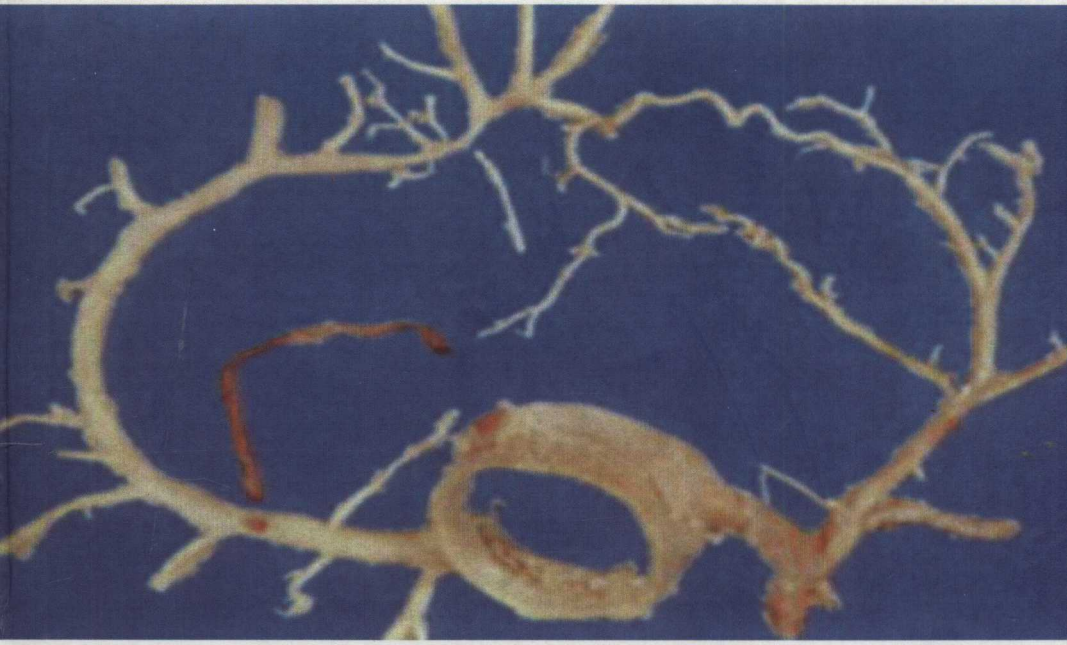
NATALIA CAPROŞ

# CORONARY ARTERY DISEASE

## ENVIRONMENTAL AND GENETIC FACTORS



**To my parents, daughter and husband this book is affectionately dedicated.**

**Reviewers:**

University Professor, MD, PhD, Head of Internal Medicine Department and Clinical Synthesis Discipline, State University of Medicine and Pharmacy N.Testemiţanu, **S. Matcovschi**

University Professor, MD, PhD, Head of Semiology Department, State University of Medicine and Pharmacy N.Testemiţanu, **V. Istrati**

University Professor, MD, PhD, Head of Cardiology Discipline, State University of Medicine and Pharmacy N.Testemiţanu, **V. Revenco**

Literary Editor, Senior lecturer, Department of Modern Languages, **Rodica Costin**

*Approved by the Ministry of Health Expert Council of the Republic of Moldova at the meeting of October, 18, 2011; Proceedings No 5.*

Descrierea CIP a Camerei Naţionale a Cărţii

**Capros, Natalia**
**Coronary artery disease environmental and genetic factors /**
Natalia Caproş - Ch.: SC Profesional Service SRL, 2012. – 244 p.
100 ex
ISBN 978-9975-4319-5-8.
616.132.2:612.17
C 21

Design & print: SC Profesional Service SRL
e-mail: dorianconea@yandex.ru
phone: +373 67 442 052; +373 79 612 988

NATALIA CAPROŞ

# CORONARY ARTERY DISEASE

## ENVIRONMENTAL AND GENETIC FACTORS

# Acknowledgements

A wish to express my gratitude to the Genetics Laboratory Staff without whose support this work would not has been possible.

I also want to thank the pharmaceutical companies:
*NEW NORDIC, SANOFI, SERVIER, GEDEON RICHTER*
whose financial assistance contributed to the appearance of this monograph and my deepest gratitude to all those who generously shared their knowledge and experience with me.

I am thankful to the *PROFESIONAL SERVICE SRL* for the editorial assistance during the preparation of this manuscript.

*Stay tuned- the future is almost here*

# Preface

Coronary artery disease (CAD) can no longer be considered a disease of the developed world, because myocardial infarction and stroke are increasingly prevalent worldwide, across all socioeconomic strata. By 2025, cardiovascular mortality on a worldwide scale will likely surpass that of every major disease group, including infection, cancer, and trauma. The prevalence of CAD is rising rapidly due to increased exposure to cardiovascular risk factors, which may lead to an expanding epidemic. CAD is the most common heart disease that is believed to be caused by multiple environmental, genetic factors and interactions among these. The purpose of this monograph is to highlight the most important discoveries made in this field in the past years. Identification of the environmental and genetic factors will provide valuable information for prevention and control of CAD.

Although we are still in the early phase of understanding the genomic basis of complex traits, there have been some remarkable recent advances in atherosclerotic CAD and myocardial infarction. The underlying pathologic spectrum is broad, ranging from accumulation of cholesterol deposits in the subendothelial arterial intima to frank inflammation of the artery wall, and, in some individuals, culminating in a plaque rupture, fissure or erosion of the wall with resultant blood clot formation. Linkage analysis, whole genome association studies and specific genetic epidemiologic studies have, in aggregate, begun to open up this field and provide insights to the genes underlying this common and important condition.

This monography introduces basic notions required to understand the language of genetics and genomics, illustrates the important insights provided by genetic research into the causes and mechanisms of CAD. It provides an exhaustive account of the numerous studies conducted on the genetics of CAD and discusses the new Genome-wide association (GWA) strategy and why this approach is likely to have a considerable impact on CAD understanding. The first chapter presents statistics on the current data about frequency, mortality/morbidity and hystory of the cholesterol controversy in atherogenesis. The second chapter enumerates underlying conventional risk factors of smoking, hypertension, hyperlipidemia, insulin resistance and diabetes, physical activity and obesity, as well as general strategies for reducing risk related to these disorders. It also briefly reviews evidence relating mental stress and depression, focusing on how they affect traditional measures of vascular risk. Not all coronary events occur in individuals with multiple traditional risk factors, however, and in some individuals abnormalities of inflammation, hemostasis, and/or thrombosis appear to contribute decisively. In particular, nearly half of all myocardial infarctions occur among individuals without hyperlipidemia. Thus, the second part of the chapter reviews atherothrombotic risk factors, including high-sensitivity C-reactive protein and other markers of inflammation, as well as homocysteine and lipoprotein (a). Also reviewed are data regarding hemostatic and thrombotic markers of risk, including fibrinogen and abnormalities of intrinsic fibrinolysis. The third chapter describes currently accepted unifying theory of atherogenesis *Response-to-vascular injury*. The fourth chapter briefly overviews the classification of genes for coronary artery disease and myocardial infarction and deals with genetic approaches, statistical issues that may contribute to risk

evaluation in the future, and views of how this new knowledge will be incorporated into practice.

The main objective of human genetic research is to discover new mechanisms of the disease. In recent years, genetics has profoundly changed our knowledge of CAD and the novel GWA strategy should further widen our understanding of the pathophysiology of these disorders. Within the last decade, several important advances have made it possible to study "modern" diseases from an evolutionary perspective. The Human Genome Project provided a reference human genome, and the subsequent International HapMap Project described genetic variations among individuals and the patterns of variation across the genome. Both projects provide raw material to study natural selection in the human genome, as reviewed in recent reports. In addition, sequencing of multiple other genomes, including those of primates, provides a framework for generating important insights into the origin and expression of human diseases. Such an avenue of investigation may help answer why humans are susceptible to CAD and why substantial differences in susceptibility to CAD are present between ethnic groups. Genetic factors underlying both "complex" and mendelian diseases may be affected by natural selection; conversely, regions of the human genome under natural selection are likely to harbor functional loci that may influence disease susceptibility. Inferences about natural selection may therefore help identify disease susceptibility loci in humans and facilitate disease mapping studies. The fifths chapter provides data on genetic basis of coronary artery disease and concludes with a discussion of a novel approach to overall risk prediction that can be applied immediately and that allows emerging concepts of inflammatory and heritable risk to play an appropriately greater role in global risk detection. In the next chapters the current hypotheses and knowledge about the evolutionary genetics of several risk factors

for CAD, including dyslipidemia, hypertension, high-sensitivity C-reactive protein and restenosis are discussed. Several candidate genes in pathways of blood pressure, lipid metabolism, blood coagulation, and inflammation that may be under natural selection are enumerated. An evolutionary perspective might explain why contemporary humans are at high risk for CAD and also helps to better understand variation in disease susceptibility. The discovery of new drug targets as a consequence of genetic research that may considerably modify the therapeutic approach of cardiovascular disorders in the middle and long terms are related in chapter seven. Directions for future research in the exciting and fast-developing realm of genetic epidemiology are outlined and the utility and clinical implications are discussed. It provides a systematic review of findings, integrated to offer a comprehensive summary and stepping stone for future research. The monograph will be of interest to students, residents, clinicians, scientists and investigators in cardiology and genetics.

*To wish to be well is a part of becoming well.*

Seneca , Roman philosopher, mid-1st century

# Chapter 1. Background

At first sight, unusual in antiquity, CAD became epidemic as population increasingly survived early mortality caused by infectious diseases and malnutrition. Such environmental factors as economic progress, diet with saturated fats and diminished physical activity have now become globalized, so that we face an epidemic of CAD.

