02-0103

Institution(s) Where Research Will be Carried Out:

St. Paul's Hospital

Co-investigators:

Bruce Forster, Catalin Taraboanta, Susanne Burns, Rajashree Devarakonda, Evelyn Wu

Sponsoring Agencies:

Project Title:

Family Atherosclerosis Counseling and Testing (FACT)

Date of Initial Approval	Term of Initial Approval	Amendment Approved:
October 23, 2002	1 Year	JUL 14 2005

Documents Included in this Approval:

Protocol (April 2005); Consent Form – First Degree Relatives and their Spouses V2 (May 2005)

The Chair/Associate Chair of the UBC/PHC REB has reviewed the amendment(s) for the above-named project and the accompanying documentation was found to be acceptable on ethical grounds for research involving human subjects.

The REB approval period for this amendment expires on the one-year anniversary date of the REB approval for the entire study.

CERTIFICATION
In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards as defined in part C Division 5 of the *Food and Drug Regulations*.
2. This Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices and
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial, which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

Approval of the Clinical Research Ethics Board by one of:
Dr. I. Fedoroff, Chair
Dr. A. McLeod, Associate Chair

Date: JUL 14 2005

The University of British Columbia
Office of Research Services,
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

Certificate of Expedited Approval: Renewal

Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
Frohlich, J.		**C04-0021**

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

Providence Health Care, Vancouver General Hospital

CO-INVESTIGATORS:

SPONSORING AGENCIES

Pfizer Canada Inc.

TITLE:

Family Atherosclerosis Counselling & Testing Project (FACT)

APPROVAL RENEWAL DATE	TERM (YEARS)	AMENDMENT:	AMENDMENT APPROVED
07 June 2006	1	First Degree Relatives/Spouses Consent Form Version 5 dd 1 Nov 2005; Optional Genetic Analysis First Degree Relatives/Spouses Consent Form Version 4 dd Dec 2005; CAC Scan First Degree Relatives/Spouses Consent Form Version 2 dd May 2005	**07 June 2006**

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.

2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.

3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

The CREB approval for renewal of this study expires one year from the date of renewal.

Approval of the Clinical Research Ethics Board by one of:

Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair
Dr. John Russell, Associate Chair
Dr. Caron Strahlendorf, Associate Chair

Family Atherosclerosis Counseling and Testing Project

This study has been completed.

Sponsored by: **University of British Columbia**
Information provided by: University of British Columbia
ClinicalTrials.gov Identifier: NCT00387595

Purpose

Family history of early atherosclerotic disease in a first-degree relative [(FDR) sibling, parent or child] is an important risk factor for coronary artery and/or vascular disease. The risk increases ~ 2 – 7 times over that of general population. Increased thickness of the intima and media of carotid arterial wall, increased rate of plaque formation is an independent predictor of cardiovascular disease. Also it is shown that increased level of calcium deposition in the arterial wall is also associated with increased level of coronary artery narrowing. We will assess the occurrence and severity of abnormalities of intima media thickness (IMT) and/or plaque formation and increased calcium deposition in the coronary arteries and their relation to the well known traditional risk factors (plasma glucose, smoking, BMI, waist circumference, blood pressure, total cholesterol, low density lipoprotein, total cholesterol/high density lipoprotein ratio) and non-traditional risk factors (C-reactive protein, Lpa, homocysteine) in FDRs of index patients with early onset of heart or vascular disease and appropriate control population. Also to determine which of the above factor can assess IMT and Ca score better. This may help to reduce the cost of investigation, and to identify the population at high risk of developing cardiovascular disease, which may help the physicians to treat early on before cardiovascular complications occur. Also this may help to reduce the cost of invasive tests, hospital admissions and medical costs overall by reducing the morbidity and mortality.