## Prevalence of cardiovascular diseases

In the developed countries (United States, Great Britain) about 80 million people, or 36.3% of the population, have existing cardiovascular diseases. In addition 795 000 people suffer new or recurrent strokes each year. The same is true of Finland and Scandinavia in general. However, in the last decade, mortality rates of CAD in these countries have decreased by two-fifths, which is attributed to better detection of hypertension. Despite consumption of rich foods, inhabitants of France and the Mediterranean region appear to have a lower incidence of CAD. This phenomenon (sometimes called the French paradox) is partly explained by greater use of alcohol and by consumption of the Mediterranean diet, which includes predominant use of monounsaturated fatty acids, such as olive oil or canola oil, as well as omega-3 fatty acids, which are less atherogenic. Russia

and many of the former states of the Soviet Union have recently experienced an exponential increase in the frequency of CAD that is likely the result of widespread economic hardships and social upheaval, a high prevalence of cigarette habituation and a diet high in saturated fats. In 2009 the prevalence of cardiovascular diseases in Moldova was 1202.7 per 10 000 population, increased as compared to 1134.1 in 2008. The frequency of CAD in the Far East is significantly lower than that documented in the West. CAD is also rare on the African continent, although growing evidence indicates that this too is changing as a result of rapid westernization and urbanization of the traditionally rural and agrarian African populations. The prevalence of cardiovascular diseases is also increasing in the Middle East, India, Central and South America. The rate of CAD in ethnic immigrant populations in the United States approaches that of the disease in whites, supporting the role of these putative environmental factors. Eskimos have been found to have a lower prevalence of cardiovascular diseases as a result of consuming fish oils containing omega-3 fatty acids (1). Ill-defined genetic reasons for this phenomenon may exist, but significant interest surrounds the role of diet and other environmental factors in the absence of clinical CAD in these populations.

## Mortality

Atherosclerosis is the leading cause of death in the developed world, and it is predicted to be the leading cause of death in the developing world within the first quarter of the next century.

- Annually, approximately 1.5 million Americans have an acute myocardial infarction, a third of whom die. In 2009, cardiovascular diseases were responsible for 8 645 000 deaths, or 35.3% of all deaths that year. They included 151 000 deaths from myocardial infarction and 143 600 deaths from stroke, in Romania- 16 215 deaths. According to statistics of 2009 the level of cardiovascular mortality

was 51.6% of all deaths registered in the Republic of Moldova (2).

- An encouraging decrease in mortality due to CAD in the developed world has occurred. Unfortunately, this decrease has not occurred in the developing world and an exponential increase in tobacco habituation and the adoption of a Western diet high in saturated fats likely predicts the continued increase in death and disability due to CAD.
- The survivors of myocardial infarction have a poor prognosis, carrying a 1.5- to 15-fold higher risk of mortality and morbidity than the rest of the population.
- For example, historically within 1 year of myocardial infarction (MI), 25% of men and 38% of women die. These rates may overstate the 1-year mortality rate today, given advances in the treatment of chronic heart failure and sudden cardiac death. Among survivors, 18% of men and 34% of women have a second MI within 6 years, 7% of men and 6% of women die suddenly, 22% of men and 46% of women are disabled and 8% of men and 11% of women have a stroke.

## Race

The incidence, prevalence, and manifestations of CAD vary significantly with race, as does the response to therapy. African Americans appear to have higher morbidity and mortality rates, even when corrected for educational and socioeconomic status. The risk-factor burden experienced by African Americans differs from that of whites. The prevalence of hypertension, obesity, dysmetabolic syndrome and lack of physical activity are much higher, whereas the prevalence of hypercholesterolemia is lower. Similar statistics can also be cited for presentation and treatment of patients with stable CAD. Asian Indians exhibit a 2- to 3-fold

higher prevalence of CAD than whites in the United States. They have greater prevalences of hypoalphalipoproteinemia, high lipoprotein(a) levels and diabetes.

## Gender

Atherosclerosis is more common in men than in women. The higher prevalence of atherosclerosis in men is thought to be due to the protective effects of the female sex hormones. Women, however, follow men by 10 years, especially after menopause. The presence of diabetes, as well as tobacco use, eliminates the protection associated with female sex. However, even in women, the most common cause of death is CAD, which accounts for more deaths than those related to breast and uterine diseases combined. Women with acute myocardial infarction are less often subjected to invasive strategies, and experience greater overall mortality. Similar statistics can also be cited for the presentation and treatment of patients with stable CAD.

## *Age*

Age is the strongest risk factor for the development of CAD. Most cases of CAD become clinically apparent in patients aged 40 and older. Elderly persons still experience higher mortality and morbidity rates from CAD. Approximately 82% of people who die of CAD are 65 years or older. Complication rates of multiple therapeutic interventions tend to be higher in the elderly, however, the magnitude of benefit from the same interventions is greater because these patients form a high-risk subgroup.

**References:**

1. Allender S., Scarborough P., Peto V., et al. European Cardiovascular Disease Statistics, 2008.
2. Ministerul sănătății. Centrul național de management în sănătate. Rapoarte și analize. Date statistice. Anuar statistic al sistemului de sănătate din Moldova. 2010.

*Superior doctors prevent the disease.*
*Mediocre doctors treat the disease before evident.*
*Inferior doctors treat the full-blown disease.*
Huang Dee Nai-Chang
(2600 BC 1st Chinese Medical Text)

# Chapter 2.
# Cardiovascular risk factors

The initial findings of the Framingham Heart Study established the basis for considering specific "cardiovascular risk factors". The risk factors can be divided into modifiable and nonmodifiable risk factors and include hyperlipidemia, hypertension, cigarette habituation, diabetes mellitus, age, and sex. More recently, a number of novel risk factors have been identified that add to the predictive value of the established risk factors and may prove to be a target for future medical interventions. To varying degrees, coronary artery atherosclerosis results from the interplay of multiple risk factors (*table* 2.1)

*Table 2.1*

**Cardiovascular risk factors**

- Family history of premature CAD
- Hypercholesterolemia (high LDL syndrome)
- Hypertension
- Cigarette smoking
- Diabetes mellitus
- Hypoalphalipoproteinemia
- Dysmetabolic syndrome
- Obesity
- Physical inactivity
- Nontraditional risk factors
    - Hyperhomocystinemia
    - High lipoprotein(a) levels
    - High iron levels
- Syndromes of accelerated atherosclerosis –
    - Graft atherosclerosis
    - CAD after cardiac transplantation
    - Restenosis
        - Infection
            - Chlamydia pneumoniae
            - Helicobacter pylori
            - Herpes simplex virus

Based on information collected from more than 24 000 women for more than a decade, the researchers created a new tool called the Reynolds risk score (1). When used on the study group, the Reynolds risk score did as well as the Framingham risk score for women at high and low risk. For those in between, it was better. The new model reclassified almost half of these women into high-risk and low-risk groups. The new assignments, done by computer, corresponded almost perfectly to what actually happened to these women over the next 10 years. Reynolds Risk Score for men improves cardiovascular (CV) risk prediction (2). Paul Ridker and team (2007) therefore assessed the predictive value of adding hsCRP and parental history of myocardial infarction before age 60 years (Reynolds Risk Score for men) to the traditional risk factors of age, blood pressure, smoking, and total and high-density lipoprotein cholesterol in a prospective cohort of 10 724 initially healthy nondiabetic men (the Physicians' Health Study).

## Prevalence

A number of large epidemiological studies in North America and Europe have identified numerous risk factors for the development and progression of atherosclerosis. The EUROASPIRE III survey on 4 366 high risk individuals shows that the prevalence of most cardiovascular risk factors in 12 European countries (Belgium, Bulgaria, Croatia, Finland, Germany, Italy, Latvia, Poland, Romania, Slovenia, Spain and the UK) was: 16% smoked cigarettes, 43% were obese and 62% centrally obese, 71% had blood pressure ≥ 140/90 mm Hg (≥130/80 in people with diabetes mellitus), 79% had total cholesterol ≥ 4.5 mmol/l and 39% reported a history of diabetes, of whom 53% had a HbA1c <6.5%. The investigators suggested that the lifestyle of high risk patients is a major cause for concern with persistent smoking and high prevalences of both obesity and central obesity. Blood pressure, lipid and glucose control are completely inadequate with most patients not achieving the targets defined in the prevention guidelines (3).

The prevalence of most cardiovascular risk factors has declined in the United States (US) over the past 40 years with the exception of diabetes and obesity (4). These favorable US trends suggest that interventions to reduce risk can be highly effective when applied in appropriate settings, as evidenced not only by reductions in coronary disease but also by reductions in stroke (5). Prevention on an international scale is thus a feasible goal. Adherence to a healthy lifestyle may prevent many cases of coronary heart disease. Therefore, targeting risk reduction by lifestyle modification for individuals who have clusters of risk factors seems a sensible primary goal for outpatient preventive cardiovascular practice (7).

## Dyslipidemia

The association between a raised plasma cholesterol and atherosclerosis is widely accepted. The mutations in the low-density lipoprotein (LDL) receptor produce human hypercholesterolemia on a monogenic basis that causes accelerated atherosclerosis as early as the first decade of life in individuals with homozygous familial hypercholesterolemia. Data from prospective cohort studies, such as that begun in Framingham in the 1950s, advocated the relationship between cholesterol and CAD. The results of the clinical trials to lower LDL cholesterol levels by various pathways (HMG-CoA reductase inhibitors, bile acid–binding resins, intestinal bypass surgery) have shown a reduction in cardiovascular events. The evidence that reducing plasma cholesterol reduces risk is unequivocal. The higher the risk, the greater the benefit. A 10% reduction in plasma total cholesterol is followed by a 25% reduction in incidence of CAD after 5 years, and reduction of LDL cholesterol of 1 mmol/l is accompanied by a 20% reduction in CAD events (7).

Substantiation of the relationship between total cholesterol and CAD risk emerged from the ALLHAT-LLT, MIRACL, PULSAR, POLARIS, ECLIPSE, EXPLORE, Multiple Risk Factor Intervention Trial

(MRFIT), studies in the United Kingdom and Europe such as the Northwick Park Study and the Prospective Cardiovascular Munster (PROCAM) Cohort and, more recently, the Atherosclerosis Risk in Communities (ARIC) study.

As demonstrated in the contemporary Heart Protection Study, the HMG-CoA reductase inhibitors can reduce stroke as well as coronary events among individuals with pre-existing vascular disease (8). More recently, several head-to-head trials comparing different statin regimens have shown that an even more aggressive LDL reduction is associated with greater clinical improvements. Such studies include the ASTEROID, REVERSAL trial, which monitored intravascular coronary ultrasound (9) and the PROVE-IT, A-to-Z, and TNT trials, all of which support a more aggressive use of statin therapy for reduction in hard clinical endpoints (10). The clinical trials MERCURY I, II have demonstrated the efficacy of statin therapy in very high-risk subjects for the treatment of familial hypercholesterolemia (11). The GALAXY Program, a series of clinical studies investigating the efficacy and tolerability of rosuvastatin in line with the hypothesis that the statin with the greatest efficacy for improving the atherogenic lipid profile and beneficially modifying inflammatory markers slowed progression of atherosclerosis and improve cardiovascular outcomes. Completed studies reported that rosuvastatin is more effective than comparator statins in reducing low-density lipoprotein cholesterol, improving the lipid profile and enabling patients to achieve lipid goals, including revised, more stringent goals, even in high-risk patients. The JUPITER and AURORA clinical trials have also reported that rosuvastatin can arrest and even regress atherosclerosis (12).

As is the case with LDL cholesterol, many large studies have demonstrated a strong inverse relationship between high-density lipoprotein (HDL) cholesterol and vascular risk. In general, each increase of HDL cholesterol by 1 mg/dl was associated with a 2 to 3 percent decrease in risk of total cardiovascular disease. The patients with angiographically-confirmed coronary artery disease

more often have low levels of HDL than high levels of LDL, as defined by current criteria. The mechanism of reverse cholesterol transport may explain in part the apparent protective role of HDL against coronary death. HDL can carry cholesterol from the vessel wall, augmenting peripheral catabolism of cholesterol (13). HDL can also carry antioxidant enzymes that may reduce the levels of oxidized phospholipids in atheromatous lesions, which might enhance atherogenesis. In a large Chinese cohort, elevated very low-density lipoprotein (VLDL) cholesterol was found to be significantly associated with elevated cardivascular risk, similar to that observed with LDL cholesterol. Cardivascular risk was further amplified when elevated VLDL cholesterol was combined with elevated LDL cholesterol and/or the presence of major CVD risk factors (16).

Overexpressing the apolipoprotein A-I gene in transgenic mice and infusing complexes of apolipoprotein A-I and phospholipids into hyperlipidemic rabbits not only increases HDL cholesterol levels but also decreases atherosclerotic development. The Adult Treatment Panel (ATP-III) guidelines support the aggressive search for agents that can directly increase HDL levels (14).

The investigators of INTERHEART suggested that measurement of apolipoproteins A-I and B-100 would predict cardiovascular risk better than HDL and LDL cholesterol in clinical practice (15).

Both these studies also found that non-HDL cholesterol (defined as total cholesterol minus HDL cholesterol) provided clinical risk information as least as strong as that of apolipoprotein B-100, because non-HDL cholesterol correlates very closely with apolipoprotein B-100 levels. Furthermore, both these studies found that the total cholesterol to HDL cholesterol ratio remained a very strong predictor of risk, superior even to the ratio of apolipoprotein B-100 to apolipoprotein A-I. Thus, despite evidence favoring apolipoproteins A-I and B-100 in univariate analyses as replacements for HDL and LDL cholesterol, there remain little clinical data that use of these measures improves overall risk

prediction compared with standard lipid testing. Apolipoproteins may have particular use in the monitoring of statin therapy (16).
Till now the role of triglycerides in atherogenesis remains controversial. Triglyceride levels tend to vary inversely with HDL cholesterol levels, demonstration of an unequivocal effect of triglycerides on cardiovascular events and mortality independent of HDL levels has proved elusive. The level of triglycerides in the blood depends exquisitely on diet. Sampling serum for triglyceride levels in the fasting state avoids some of the variability in this measurement. The actual exposure of the artery wall to triglyceride-rich lipoprotein particles, such as very-low-density lipoproteins, may constitute a factor that promotes atherosclerosis that would be missed by the fasting lipid profile. For these reasons, among others, current guidelines do not establish a target value of triglycerides. However, some studies have suggested that triglycerides do provide important information about risk in certain populations (17). In view of the tight link of triglyceride levels with known risk factors for atherosclerosis (e.g., low HDL cholesterol level, uncontrolled diabetes, hypothyroidism), the finding of marked and persistently elevated triglyceride levels should enter into the overall risk assessment for an individual and stimulate consideration of the reason for triglyceride level elevation (18). A cautious approach to triglyceride reduction would seem prudent, because randomized trial data using fenofibrate among diabetic patients with elevated triglyceride levels have failed to find significant reductions in risk using this approach (19).

## Smoking

Cigarette smokers are two to four times more likely to develop coronary heart disease than non-smokers and they have double the risk for stroke. The mechanisms are complex and likely multifactorial and result in endothelial dysfunction and a relatively hypercoagulable state. It is known that after smokers give up smoking, their risk of mortality and future cardiac events declines,

although whether cardiovascular risk for former smokers ever reaches that of never smokers. Using data from the Third National Health and Nutrition Examination Survey (NHANES III), researchers found that the smoking-associated inflammatory response subsides within 5 years after smoking cessation, suggesting that the cardiovascular risk subsides gradually with reduced exposure.

There is evidence for an adverse effect of smoking on CAD (20). This adverse effect of smoking is related to the amount of tobacco smoked daily and to the duration of smoking. The effect of smoking on CAD interact synergically in the presence of other cardiovascular risk factors such age, gender, arterial hypertension and diabetes (21-25). Passive smoking has been shown to increase the risk of CAD and other smoking-related diseases (26-31).

Recent prospective studies have documented that, compared with nonsmokers, persons who consume 20 or more cigarettes daily have a two- to threefold increase in total coronary heart disease. Moreover, these effects depend on dose; consumption of as few as one to four cigarettes daily increases coronary artery disease risk. Such "light" levels of smoking have a major impact on myocardial infarction and all-cause mortality, even among smokers who do not report inhalation. In addition to myocardial infarction, cigarette consumption directly relates to increased rates of sudden death, aortic aneurysm formation, symptomatic peripheral vascular disease, and ischemic stroke.

Beyond acute unfavorable effects on blood pressure and sympathetic tone, and a reduction in myocardial oxygen supply, smoking affects atherothrombosis by several other mechanisms. In addition to accelerating atherosclerotic progression, long-term smoking may enhance oxidation of low-density lipoprotein cholesterol and impair endothelium-dependent coronary artery vasodilation. This latter effect has been linked to dysfunctional endothelial nitric oxide biosynthesis following chronic as well as acute cigarette consumption (32). In addition, smoking has adverse

hemostatic and inflammatory effects, including increased levels of C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (ICAM-1), fibrinogen, and homocysteine (33). Additionally, smoking is associated with spontaneous platelet aggregation, increased monocyte adhesion to endothelial cells, and adverse alterations in endothelial derived fibrinolytic and antithrombotic factors, including tissue-type plasminogen activator and tissue pathway factor inhibitor. Compared with nonsmokers, smokers have an increased prevalence of coronary spasm and reduced thresholds for ventricular arrhythmia. Increasing evidence has suggested that insulin resistance represents an additional mechanistic link between smoking and premature atherosclerosis (34).

## Hypertension

Hypertension is a risk factor for the development of atherosclerosis, atherosclerotic cardiovascular disease, and stroke. Elevated blood pressure (BP) decreases cognitive function and is associated with heart failure, peripheral vascular disease and renal failure (35). CAD and stroke mortality increases progressively and linearly from BP levels as low as 115mmHg systolic and diastolic upward (36). High blood pressure often confers silent cardiovascular risk, and its prevalence is steadily increasing. Longitudinal data obtained from the Framingham Heart Study indicated that BP values (systolic blood pressure, 130 to 139 mmHg, diastolic blood pressure 85 to 89 mmHg, or both) augment the risk of cardiovascular disease twofold compared with lower levels (37). The mechanism by which hypertension causes these effects is not known, and some uncertainty exists as to what the primary and secondary factors are in a typically multifactorial syndrome. These factors may include hyperlipidemia, hypertension, diabetes mellitus, obesity, and physical inactivity. Hypertension is associated with morphologic alterations of the arterial intima and functional alterations of the endothelium that are similar to the changes observed in hypercholesterolemia and

established atherosclerosis. Endothelial dysfunction is a feature of hypertension, hyperlipidemia, and atherosclerosis and is known to represent and contribute to the procoagulant, proinflammatory, and proliferative components of atherogenesis. Hypertension has been shown, in both epidemiologic and experimental studies, to accelerate atherosclerotic vascular disease and increase the incidence of clinical complications (38).

## The metabolic syndrome and diabetes

Diabetes mellitus is an important risk factor for hyperlipidemia and atherosclerosis and commonly associated with hypertension, abnormalities of coagulation, platelet adhesion and aggregation, increased oxidative stress, and functional and anatomic abnormalities of the endothelium and endothelial vasomotion. The metabolic syndrome describes the association of cardiovascular risk factors in individuals with obesity or insulin resistance. It identifies subjects with increased risk of developing CAD in accordance with clustering of risk factors, but does not indicate the risk of CAD over and above the effect of the risk factors involved. Patients with diabetes have two-to eightfold higher rate of future cardiovascular events as compared with age- and ethnically-matched nondiabetic individuals, and 75 percent of all deaths in diabetic patients result from coronary heart disease. Compared with unaffected individuals, diabetic patients have a greater atherosclerotic burden, both in the major arteries and in the microvascular circulation. Not surprisingly, diabetic patients have substantially increased rates of atherosclerotic complications in the settings of primary prevention and after coronary interventional procedures. Insulin resistance alone confers an elevated risk of congestive heart failure and probably explains the association of obesity with this common vascular complication (39). Moreover, the risk of cardiovascular disease starts to increase long before the onset of clinical diabetes. Although hyperglycemia

is associated with microvascular disease, insulin resistance itself promotes atherosclerosis even before it produces frank diabetes, and available data corroborate the role of insulin resistance as an independent risk factor for atherothrombosis (40).

The diagnosis of metabolic syndrome is of greatest importance in non-diabetic subjects as an indicator of an increased risk of developing type 2 diabetes and CAD. Among different definitions for the metabolic syndrome formulated by international and national expert groups, the original National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) definition and its revision recommended by the American Heart Association (AHA) and National Heart, Lung and Blood Institute (NHLBI) and the definition created by the Consensus Panel of the International Diabetes Federation (41,42) have been developed for clinical use. Insulin resistance and diabetes rank among the major cardiovascular risk factors; in one major survey, the presence of diabetes conferred an equivalent risk to aging 15 years, an impact greater than that of smoking (43).

## Obesity

From an epidemiological perspective, obesity alone associates with elevated vascular risk regardless of activity levels, and the waist-to-hip ratio independently predicts vascular risk in women and in older men. Fat is associated with increased secretion of free fatty acids and dyslipidemia (44). Excess central obesity has been shown to be strongly associated with metabolic and cardiovascular risk. Increased visceral adipose tissue area was significantly associated with noncalcified coronary plaques burden and vulnerable characteristics (positive remodeling index > 1.05) identified by 64-slice computed tomography angiography (45). Body Mass Index (BMI)- waist-hip circumference ratio has been used to define groups of body weight classified by the National Institutes of health and the World Health Organization (WHO).