Condition	Phase
Coronary Artery Disease	Phase I Phase II

MedlinePlus related topics: Coronary Disease
Genetics Home Reference related topics: Coronary Disease

Study Type: Observational
Study Design: Screening, Cross-Sectional, Random Sample, Retrospective/Prospective Study

Official Title: Family Atherosclerosis Counseling and Testing Project (FACT)

Further study details as provided by University of British Columbia:

Study start: February 2003; Study completion: December 2008
Last follow-up: September 2008; Data entry closure: September 2008

Objective: (i) To identify the differences between the "intervention" and "usual care" groups in Framingham risk scores, anthropometrical, biochemical parameters at baseline and at 24 months and compliance to prescribed regimens. Prevalence of maternal and paternal risk factors, DNA/mRNA profile and other risk factors may also be examined. (ii) To assess the relation between CIMT and psychosocial status with other conventional cardiovascular risk factors and serum levels of inflammatory markers in the "intervention" versus "usual care" groups over 2 years of follow up. (iii) Subjects that scored high on the emerging risk factors and IMT score will undergo CAC scanning. This will allow us to correlate the values of the CAC score in FDR's of patients with

premature atherosclerosis to the IMT score and to the biochemical markers already captured in this study.

Method: (i) Index patients with premature atherosclerosis (angina with ECG changes, myocardial infarction, Coronary artery bypass grafting, Percutaneous transluminal coronary angioplasty, Peripheral vascular disease, Cerebro vascular disorders) in men below age 50yrs and women below age 60yrs at the time of the above mentioned adverse events will be identified from cardiac cath lab, healthy heart program St.Paul's and VGH, wards of St. Paul's hospital, heart function and transplant clinic and also from Cardiologists' offices, and will be asked to participate in the study. Those who volunteer to participate will be assessed for both the classical and emerging cardiovascular risk factors and asked for permission to approach their FDRs. The family of these index patients will be randomized using block randomization method (block of 4) in to usual care or intervention group. After recruitment of the patient, recruitment of their first degree relative will be undertaken. If present in the hospital during visiting hours, relatives will be approached in person by the study coordinator for participation in the study. Alternatively, relatives will be contacted by letter for participation, followed up by a telephone call.

FDRs and their spouses will undergo a comprehensive risk factor assessment including dietary, smoking, drinking and exercise habits. Data on past medical history, family history and treatment history will be collected. Anthropometric measurements (height, weight, waist circumference), blood pressure, heart rate and lipidemia markers will be recorded. Blood sample will be collected for biochemical measurements, which will include total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, Apoprotein-B and A1, lipoprotein (a), homocysteine, C-reactive protein and glucose. Blood will be stored for future genetic analysis. Risk for future events will be estimated according to Framingham and or procam risk score. The same procedure will be repeated at the beginning and at the end of the 2-year period.

The participants will be divided into "intervention" and "usual care" groups. The intervention group will receive a risk assessment summary, with a copy to their family physician. Recommendations for treatment of modifiable risk factors to targets (blood pressure control, lipids, glucose, and smoking cessation) and counseling will be carried out. Regular follow up of study participants at 3 and 6 months will be made and patient will be called over phone at 4 and 8 months for lifestyle counseling and all participants will be reassessed at 2 year. The usual care group will receive an initial risk assessment summary along with their diet, exercise and other risk assessments using the questionnaire. The risk assessment summary will be mailed to the patient and to their family physicians with a note highlighting the abnormal results and will be asked to discuss this with them. The same procedure will be repeated at the end of the 2-year period. The comparison of intervention and usual care groups will be made at 24 months after their initial assessment for their health and risk factor profile.

(ii) Those participants older than 30 yrs with >10% risk score who volunteer to participate in FACT phase 2 study will be asked to sign a consent for the IMT study and be given the psychosocial questionnaire to complete and asked to bring it back at the time of their IMT visit. IMT will be measured by experienced technicians using HP Pointrex ultrasound scanner. This takes about 45 minutes. The data will be analysed by the experienced sonographer and stored offline for future use. A second scan will be performed two years later.