Overweight is defined by an increased BMI ranging from 25 to 29,9 kg/m$^2$ and obesity>30 kg/m$^2$. Midlife obesity strongly presages hospitalization and future complications of coronary heart disease, even among those with few or no other major risk factors (46). Thus, weight control must play a fundamental role in all preventive cardiology practices, preferably in conjunction with advice regarding diet and exercise. In the Women's Health Study, high BMI was more strongly associated with adverse cardiovascular biomarkers than physical activity, yet within BMI categories, physical activity directly influenced overall levels of risk. As also made clear from recent randomized trial data, life style modification, including exercise, is a crucial adjunct to any pharmacological weight loss program (47).

## Physical activity

Physical inactivity is a significant public health problem overworld. Physical exercise reduces myocardial oxygen demand and increases exercise capacity, both of which correlate with lower levels of coronary risk. The cardioprotective effects of exercise include reduced adiposity and diabetes incidence, lowered blood pressure, and improvement of dyslipidemia, as well as vascular inflammation. Exercise also enhances endothelial dysfunction, insulin sensitivity, and endogenous fibrinolysis. Prospective epidemiological studies almost universally demonstrate strong graded associations between levels of physical activity and reduced rates of cardiovascular morbidity and all-cause mortality (48).

In the Women's Health Initiative, walking briskly for 30 minutes five times/week was associated with a 30 percent reduction in vascular events over a 3.5-year follow-up, an effect that persisted after adjustment for body mass index, age, and ethnicity (49). In men participating in the Health Professional Follow-Up Study, 30 minutes of daily walking was associated with an 18 percent

reduction in coronary risk. In that study, contrary to commonly given medical advice, resistance exercise and weight training also contributed to cardiovascular benefit (50).

Data that exercise and life style interventions will remain crucial for risk reduction programs is also showed by the outcome of several recent dietary intervention studies that have failed to demonstrate sustained vascular benefit. Despite cohort studies suggesting benefit (51) in the Women's Health Initiative Dietary Modification trial, interventions that reduced total fat intake and increased intake of vegetables, fruits, and grains did not significantly reduce event rates in postmenopausal women and achieved only modest effects on traditional risk factors (52). By contrast, when adherence rates were higher, as in a study of both Mediterranean diet and life style interventions conducted in the elderly, almost 50 percent reductions in vascular risk were observed (53).

## Psychosocial factors

The following psychosocial risk factors have been shown to influence both the risk of contracting CAD and the worsening of clinical course and prognosis in patients with CAD:

- Low socio-economic status
- Social isolation and lack of social support
- Stress at work and in family life
- Negative emotions including depression and hostility.

The adrenergic stimulation of mental stress can augment myocardial oxygen requirements and aggravate myocardial ischemia. Mental stress can cause coronary vasoconstriction, particularly in atherosclerotic coronary arteries, and hence can influence myocardial oxygen supply as well. Studies have further linked mental stress to platelet and endothelial dysfunction, the metabolic syndrome, and the induction of ventricular arrhythmias.

Acute stress, such as that associated with natural disasters, is recognized as a risk factor for acute coronary events. More recently, work-related stress has gained recognition as a source of vascular risk. Work stress has two components- job strain, which combines high work demands and low job control, and effort-reward imbalance, which more closely reflects economic factors in the workplace. Both components are associated with an approximate doubling of risk for myocardial infarction and stroke (54). Other psychological metrics, including anger and hostility scales, have also been associated with elevated vascular risk. In the INTERHEART study evaluating postinfarction patients from 52 countries, psychosocial stress was found to be associated with vascular risk, having a magnitude of effect similar to that of the major coronary risk factors (55). The impact of stress reduction has also undergone evaluation in randomized trials. Among patients with stable ischemic heart disease and exercise-induced myocardial ischemia, random allocation to a stress management program reduced emotional distress and improved biomarkers of vascular risk significantly more than usual care (56). Clinical depression strongly predicts coronary heart disease (57). In a meta-analysis of 11 studies involving initially healthy individuals, those with depression had a significantly higher risk of developing coronary disease during follow-up (58). Although depression is also associated with an increased prevalence of hypertension, smoking, and lack of physical activity, the effects of depression on overall risk remain after adjusting for these and other traditional risk factors.

## Inflammation markers

Prospective epidemiological studies have linked the novel risk factors, including high-sensitivity C-reactive protein (hsCRP) and other markers of inflammation, lipoprotein(a), homocysteine, and

markers of fibrinolytic and hemostatic function, such as fibrinogen, D-dimer, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor 1 (PAI-1) antigens (59-61).

## High-sensitivity C-reactive protein

C-reactive protein is a circulating member of the pentraxin family that plays a major role in the human innate immune response. Although derived primarily from the liver, studies have found that cells within human coronary arteries, particularly in the atherosclerotic intima, can also elaborate CRP. More than simply a marker of inflammation, CRP may influence directly vascular vulnerability through several mechanisms, including enhanced expression of local adhesion molecules, increased expression of endothelial PAI-1, reduced endothelial nitric oxide bioactivity, altered LDL uptake by macrophages, and colocalization with complement within atherosclerotic lesions. Moreover, expression of human CRP in CRP-transgenic mice was found to directly enhance intravascular thrombosis (62). The evidence for direct proinflammatory effects of CRP has less strength at present than the consistent data regarding its ability to predict risk.

A large series of prospective epidemiological studies (63), has demonstrated convincingly that CRP is a strong predictor for future cardiovascular events. Most importantly, high-sensitivity C-reactive protein (hsCRP) adds prognostic information at all levels of LDL cholesterol and at all levels of risk, as determined by the Framingham Risk Score (64). In clinical terms, absolute vascular risk is higher in individuals with elevated hsCRP levels and low levels of LDL cholesterol than in those with elevated levels of LDL cholesterol but low levels of hsCRP, but current guidelines consider only the latter group to be at high risk. Because hsCRP levels are stable over long periods, having no circadian variation and these do not depend on prandial state, screening can easily be done on an outpatient basis. High-sensitivity C-reactive protein levels less

than 1, 1 to 3, and higher than 3 mg/liter should be interpreted as lower, moderate, and higher relative vascular risk, respectively, when considered along with traditional markers of risk (65). Levels of hsCRP greater than 3 mg/liter also predict recurrent coronary events, thrombotic complications after angioplasty, poor outcome in the setting of unstable angina and vascular complications after bypass surgery. All these data support the concept that inflammation plays a critical role throughout the atherothrombotic process.

It has long been known that CRP levels vary a bit between ethnic groups. The vascular event rates vary between ethnic groups and this variation tracks with CRP levels. A proportion of African Americans have higher CRP levels, since they also have higher vascular-event rates than do age-matched whites. Similarly, those of Japanese or Chinese descent have somewhat lower CRP levels and also have somewhat lower vascular risk (65).

The hsCRP provides a useful measure of inflammation in the atherosclerotic process. It remains uncertain as to what stimulus initiates the underlying proinflammatory response. Patients with low-grade infections such as gingivitis or those who chronically carry Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus, and cytomegalovirus may also have higher vascular risk on the basis of a chronic systemic inflammatory response. However, careful prospective studies of antibody titers directed against these agents have not consistently found evidence of association, and large-scale antibiotic trials have not shown reduced recurrent events in myocardial infarction survivors (66).

## Other markers of inflammation

Several markers of inflammation have shown promise in terms of predicting vascular risk. These include cytokines such as

interleukin-6, soluble forms of certain cell adhesion molecules such as intercellular adhesion molecule (sICAM-1), P-selectin, or the mediator CD40 ligand, as well as markers of leukocyte activation such as myeloperoxidase. The inflammatory markers associated with lipid oxidation such as lipoprotein-associated phospholipase $A_2$ and pregnancy-associated plasma protein A have also shown promise (67). However, each of these biomarkers has analytical issues that need careful evaluation before routine clinical use. Continued evaluation of other inflammatory biomarkers may well provide targets for or monitors of therapy, particularly in the setting of acute coronary ischemia (68).

## Homocysteine

Patients with rare inherited defects of methionine metabolism can develop severe hyperhomocysteinemia and have markedly elevated risk of premature atherothrombosis. Mechanisms suggested to account for these effects include endothelial dysfunction, accelerated oxidation of LDL cholesterol, impairment of flow-mediated endothelium-derived relaxing factor with subsequent reduction in arterial vasodilation, platelet activation, and oxidative stress (69). In contrast to severe hyperhomocysteinemia, mild to moderate elevations of homocysteine (plasma levels higher than 15μmol/liter) are more common in the general population, primarily because of insufficient dietary intake of folic acid. Other patient groups who tend to have elevated levels of homocysteine include those receiving folate antagonists such as methotrexate and carbamazepine and those with impaired homocysteine metabolism caused by hypothyroidism or renal insufficiency.

With regard to clinical trials of homocysteine reduction, several major studies have been completed and none have shown substantive benefit. In the Vitamin Intervention for Stroke

Prevention (VISP) trial conducted among 3 680 patients with prior stroke allocated to high-dose or low-dose vitamin regimens containing folate and pyridoxine, there was no evidence of differential benefit in the high-dose group, despite greater homocysteine level reduction (70). In a second trial, of 636 postangioplasty patients treated with folate, vitamin $B_6$ and vitamin $B_{12}$, rates of in-stent stenosis were actually higher in most intervention groups compared with those allocated to placebo. This negative trial is clinically important, because it conflicts with prior work that had suggested benefit in this setting.

## Fibrinogen

In a recent meta-analysis, there was an approximately linear logarithmic association between usual fibrinogen level and the risk of coronary heart disease and stroke. In one analysis, the age- and gender-adjusted hazard ratio per 1g/liter increase in fibrinogen was 2.4 for coronary heart disease and 2.1 for stroke (71). In more recent studies, hsCRP and fibrinogen levels appeared to be additive in their ability to predict risk, although the absolute effect of hsCRP appeared to be larger. Other studies have suggested that the predictive usefulness of fibrinogen is highest in those with other concomitant elevations of lipoprotein(a) or homocysteine (72).

The Bezafibrate Infarction Prevention Trial showed no reduction in event rates with active therapy despite a significant reduction in fibrinogen levels and despite evidence that within the study population, baseline fibrinogen levels predicted vascular risk (73). In a second trial of more than 1 500 patients with peripheral vascular disease, bezafibrate reduced fibrinogen levels by 13 percent but again had no significant effect on clinical outcomes.

# Markers of fibrinolytic function

Impaired fibrinolysis can result from an imbalance between the clot-dissolving enzymes
t-PA or urokinase-type plasminogen activator and their endogenous inhibitors, primarily plasminogen activator inhibitor 1 (PAI-1). Visceral obesity yields enhanced PAI-1 production from adipocytes, and thus impaired fibrinolysis may help explain how weight gain and obesity influence atherothrombosis (74). Prospective associations exist between PAI-1 antigen and activity levels and the risk of arterial thrombosis and recurrent myocardial infarction. Iindividuals at risk for future coronary as well as cerebral thrombosis consistently also have elevated levels of circulating t-PA antigen. It may represents evidence of underlying endothelial dysfunction in individuals at risk or a direct relationship between t-PA and PAI-1, or a biological response to impaired fibrinolysis. In this regard, reduced clot lysis time, an overall indicator of net fibrinolytic function, also predicts coronary risk. As reviewed earlier, several studies have indicated that levels of D-dimer, a peptide released by plasmin's action on fibrin, also predicts myocardial infarction, peripheral atherosclerosis, and recurrent coronary events. These observations have been confirmed in studies of women taking and not taking hormone replacement therapy, an important issue because conjugated estrogens decrease PAI-1 antigen concentrations. Despite these data, the clinical use of fibrinolytic markers to determine coronary risk offers only marginal value, and no data available suggest that measures of fibrinolysis add to traditional risk scores. Direct measurement of PAI-1 activity is difficult in clinical settings and requires special anticoagulants and precise phlebotomy techniques to avoid degranulation of platelets, a rich source of PAI-1. In addition, markers of fibrinolytic function such as PAI-1 have a wide circadian variation, limiting use in outpatient settings as a risk determinant (75).

## References:


1. Paul M Ridker, Julie E. Buring, Nader Rifai, Nancy R. Cook. Development and Validation of Improved Algorithms for the Assessment of Global Cardiovascular Risk in Women.The Reynolds Risk Score. JAMA. 2007;297(6):611-619.
2. Khot UN, *Khot MB, Bajzer CT, et al. Prevalence of conventional risk factors in patients with coronary heart disease.* JAMA. 2003;290:898-904. pmid:12928466.
3. Group EUROASPIREIIS. Lifestyle and risk management and use of drug therapies in coronary patients from 15 countries. Principal results from EUROASPIRE II. Eur Heart J. 2001;22:554–572. doi: 10.1053/euhj.2001.2610.
4. Gregg EW, Cheng YJ, Cadwell BL, et al: Secular trends in cardiovascular disease risk factors according to body mass index in U.S. adults. JAMA 2005; 293:1868.
5. Rothwell PM, Coull AJ, Giles MF, et al: Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). Lancet 2004; 363:1925.
6. Chiuve SE, McCullough ML, Sacks FM, Rimm EB: Healthy life style factors in the primary prevention of coronary heart disease among men: Benefits among users and nonusers of lipid-lowering and antihypertensive medications. Circulation 2006; 114:160.
7. ACCF/AHA/ACP 2009 Competence and Training Statement: A Curriculum on Prevention of Cardiovascular Disease. J Am Coll Cardiol, 2009; 54:1336-1363.
8. Collins R, Armitage J, Parish S, et al: Effects of cholesterol-lowering with simvastatin on stroke and other major vascular events in 20536 people with cerebrovascular disease or other high-risk conditions. Lancet 2004; 363:757.
9. Nissen SE, Tuzcu EM, Schoenhagen P, et al: Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: A randomized controlled trial. JAMA 2004; 291:1071.
10. Cannon CP, Braunwald E, McCabe CH, et al: Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med. 2004; 350:1495.

11. Schuster H. The GALAXY Program: an update on studies investigating efficacy and tolerability of rosuvastatin for reducing cardiovascular risk. Expert Rev Cardiovasc Ther. 2007, 5:177-93.

12. Venkata Narla, Michael J Blaha, Roger S Blumenthal, and Erin D Michos Vasc Health Risk manage. The JUPITER and AURORA clinical trials for rosuvastatin in special primary prevention populations: perspectives, outcomes, and consequences. 2009,5:1033-1042.

13. Brewer Jr HB: Increasing HDL cholesterol levels. N Engl J Med. 2004; 350:1491.

14. Forrester JS, Makkar R, Shah PK: Increasing high-density lipoprotein cholesterol in dyslipidemia by cholesteryl ester transfer protein inhibition: An update for clinicians. Circulation.2005;111:1847.

15. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004 Sep 11-17;364(9438):937-52.

16. Sniderman AD, Furberg CD, Keech A, et al: Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. Lancet 2003; 361:777.

17. Patel A, Barzi F, Jamrozik K, et al: Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region. Circulation 2004; 110:2678.

18. Abdel-Maksoud MF, Hokanson JE: The complex role of triglycerides in cardiovascular disease. Semin Vasc Med 2002; 2:325.

19. Keech A, Simes RJ, Barter P, et al: Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): Randomised controlled trial. Lancet 2005; 366:1849.

20. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G,Wouters EF. Systemic effects of smoking. Chest. 2007;131(5):1557-1566.

21. Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: A systematic review and metaanalysis.JAMA. 2007;298(22):2654-2664.

22. Miyatake N, Wada J, Kawasaki Y, Nishii K, Makino H, Numata T.Relationship between metabolic syndrome and cigarette smoking in the Japanese population. Intern Med. 2006;45(18):1039-1043.

23. Halperin RO, Gaziano JM, Sesso HD. Smoking and the risk of incident hypertension in middle aged men. Am J Hyperten.2008;21(2):148-152.

24. Oncken CA, White WB, Cooney JL, van Kirk JR, Ahluwalia JS,Giacco S. Impact of smoking cessation on ambulatory blood pressure and heart rate in postmenopausal women. Am JHyperten. 2001;14(9 Pt 1):942-949.

25. Janzon E, Hedblad B, Berglund G, Engstrom G. Changes in blood pressure and body weight following smoking cessation in women. J Intern Med. 2004;255(2):266-272.

26. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relationto smoking: 50 years' observations on male British doctors. BMJ. 2004;328(7455):1519-1528.

27. Kenfield SA, Stampfer MJ, Rosner BA, Colditz GA. Smoking and smoking cessation in relation to mortality and women. JAMA. 2008;299(17):2037-2047.

28. The Multiple Risk Factor Intervention Trial Research Group (MRFIT). Mortality after 16 years for participants randomized to the multiple risk factor intervention trial. Circulation. 1996;94(5):946-951.

29. Anthonisen NR, Skeans MA, Wise RA, et al. The effects of a smoking cessation intervention on 14.5-year mortality. Ann Intern Med. 2005;142(4):233-239.

30. Law MR, Morris JK, Wald NJ. Environmental tobacco smoke exposure and ischaemic heart disease: an evaluation of the evidence. BMJ. 1997;315(7114):973-980.

31. Canadian Tobacco Use Monitoring Survey. Smoking prevalence, Canada, 1999-2007. Available at: www.hc-sc.gc.ca/hl-vs/tobactabac/research-recherche/stat/ctums-esutc 2007-eng.php.

32. Barua RS, Ambrose JA, Srivastava S, et al: Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: An in vitro demonstration in human coronary artery endothelial cells. Circulation 2003; 107:2342.

33. Bazzano LA, He J, Muntner P, et al: Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 2003; 138:89.

34. Reaven G, Tsao PS: Insulin resistance and compensatory hyperinsulinemia: the key player between cigarette smoking and cardiovascular disease? J Am Coll Cardiol 2003; 41:1044.

35. Lithell H, Hansson L, Skoog I, et al. SCOPE Study Group The Study on Cognition and Prognosis in the Elderly (SCOPE): principal results of a randomized double-blind intervention trial Hypertension 2003;21:875-876.

36. Truelsen T, Mähönen M, Tolonen H, Asplund K, Bonita R, Vanuzzo D, for the WHO MONICA Project. Trends in stroke and coronary heart disease in the WHO MONICA Project. *Stroke* 2003;34:1346-1352.

37. Vasan RS, Larson MG, Leip EP, et al: Impact of high-normal blood pressure on the risk of cardiovascular disease. N Engl J Med 2001; 345:1291.

38. Blood Pressure Lowering Treatment Trialists Collaboration Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials Lancet 2003;362:1527-1545.

39. Ingelsson E, Sundstrom J, Arnlov J, et al: Insulin resistance and risk of congestive heart failure. JAMA 2005; 294:334.

40. Grosu A., David L.,Ţurcan V. Mortality in non-ST-elevated ecute coronary syndrome associated with diabetes. BULETIN OF THE ACADEMY OF SCIENCES OF MOLDOVA, 4(18)2008,10-14.

41. Scott M. Grundy, Barbara Hansen, Sidney C. Smith, James I. Cleeman, Richard A. Kahn. Clinical Management of Metabolic Syndrome Report of the American Heart Association/National Heart, Lung, and Blood Institute/ American Diabetes Association Conference on Scientific Issues Related to Management.. *Circulation* 2004;109:551-556.

42. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome-a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med, 2006 May;23(5):469-80.

43. Booth GL, Kapral MK, Fung K, Tu JV: Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: A population-based retrospective cohort study. Lancet 2006; 368:29.

44. Silventoinen K, Sans S, Tolonen H, Monterde D, Kuulasmaa K, Kesteloot H, Tuomilehto J, The WHO MONICA Project. Trends in obesity and energy supply in the WHO MONICA Project. *Int J Obes* 2004;28:710-718.

45. Norihiko Ohashi, Hideya Yamamoto, Jun Horiguchi, Toshiro Kitagawa, Eiji Kunita, Hiroto Utsunomiya, Toshiharu Oka, Nobuoki Kohno, Yasuki Kihara. Association Between Visceral Adipose Tissue Area and Coronary Plaque Morphology Assessed by CT Angiography. J Am Coll Cardiol Img, 2010; 3:908-917.

46. Yan LL, Daviglus ML, Liu K, et al: Midlife body mass index and hospitalization and mortality in older age. JAMA 2006; 295:190.