(iii) Subjects already enrolled in the study that underwent IMT scan for the baseline visit will be selected as candidates for this procedure. Patients who meet the above criteria will be selected form both the "intervention" and "usual care" group; contacted by mail/phone and asked to participate in this portion of the study. If, they agree, patients will be given a new consent form to sign and after the consenting is done, an appointment for the CAC scan is made.

The subject will be asked to abstain from caffeine or caffeine-containing products the day of the scan in order to ensure an optimal rest heart beat < 80bpm. No other preparation is necessary for this procedure. The total length of this appointment is about 30 minutes, and most of this time is required to place ECG patches and to properly position the subject. The actual scan takes only 20 seconds. The technician will ask the patient to hold his breath for approximately 20 seconds while the scan is performed. The CAC scan will be administered by a trained technician at the Canada Diagnostic Centre located at 136-55 West 12th Avenue in Vancouver, BC. Except for the small amount of radiation the patient will be exposed to during this procedure there are no other known risks associated with this scan.

Eligibility

Ages Eligible for Study: 16 Years and above, Genders Eligible for Study: Both

Criteria

Inclusion Criteria:

- First degree relatives of patients with early onset of cardiovascular disease (men<50 and women<60 years of age).

Location Information

Study chairs or principal investigators

Jiri Frohlich, Principal Investigator, University of British Columbia

More Information

Study ID Numbers: P02-0103
Last Updated: October 12, 2006
Record first received: October 11, 2006
ClinicalTrials.gov Identifier: NCT00387595
Health Authority: Canada: Health Canada
ClinicalTrials.gov processed this record on 2006-10-31

Appendix 3. FDRs Information and consent form;

Providence HEALTH CARE
St. Paul's Hospital

UBC/PROVIDENCE HEALTHCARE
1081-BURRARD STREET
VANCOUVER, BC, V6Z 1Y6
TEL: (604)-806-8591
FAX: (604)-806-8590

Patient information and informed consent

First degree relatives and their spouses

Title of project: Family **A**therosclerosis **C**ounseling and **T**esting (FACT) Study-IMT part.

Investigator: Dr. Jiri Frohlich.

Telephone number: (604)-806-8612

Research Co-ordinator: Rajashree M. Devarakonda

Telephone number: (604)-806-9251 **Pager number (24 hour):**(604)- 268-0871

Introduction: The combined thickness of the inner layer of the blood vessel (intima) and the middle layer of the blood vessel (media) of the blood vessel in the neck (carotid artery) is associated with the likelihood of developing cardiovascular disease. Number of studies have shown that the associations between the combined thickness of the inner and middle layer of carotid-artery (IMT) and new myocardial infarction (heart attack) or stroke in persons without clinical cardiovascular disease. The studies also show that the rate of increase in the thickness of the intima and media of the carotid artery, as measured non-invasively by ultrasonography, are directly associated with an increased risk of myocardial infarction and stroke in older adults without a history of cardiovascular disease.

Purpose of the study: You and your spouse are being invited to participate in this part of the study because you have a first-degree relative who has had heart or vascular disease at a relatively early age, which puts you at higher risk of developing a vascular disease. You and your spouse are being invited to participate in this part of the FACT study to evaluate the sub-clinical (no signs and symptoms of disease) atherosclerosis (thickening of the artery), and frequency and nature of the risk factors for atherosclerosis in your family.

Description of the research: Your participation in this is entirely voluntary. You have the right to refuse to participate in this part of the study. If you decide to participate, your decision is not binding and you may choose to withdraw from the IMT part of the study at any time without any negative consequences to the medical care, education, or other services you or your family may receive from this hospital.

1. You will be asked to come in for noninvasive (a probe that moves over the skin of your neck is used) measurements of the intima and media of the common and internal carotid artery (the arteries in the neck). This will be made using high-resolution ultrasonography and it takes approximately about 15 minutes. This will be repeated 2 years later.