47. Mora S, Lee IM, Buring JE, Ridker PM: Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. JAMA 2006; 295:1412.

48. Thompson PD, Buchner D, Pina IL, et al: Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: A statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). Circulation 2003; 107:3109.

49. Manson JE, Greenland P, La Croix AZ, et al: Walking compared with vigorous exercise for the prevention of cardiovascular events in women. N Engl J Med 2002; 347:716.

50. Tanasescu M, Leitzmann MF, Rimm EB, et al: Exercise type and intensity in relation to coronary heart disease in men. JAMA 2002; 288:1994.

51. He FJ, Nowson CA, MacGregor GA: Fruit and vegetable consumption and stroke: Meta-analysis of cohort studies. Lancet 2006; 367:320.

52. Howard BV, Van Horn L, Hsia J, et al: Low-fat dietary pattern and risk of cardiovascular disease: The Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 2006; 295:655.

53. Knoops KT, de Groot LC, Kromhout D, et al: Mediterranean diet, life style factors, and 10-year mortality in elderly European men and women: The HALE project. JAMA 2004; 292:1433.

54. Kivimaki M, Leino-Arjas P, Luukkonen R, et al: Work stress and risk of cardiovascular mortality: prospective cohort study of industrial employee s. BMJ 2002; 325:857.

55. Rosengren A, Hawken S, Ounpuu S, et al: Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): Case-control study. Lancet 2004; 364:953.

56. Blumenthal JA, Sherwood A, Babyak MA, et al: Effects of exercise and stress management training on markers of cardiovascular risk in patients with ischemic heart disease: A randomized controlled trial. JAMA 2005; 293:1626.

57. Habra ME, Baker B et al. First episode of major depressive disorder and vascular factors in coronary artery disease patients: Baseline characteristics and response to antidepressant treatment in the CREATE trial; Journal of Psychosomatic Research 69 (2), 133-14, 2010.

58. Rugulies R: Depression as a predictor for coronary heart disease. A review and meta-analysis. Am J Prev Med 2002; 23:51.

59. Strandberg TE, Tilvis RS. C-reactive protein, cardiovascular risk factors, and mortality in a prospective study in the elderly. Arterioscler Thromb Vasc Biol 2000;20:1057-60.

60. Volpato S, Guralnik JM, Ferrucci L, Balfour J, Chaves P, Fried LP, et al. Cardiovascular disease, interleukin-6, and risk of mortality in older women: the Women's Health and Aging study. Circulation 2001;103:947-53.

61. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol 2007;49: 2129-38.

62. Danenberg HD, Szalai AJ, Swaminathan RV, et al: Increased thrombosis after arterial injury in human C-reactive protein-transgenic mice. Circulation 2003; 108:512.

63. Peters S. A. E., Palmer M. K., Grobbee D. E., Crouse III J. R., O'Leary D. H., Raichlen J. S., Bots M. L. C-reactive protein lowering with rosuvastatin in the METEOR study. Journal of Internal Medicine.Volume 268, Issue 2, 2010; pages 155–161.

64. Ridker PM, Rifai N, Rose L. et al: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002; 347:1557.

65. Koenig W, Lowel H, Baumert J, Meisinger C: C-reactive protein modulates risk prediction based on the Framingham Score: Implications for

future risk assessment: Results from a large cohort study in southern Germany. Circulation 2004; 109:1349.

66. Andraws R, Berger JS, Brown DL: Effects of antibiotic therapy on outcomes of patients with coronary artery disease: A meta-analysis of randomized controlled trials. JAMA 2005; 293:2641.
67. Zalewski A, Macphee C: Role of lipoprotein-associated phospholipase A2 in atherosclerosis: Biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol 2005; 25:923.
68. Apple FS, Wu AH, Mair J, et al: Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem 2005; 51:810.
69. Ford ES, Smith SJ, Stroup DF, et al: Homocysteine and cardiovascular disease: A systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. Int J Epidemiol 2002; 31:59.
70. Lange H, Suryapranata H, De Luca G, et al: Folate therapy and in-stent restenosis after coronary stenting. N Engl J Med 2004; 350:2673.
71. Danesh J, Lewington S, Thompson SG, Fibrinogen Studies Collaboration et al: Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: An individual participant meta-analysis. JAMA 2005; 294:1799.
72. Menown IB, Mathew TP, Gracey HM, et al: Prediction of Recurrent Events by D-Dimer and Inflammatory Markers in Patients with Normal Cardiac Troponin I (PREDICT) Study. Am Heart J 2003; 145:986.
73. Tanne D, Benderly M, Goldbourt U, et al: A prospective study of plasma fibrinogen levels and the risk of stroke among participants in the bezafibrate infarction prevention study. Am J Med 2001; 111:457.
74. Festa A, D'Agostino Jr R, Tracy RP, Haffner SM: Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: The insulin resistance atherosclerosis study. Diabetes 2002; 51:1131.
75. Pradhan AD, LaCroix AZ, Langer RD, et al: Tissue plasminogen activator antigen and D-dimer as markers for atherothrombotic risk among healthy postmenopausal women. Circulation 2004; 110:292.

*When man is serene, the pulse of the heart flows and connects, just as pearls are joined together or like a string of red jade, then one can talk about a healthy heart.*

The Yellow Emperor's Canon of Internal Medicine, 2500 B.C.

# Chapter 3. The pathogenesis of atherosclerosis

Atherosclerosis, the primary cause of coronary artery disease, is a disease of large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow and diminished oxygen supply to target organs (1).

The 3rd millennium assists a significant evolution in views related to the pathogenesis of atherosclerosis. This disease has a respected history, including left evidences in the arteries of Egyptian mummies. A century has passed since the word "atherosclerosis" was introduced by Felix Marchand in 1904, and he suggested that atherosclerosis was responsible for almost all obstructive processes in the arteries. Then, in 1913, Nikolai N. Anitschkow showed that cholesterol alone caused the atheromatous changes

in the vascular wall. Elucidation of the role of cholesterol in the pathogenesis of atherosclerosis is often referred to as one of the greatest discoveries of the 20th century. This discovery introduced a new era in the studies of atherosclerosis. His classic experiments in 1913 paved the way to our current understanding of the role of cholesterol in cardiovascular disease.

## Response-to-vascular injury theory

Over the past decade, Ross R, and Fuster V. (1996) and colleagues have proposed the currently accepted unifying theory of atherogenesis *Response-to-vascular injury (table 3.1)*, in that vascular injury starts the atherosclerotic process (2).

*Table 3.1*

**Vascular injury** (Ross R, Fuster V. *The pathogenesis of atherosclerosis):*

| |
|---|
| **Type I** - Vascular injury involving functional changes in the endothelium with minimal structural changes, (ie, increased lipoprotein permeability and white blood cell adhesion) |
| **Type II** - Vascular injury involving endothelial disruption with minimal thrombosis |
| **Type III** - Vascular injury involving damage to media, which may stimulate severe thrombosis, resulting in unstable coronary syndromes. |

Termed the response-to-injury hypothesis, it postulates that atherosclerosis begins with endothelial injury, making the endothelium susceptible to the accumulation of lipids and the deposition of thrombus (*table 3.1*).

## Endothelial dysfunction

The intimal layer of arteries in normal human adults contains some resident smooth muscle cells embedded in extracellular matrix and is covered with a monolayer of vascular endothelial cells. Endothelium is the monolayered inner lining of the vascular system. It covers almost 700 $m^2$ and weighs 1.5 kg. Endothelial dysfunction is the initial step that allows diffusion of lipids and inflammatory cells (ie, monocytes, T lymphocytes) into the endothelial and subendothelial spaces (*figure 3.1*-3.3).

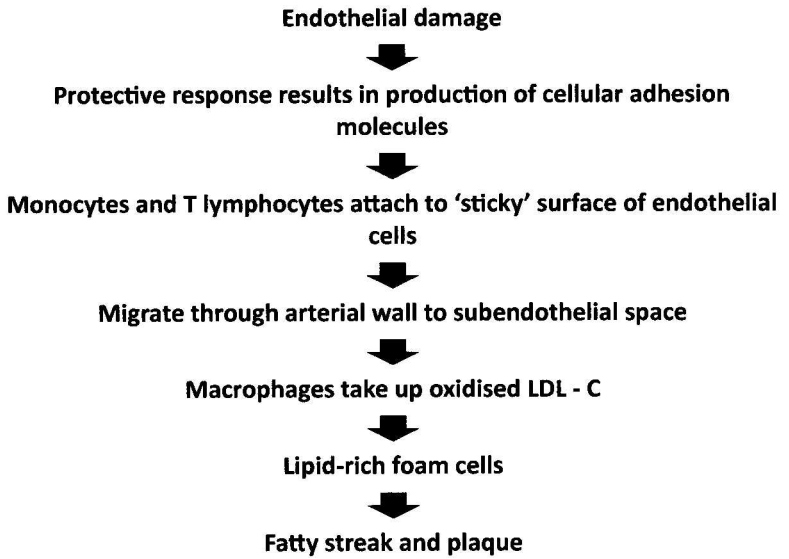



*Figure 3.1.* **Pathogenesis of atherosclerotic plaques**
(Koenig W, *Eur. Heart J Suppl* 1999).

According to the *response-to-vascular injury* theory, injury to the endothelium by local disturbances of blood flow at angulated or branch points, along with systemic risk factors (eg, hypertension, hyperglycemia, dyslipidemia, cigarette smoking, possible infection) perpetuates a series of events that culminate in the development of atherosclerotic plaque. Experimental and population studies have shown that endothelial damage may be reversed if the underlying cause is attenuated (3*).* Endothelial damage may cause changes that are localized or generalized and transient or persistent, as follows: increased permeability to lipoproteins, decreased nitric oxide production, increased leukocyte migration and adhesion, prothrombotic dominance and vascular growth stimulation and vasoactive substance release (*figure 3.2*).

Secretion of cytokines and growth factors promotes intimal migration. Taken together, experimental results in animals and studies on human atherosclerosis suggest that the "fatty streak" represents the initial lesion of atherosclerosis. The formation of these early lesions results from focal accumulation of lipoproteins in regions of the intimal layer of the artery (4).