Confidentiality: Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the sponsor of the study, Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records, which identify you by name or initials, will be allowed to leave the Investigators' offices. All data will be kept in a secure database, where only authorized individuals involved in the study can access it. No personal information such as your name or address will be included in the database; only a code will identify you. The documents relating your name and code will be kept in a separate location in a secure place.

All resulting documents and computer files will be identified by this code number and will be kept in a secure collection in an access-controlled database. Risk assessment summary will be given to you and will be sent to your family doctor. Every effort will be made to keep your records confidential and your information will only be used for the purposes described in this consent form.

Costs and compensation: There is no cost to you for study procedures however we will compensate you for your parking expense of ~5 dollars per visit. There is no monetary compensation for your participation. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

Potential risks and benefits: You and your family members may benefit from the risk assessment and counseling that will be given if you have significant sub-clinical (without signs and symptoms of the disease) atherosclerosis. You will benefit from early intervention and treatment either by your family physicians or by physicians at Healthy Heart Program, St.Paul's Hospital. Also, you will be helping us advance the knowledge of potential risk factors and possibly finding new treatable risk factors for heart disease. As previously stated, every effort will be made to protect your confidentiality.

Questions about your rights as a research subject: The UBC/Providence Health Care Ethics Board has approved this project. If you have any questions about your right as a research subject, you can contact the chairman of the Ethics Board, Dr. Steve Shalansky at (604)-806-9013 or you can contact the 'Research Subject Information Line in the UBC Office of Research Services' at 604-822-8598

Questions about this study: If you have any questions about this study itself, you may contact the Principal investigator, Dr. Jiri Frohlich at (604)-806-8612 or page the study research coordinator at (604)-268-0871.

Informed Consent

I acknowledge that the study protocol has been explained to me and that I have had my questions answered to my satisfaction to help me understand what my participation will involve. I have received a copy of this form. It has also been explained to me that if I have any further questions about any of these procedures I can contact the Principal investigator at the telephone number shown on this form. I recognize that it is my right to withdraw from any of these procedures at any time without any consequences to my medical treatment and that all of the information obtained will be kept in a confidential file. I consent to participate for the IMT measurement of the study.