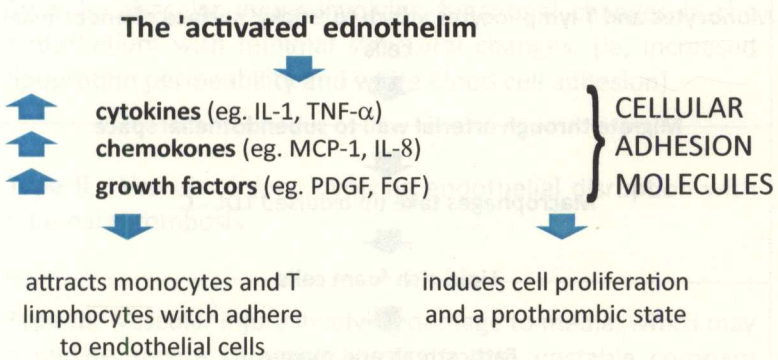



*Figure 3.2.* **Endothelial damage**
(Koenig W, *Eur Heart J Suppl* 1999).

# Role of LDL - Oxidative stress

The most atherogenic type of lipid is the low-density lipoprotein (LDL) component of total serum cholesterol. The endothelium's ability to modify lipoproteins may be particularly important in atherogenesis. LDLs appear to be modified by a process of low-level oxidation when bound to the LDL receptor, internalized, and transported through the endothelium. LDLs initially accrue in the subendothelial space and stimulate vascular cells to produce cytokines for recruiting monocytes, which causes further LDL oxidation. Extensively oxidized LDL (oxLDL) is picked up by the scavenger receptors on macrophages, which absorb the LDL and turn into foam cells. Oxidized LDL is exceedingly atherogenic and is responsible for the following (5):

- Promoting cholesterol accumulation in macrophages, which then become foam cells: All macrophages are derived from circulating monocytes. When the monocyte enters a tissue, it appears to take on characteristics peculiar to the host tissue. In most inflammatory sites, the macrophage acts as a scavenger cell to remove foreign substances by phagocytosis and intracellular hydrolysis. As a scavenger cell, the macrophage attempts to remove injurious materials (eg, oxLDL) via scavenger receptors and can oxidize LDL by such means as lipoxygenase enzymes (eg, 15-lipoxygenase) forming foam cells.
- Enhancing endothelial production of leukocyte adhesion molecules(eg, vascular cell adhesion molecule-1, intercellular cell adhesion molecule-1), cytokines and growth factors that regulate SMC proliferation, collagen degradation, and thrombosis
- Inhibiting nitric oxide synthase activity and increasing reactive oxygen species generation (eg, superoxide, hydrogen peroxide), thus reducing endothelium-dependent vasodilation.

Substantial evidence suggests that oxLDL is the prominent component of atheromas. Antibodies against oxLDL react with atherosclerotic plaques, and plasma levels of immunoreactive altered LDL are greater in persons with acute myocardial infarction than in controls. Oxidative stress has therefore been recognized as the most significant contributor to atherosclerosis by causing LDL oxidation and increasing nitric oxide breakdown.

Specified studies of labeled lipoprotein particles indicate that a prolonged residence time characterizes sites of early lesion formation in rabbits. The binding of lipoproteins to proteoglycans in the intima captures and retains these particles, accounting for their prolonged residence time. Lipoprotein particles bound to proteoglycans have increased susceptibility to oxidative or other chemical modifications, considered by many investigators to be an important component of the pathogenesis of early atherosclerosis (6). Other studies suggest that permeability of the endothelial monolayer increases at sites of lesion predilection to LDL. Contributors to oxidative stress in the nascent atheroma could include nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidases expressed by vascular cells, lipoxygenases expressed by infiltrating leukocytes, or the enzyme myeloperoxidase (7).

## Leukocyte Recruitment

Recent studies demonstrated that endothelial activation by inflammatory mediators is critical for initiating platelet adhesion and platelet-dependent leucocyte recruitment resulting in augmented increases in microvessel permeability (8).

Very early after initiation of hypercholesterolemia, leukocytes adhere to the endothelium and diapedese between endothelial cell junctions to enter the intima (*figure 3.3*), where they begin

to accumulate lipids and become foam cells (9,10). In addition to the monocyte, T-lymphocytes also tend to accumulate in early human and animal atherosclerotic lesions. The recruitment of these inflammatory cells and their transendothelial migration is mediated by the cellular adhesion molecules (CAMs), which are expressed by endothelial cells and circulating leukocytes (monocytes and limfocytes) in response to different inflammatory stimuli. Three families of CAMs participate in the leukocyte-endothelium interaction: a) the selectines that facilitate rolling of leukocytes over the endothelial surface; b) the integrines, important for the adherence of the leukocytes to the endothelium and c) the superfamily of immunoglobulines that have importance in the adhesion and transmigration of leukocytes (11).

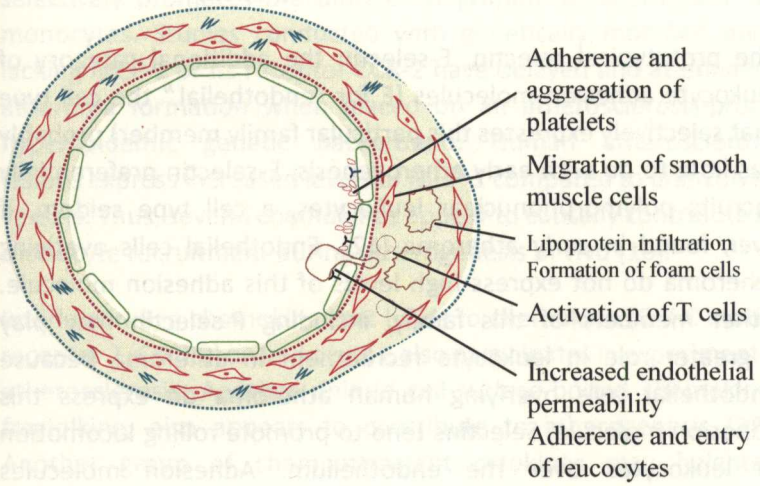



*Figure 3.3.* **Endothelial dysfunction and fatty streak formation** (Koenig W, *Eur Heart J Suppl* 1999).

The simulating role of connexin 37 in transendothelial migration of macrophages has recently been shown. Members of the immunoglobulin superfamily include such structures as vascular cell adhesion molecule-1 (VCAM-1). This adhesion molecule has particular interest in the context of early atherogenesis because it interacts with very late antigen-4 (VLA-4), an integrin, characteristically expressed by those classes of leukocytes that accumulate in nascent atheroma, monocytes, and T-cells. Studies in rabbits and mice have shown expression of VCAM-1 on endothelial cells overlying the very early atheromatous lesions. Other members of the immunoglobulin family of leukocyte adhesion molecules include intercellular adhesion molecule-1 (ICAM-1). This molecule is heterogeneous, both in the types of leukocytes it binds and because of its wide and constitutive expression at low levels by endothelial cells in many parts of the circulation.

The prototypical selectin, E-selectin the additional category of leukocyte adhesion molecules (E for "endothelial," the cell type that selectively expresses this particular family member) probably has little to do with early atherogenesis. E-selectin preferentially recruits polymorphonuclear leukocytes, a cell type seldom, if ever, found in early atheroma (12). Endothelial cells overlying atheroma do not express high levels of this adhesion molecule. Other members of this family, including P-selectin may play a greater role in leukocyte recruitment in atheroma because endothelial cells overlying human atheroma do express this adhesion molecule. Selectins tend to promote rolling locomotion of leukocytes over the endothelium. Adhesion molecules belonging to the immunoglobulin superfamily tend to promote tighter adhesive interactions and immobilization of leukocytes. Studies in genetically altered mice have proven roles for VCAM-1 and P-selectin (including both platelet- and endothelial-derived P-selectin) in experimental atherosclerosis (13). Increasing evidence

supports the accumulation in atheromas of mononuclear phagocytes of distinct subtypes (14).

To penetrate the endothelial cells and enter the arterial wall once adherent to the endothelium leukocytes must receive a signal (15). The current concept of directed migration of leukocytes involves the action of protein molecules known as chemoattractant cytokines, or chemokines. Two groups of chemokines have particular interest in recruiting the mononuclear cells characteristic of the early atheroma. One such molecule, known as monocyte chemoattractant protein-1 (MCP-1), is produced by the endothelium in response to oxidized lipoprotein and other stimuli. Cells intrinsic to the normal artery, including endothelium and smooth muscle cells, can produce this chemokine when stimulated by inflammatory mediators, as do many other cell types. MCP-1 selectively promotes the directed migration, or chemotaxis, of monocytes. Studies conducted with genetically modified mice lacking MCP-1 or its receptor CCR-2 have delayed and attenuated atheroma formation when placed on an atherosclerosis-prone hyperlipidemic genetic background. Human atherosclerotic lesions express increased levels of MCP-1 compared to uninvolved vessels. Thus, several chemokines appear to causally contribute to monocyte recruitment during atherogenesis in vivo (16).

Interleukin-8, a chemokine that binds to chemokine (C-X-C motif) receptor 2 (CXCR2) on leukocytes, also participates in experimental atherosclerosis. Another unique cell-surface-bound chemokine, fractalkine, also appears to contribute to atherogenesis (17). Another group of chemoattractant cytokines may heighten lymphocyte accumulation in plaques as well. Atheromas express a trio of lymphocyte-selective chemokines (IP-10, I-TAC, and MIG). Gamma interferon, a cytokine known to be present in atheromatous plaques, induces the genes encoding this family of T-cell chemoattractants.

## Lesion formation

As revealed by studies of morphology (*table 3.2*), atheromas typically form focally lipid accumulation and adhesion molecule expression. The location of sites of lesion predilection at proximal portions of arteries after branch points or bifurcations at flow dividers suggests a hydrodynamic basis for early lesion development.

Arteries without many branches tend not to develop atherosclerosis.

To understand how local flow disturbances might render certain foci sites of lesion predilection can help two concepts. Locally disturbed flow could induce alterations that promote the steps of early atherogenesis. Alternatively, the laminar flow that usually prevails at sites that do not tend to develop early lesions may elicit antiatherogenic homeostatic mechanisms (19). The endothelial cell experiences the laminar shear stress of normal flow and the disturbed flow at predilected sites. *In vitro* data suggest that laminar shear stress can augment the expression of genes that may protect against atherosclerosis, including forms of the enzymes superoxide dismutase, or nitric oxide synthase. Superoxide dismutase can reduce oxidative stress by catabolizing the reactive and injurious superoxide anion. Endothelial nitric oxide synthase produces the well-known endogenous vasodilator, nitric oxide. Beyond its vasodilating actions, nitric oxide can resist inflammatory activation of endothelial functions such as expression of the adhesion molecule VCAM-1. Nitric oxide appears to exert this antiinflammatory action at the level of gene expression by interfering with the transcriptional regulator nuclear factor kappa

B (NFkB). Nitric oxide actually increases the production of IkBa, an intracellular inhibitor of this important transcription factor. NFkB regulates numerous genes involved in inflammatory responses in general, and in atherogenesis in particular (20).