_____ _____

Name of subject (Please print) Signature of subject

_____ _____

Date

_____ _____

Name of witness (Please print) Signature of witness

_____

Date

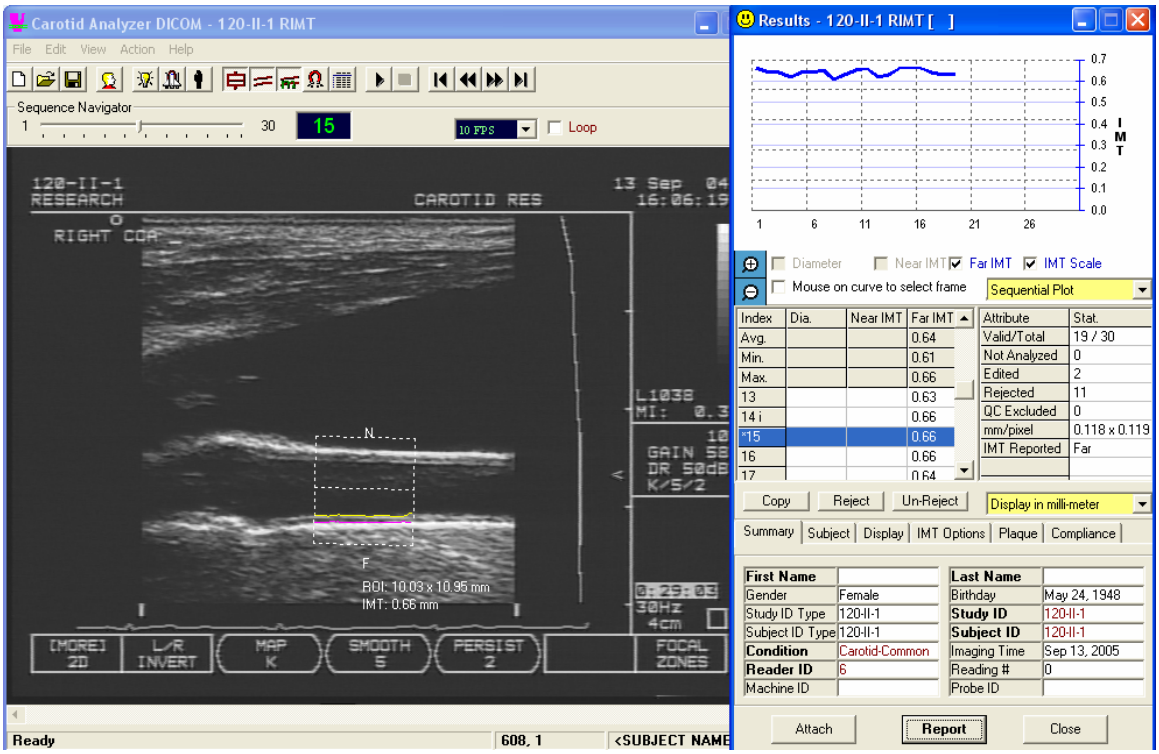
_____ _____

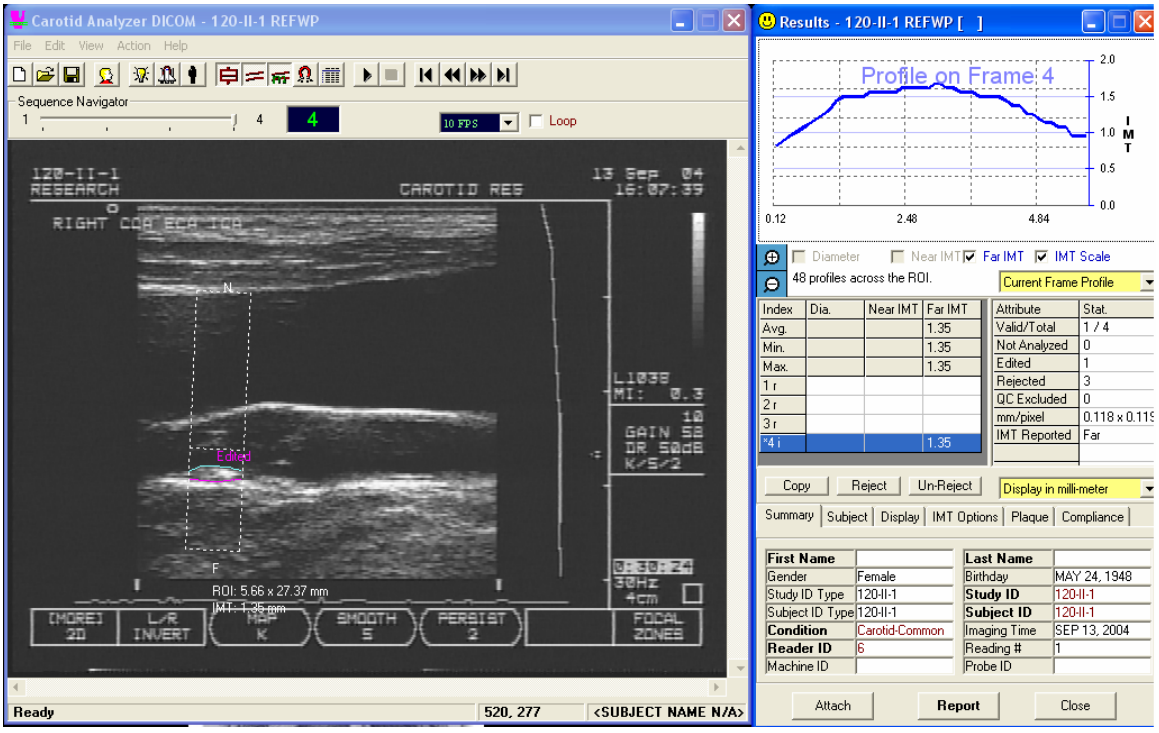
Name of person explaining the consent (Please print) Signature of person explaining the consent

_____

Date

Appendix 4. Still images of IMT and plaque measurements using edge detection software – Vascular Imager 5.0 Medical Imaging Applications, LLC)





SDC-CPAP Study

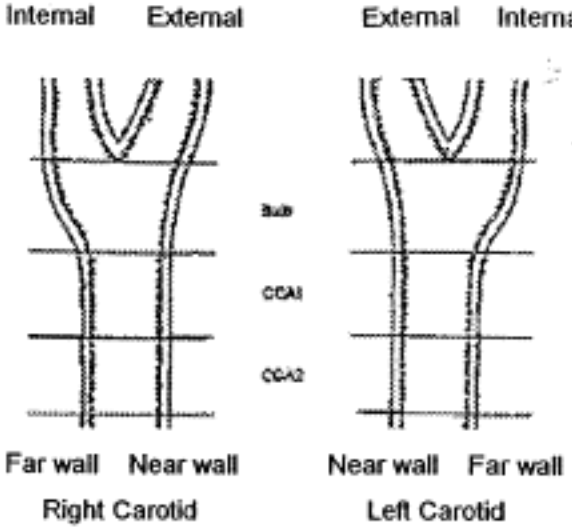
CIRCL

Patient ID: _____ Patient Initials :_____

Patient Birthdate: _____ Patient Age :_____ Gender: M / F

Date of scan: _____ Date of Analysis: _____

Scan # : 1 / 2 / 3



				Lesion				
Side	Seg.	Wall	IMT (mm)	Seg.	Wall	Avg. Lesion Thickness (mm)	Plaque Area (mm^2)	%DS
Right								
Left								

	Expected	Observed	Expected Δ/year	Observed Δ/year
Average IMT (mm)				
Total plaque area (mm^2)				
Average Total Thickness (mm)				
Total Area (mm^2)				

Video quality: excellent / good / fair: _____

Comments and conclusions:

_____

_____

_____

Technician Signature: _____ Date: _____

(Tech #:____)

I have reviewed these analyses and found them to be accurate and complete.

_____ _____

G. B. John Mancini, MD Date

Appendix 6. CAC Information and consent form;

Providence HEALTH CARE
St. Paul's Hospital

UBC/PROVIDENCE HEALTHCARE
1081-BURRARD STREET
VANCOUVER, BC, V6Z 1Y6
TEL: (604)-806-8591
FAX: (604)-806-8590

Patient information and informed consent

First degree relatives and their spouses

Title of project: **F**amily **A**therosclerosis **C**ounseling and **T**esting (FACT) Study - Coronary Artery Calcium Score Scan.

Investigator: Dr. Jiri Frohlich. **Telephone number:** (604)-806-8612

Co-investigator: Dr. Bruce Forster **Telephone number:** (604)-822-7976

Telephone number: (604)-806-9251 **Pager number (24 hour):**(604)- 268-0871

Introduction: You are being invited to participate in this study because you are a participant in the FACT study. Calcium deposits in the blood vessels that supply the heart (Coronary Arteries) can be detected by non-invasive computerized tomography (CT) (Coronary Artery Calcium Scan-CAC), which scans the heart (from the base of the aorta (major blood vessel) down 12 cm towards the tip (apex) of the heart). This scan can detect very small calcium deposits within the coronary arteries. These calcium deposits can identify arterial plaques, and are early markers of coronary artery disease (CAD). Studies have shown that CAC scans can accurately detect the level of coronary clacification, which has been shown to be directly related to the frequency of myocardial infarction (heart attack) in younger patients.

Purpose of the study: You and your spouse are being invited to participate in this part of the study because you have a first-degree relative who has had heart or vascular disease at a relatively early age; this increases your risk of developing a vascular disease. You and your spouse are being invited to participate in this part of the FACT study to evaluate the sub-clinical (no signs and symptoms of disease) atherosclerosis (thickening of the artery), and frequency and nature of the risk factors for atherosclerosis in your family. Also we would like to compare your calcium score with other emerging risk factors.

Description of the research: Your participation in this is entirely voluntary. You have the right to refuse to participate in this part of the study. If you decide to participate, your decision is not binding and you may choose to withdraw from the CACS part of the study at any time without any negative consequences to the medical care, education, or other services you or your family may receive from this hospital.

You will be asked to come in for a CT scan for measurement of any calcium deposits in the coronary arteries. The procedure is non invasive. The entire procedure takes about 30 minutes. A trained technician will guide you to make sure you are in correct position and will prepare you with ECG

patches. This takes about 15-20 minutes. The actual CAC scan requires about 20 seconds to complete. A CAC scan generates minimal radiation, close to about ½ of the annual environmental exposure in Canada. Breathing during the scan is very important, and you will be required to hold your breath for approximately 20 seconds during the CT scan. There is no special preparation required for a CAC scan, other than avoidance of caffeine the day of the scan, to ensure an optimal resting heart rate (<80bpm).

Confidentiality: Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the sponsor of the study, Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records, which identify you by name or initials, will be allowed to leave the Investigators' offices except to members of the research team, or published without consent, unless required by law. All data will be kept in a secure database, where only authorized individuals involved in the study can access it. No personal information such as your name or address will be included in the database; only a code will identify you. The documents relating your name and code will be kept in a separate location in a secure place.

All resulting documents and computer files will be identified by this code number and will be kept in a secure collection in an access-controlled database. Every effort will be made to keep your records confidential and your information will only be used for the purposes described in this consent form.

Costs and compensation: There is no cost to you for study procedures however we will compensate you for parking expense. There is no monetary compensation for your participation. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

Potential risks and benefits: Except the small amount of radiation the patient will be exposed to during this procedure (about ½ of the annual environmental exposure in Canada), there are no other known risks associated with this procedure. You and your spouse may benefit from the assessment and counseling that may be given if you have significant sub-clinical (without signs and symptoms of the disease) atherosclerosis. If this is the case you will benefit from early treatment either by your family physician or by physicians at Healthy Heart Program, St.Paul's Hospital. Also, you will be helping us advance the knowledge of potential risk factors and possibly finding new treatable risk factors for heart disease. As previously stated, every effort will be made to protect your confidentiality.

Questions about your rights as a research subject:
The UBC/Providence Health Care Ethics Board has approved this project. If you have any questions about your right as a research subject, you can contact the chairman of the Ethics Board, Dr. Ingrid Fedoroff at 604-682-2344 Ext-62325 or you can contact the 'Research Subject Information Line in the UBC Office of Research Services' at 604-822-8598

Questions about this study:
If you have any questions about this study itself, you may contact the investigator, Dr. Jiri Frohlich at (604)-806-8612 or the study research coordinator at (604)-806-9251.

Informed Consent

I acknowledge that the study protocol has been explained to me and that I have had my questions answered to my satisfaction to help me understand what my participation will involve. I have been informed of the alternatives to participation in this study. The potential risks, harms and discomforts have been explained to me and I also understand the benefits (if any) of participating the research study. I have received a copy of this form. It has also been explained to me that if I have any further questions about any of these procedures I can contact the Principal investigator at the telephone number shown on this form. I know that the study participation is voluntary and I recognize that it is my right to withdraw from any of these procedures at any time without any consequences to my medical treatment and that all of the information obtained will be kept in a confidential file. I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties. I have been assured that records relating to me and my care, will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law. I have been given sufficient time to read and understand the above information.

_____ _____

Name of subject (Please print) Signature of subject

_____ _____

Date

_____ _____

Name of witness (Please print) Signature of witness

_____

Date

_____ _____

Name of person explaining the consent (Please print) Signature of person explaining the consent

_____

Date

Appendix 7. CAC pre-scan questionnaire

(Full Name) **CONFIDENTIAL:**
(Address, City, Province, Postal Code, Phone#)

(Gender)DOB: **Age: _____ Height: _____ Weight: _____**

Dr. _____ Fax: _____

Medical History	**Please circle the correct answer:**	
1. Do you have high blood pressure?	Yes / No	Do you know your last reading? (e.g. 120/80)_____
2. Have you been treated for high BP?	Yes / No	If yes, which medication are you taking? _____
3. Do you currently smoke?	Yes / No	Have you ever smoked? Yes / No
If yes, how many packs per day? _____		If yes, for how long?_____ Which year did you quit?_____
4. Do you have high cholesterol?	Yes / No	Values? Total Cholesterol _____ LDL (bad) _____ HDL (good) _____ TG _____
5. Are you treated for high cholesterol?	Yes / No	If yes, which medication are you taking? _____
6. Females, are you postmenopausal?	Yes / No	If yes, are you taking any hormones? Yes / No
Have you ever had:		
7. A heart attack?	Yes / No	If yes, when? _____
8. A stroke?	Yes / No	If yes, when? _____
9. Chest Pain / Angina / Claudication?	Yes / No	If yes, when? _____
10. Heart Catheterization?	**Yes / No**	If yes, when? _____
11. STENT insertion?	**Yes / No**	If yes, when? _____
12. Diabetes? High fasting blood sugar?	Yes / No	Do you know your last blood sugar level? _____
13. Cancer?	Yes / No	If yes, what type of cancer? _____
14. Blood in the stool?	Yes / No	15. Significant unintended weight loss? Yes / No
16. Family history of: angina / heart attack / stroke in mother / father / brother / sister at what age? _____		(circle the answers)
17. Family history of cancer in mother / father / brother / sister (circle)? – at what age _____		
18. A test for osteoporosis?	Yes / No	If yes, did you have osteoporosis? Yes / No
19. Is there any specific health concern that you hope this screening exam will address? _____		
20. Have you ever had a reaction to any sort of X-ray dye?	Yes / No	If yes, please describe _____
21. Please list any previous surgeries: _____		

A dictated report of your assessment will be sent to you and your physician. **It is your responsibility to follow-up with your physician regarding any abnormal results.** Depending on the results of your scan, other imaging or laboratory tests may be recommended to you and your doctor.

I certify the above to be correct

_____ _____

Signature: Date:

Appendix 8. CAC scoring age and gender adjusting table

Nomogram of Over 21,000 Asymptomatic Patients Scanned at Harbor-UCLA Medical Center

Asymptomatic Women (N=6,027)

	</=40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	>75
10%	0	0	0	0	0	0	0	0	0
20%	0	0	0	0	0	0	0	0	6
25%	0	0	0	0	0	0	0	4	25
30%	0	0	0	0	0	0	0	10	40
40%	0	0	0	0	0	0	3	29	86
50%	0	0	0	0	0	2	17	67	157
60%	0	0	0	0	2	17	48	120	314
70%	0	0	0	3	12	55	114	217	403
75%	0	0	2	7	29	81	163	310	577
80%	0	0	3	16	56	114	215	398	775
90%	2	5	35	79	166	273	481	738	1,193

Asymptomatic Men (N=15,238)

	</=35	35-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	>75
10%	0	0	0	0	0	0	0	1	8	17
20%	0	0	0	0	0	0	5	23	51	91
25%	0	0	0	0	0	1	12	41	81	148
30%	0	0	0	0	0	4	25	66	121	233
40%	0	0	0	0	4	15	59	128	216	358
50%	0	0	0	2	14	42	114	211	328	562
60%	0	0	1	8	36	89	206	351	493	816
70%	0	1	4	26	80	166	335	554	749	1,223
75%	0	3	8	41	116	227	421	709	918	1,409
80%	2	5	14	67	161	314	543	888	1,119	1,658
90%	12	23	56	174	379	654	996	1,484	1,667	2,396