*Table 3.2*

**Classification of human atherosclerotic lesions (AHA,1995)** (18)

<table>
<tr><th>Nomenclature and main hystology</th><th>Main growth mechanism</th><th>Earliest onset</th><th>Clinical correlation</th></tr>
<tr><td>Type I (initial) lesion<br>isolated macrophage foam cells</td><td rowspan="4">Growth mainly lipid accumulation</td><td rowspan="2">From first decade</td><td rowspan="3">Clinicaly silent</td></tr>
<tr><td>Type II (fatty streak) lesion<br>mainly intracellular lipid accumulation</td></tr>
<tr><td>Type III (intermediate) lesion<br>Type II changes and small extracelular lipid pools</td><td rowspan="2">From third decade</td></tr>
<tr><td>Type IV (atheroma) lesion<br>Type II changes and core of extracelular lipid</td><td rowspan="3">Clinicaly silent or overt</td></tr>
<tr><td>Type V (fibroatheroma) lesion lipid core and fibrotic layers or mainly calcific or mainly fibrotic</td><td>Accelerated smooth muscle and collagen increase</td><td rowspan="2">From first decade</td></tr>
<tr><td>Type VI (complicated) lesion<br>surface defect, hematoma-hemorrhage, thrombus</td><td>Thrombosis, hematoma</td></tr>
</table>

The nitric oxide (NO) cascade and endothelial NO synthase (eNOS) are best known for their role in endothelium-mediated relaxation of vascular smooth muscle. Activation of eNOS by certain inflammatory stimuli and enhanced NO release have also been shown to promote increased microvascular permeability (21). Endothelial nitric oxide bioavailability is thought to be one of several factors that play a role in the progression of atherosclerosis. Current studies also implicate a transcription Krupple-like factor-2 (KLF-2) as an important regulator of endothelial antiinflammatory properties (22). Tetrahydrobiopterin (BH4) plays a role in eNOS regulation. BH4 deficient mice develop significantly more atherosclerotic plaques. The Krupple-like factor-2 can induce eNOS expression and inhibits NFkB function by sequestering co-factors needed to boost NFkB transcriptional activity, resulting in inhibition of the expression of the cassette of KFkB-dependent genes involved in the inflammatory pathways that operate during atherogenesis. Laminar shear stress can also limit expression of another regulator of endothelial function: thioredoxin-interacting (Txnip) protein (23). Increased thioredoxin in regions of laminar shear stress caused by decreased Txnip levels in turn augments activity of apoptosis signal-regulating kinase 1 (ASK1), an activator of c-Jun N-terminal kinase (JNK). This pathway attenuates JNK activation by proinflammatory cytokines including tumor necrosis factor. Thus, several separate athero-protective mechanisms operate so that under usual conditions of laminar shear stress in normal arteries, the endothelium tonically expresses locally acting antiinflammatory functions *(24).*

## Foam-Cell Formation

Once recruited to the arterial intima, the monocyte can there imbibe lipid and become a foam cell (*figure 3.4*), or lipid-laden macrophage (25).

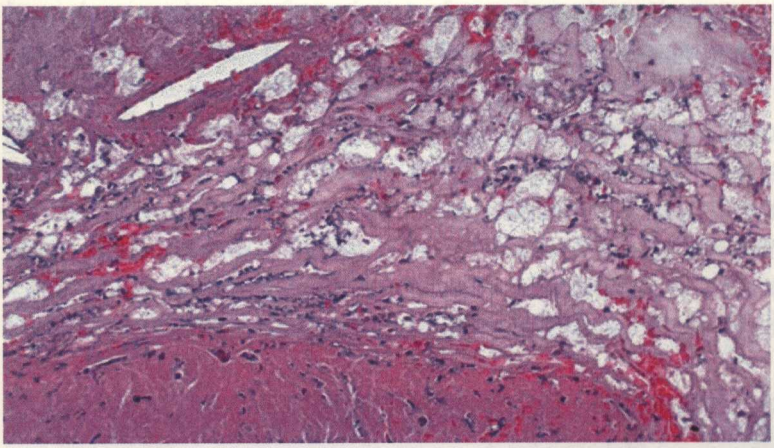


*Figure 3.4.* **Foam cells (macrophages full of lipid material) and a cholesterol cleft at higher magnification in the**

While most cells can express the classic cell surface receptor for low-density lipoprotein, that receptor does not mediate foam cell accumulation. This is evident clinically because patients lacking functional LDL receptors still develop tendinous xanthomata filled with foamy macrophages. The LDL receptor does not mediate foam cell formation because of its exquisite regulation by cholesterol. As soon as a cell collects enough cholesterol from low-density lipoprotein capture for its metabolic needs, an elegant transcriptional control mechanism quenches expression of the

receptor. Lipoprotein particles transport lipids such as cholesterol and triglycerides in association with proteins and phospholipids that render the lipids soluble in blood. Low-density lipoprotein particles, rich in cholesterol, are an example of an atherogenic lipoprotein. The accumulation of lipoprotein particles in the arterial intima during early atherogenesis may not result simply from an increase in the permeability or leakiness of the overlying endothelium.

Lipoproteins that accumulate in the extracellular space of the arterial intima often associate with proteoglycan molecules of the arterial extracellular matrix. At sites of lesion formation, the balance of different matrix constituents may vary in important ways. Various molecules known as "scavenger" receptors appear to mediate the excessive lipid uptake characteristic of foam cell

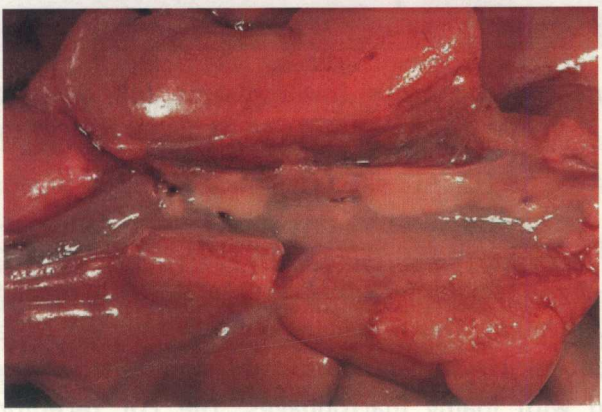


*Figure 3.5.* **Mild coronary atherosclerosis. A few scattered yellow lipid plaques are seen on the intimal surface of the opened coronary artery traversing the epicardial surface of a heart** *(Image Contrib. by:T.V. Rajan).*

formation alternatively of the classic low-density lipoprotein receptor (26). The longest studied of these receptors belongs to the scavenger receptor-A family. These surface molecules bind modified, rather than native, lipoproteins and apparently participate in their internalization. Atherosclerosis-prone mice with mutations that delete functional scavenger receptor-A have less exuberant fatty lesion formation than those with functional scavenger receptor-A molecules. Other receptors that bind modified lipoprotein and that may participate in foam cell formation include CD36 and macrosialin, the latter exhibiting preferential binding specificity for oxidized forms of LDL. Once macrophages have taken up residence in the intima and become foam cells *(figures 3.5,3.6)*, they replicate. The factors that trigger macrophage cell division in the atherosclerotic plaque likely include macrophage-colony stimulating factor (M-CSF).

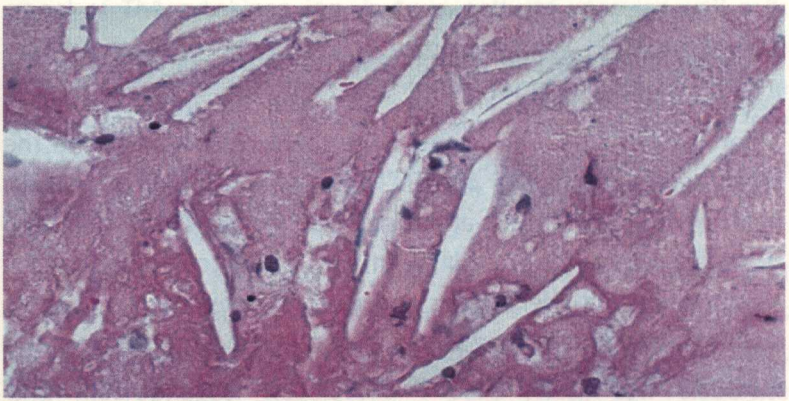


*Figure 3.6.* **The cholesterol clefts of lipid, along with a few scattered foam cells and a couple of lymphocytes, at high magnification in the atheromatous plaque** *(Image Contrib. by:T.V. Rajan).*

This co-mitogen and survival factor for mononuclear phagocytes exists in human and experimental atheromatous lesions. Again, atherosclerosis-prone mice that lack functional M-CSF have retarded fatty lesion development as well. Other candidates for macrophage mitogens or co-mitogens include interleukin 3 (IL-3) and granulocyte-macrophage colony stimulating factor (GM-CSF). Up to this point in the development of the nascent atheroma, the lesion consists primarily of lipid-engorged macrophages. Complex features such as fibrosis, thrombosis, and calcification do not characterize the fatty streak-the precursor lesion of the complex atheroma. Several lines of evidence suggest that such fatty streaks may be reversible, at least to some extent.

## Inflammation

Basic evidence and clinical evidence have converged in the last decade, showing an important role for inflammation in atherogenesis (27). The macrophage foam cells recruited to the artery wall early in this process serve as a reservoir for excess lipid. In the established atherosclerotic lesion, these cells also provide a rich source of proinflammatory mediators- proteins such as cytokines and chemokines and various eicosanoids and lipids such as platelet-activating factor. These phagocytic cells can also elaborate large quantities of oxidant species such as superoxide anion in the milieu of the atherosclerotic plaque. This ensemble of mediators can promote inflammation in the plaque and thus contribute to the progression of lesions. Mounting evidence supports a prominent role for antigen-specific or adaptive immunity in plaque progression in accession to innate immunity (28).

In addition to the mononuclear phagocytes, dendritic cells in atherosclerotic lesion can present antigens to the T cells that constitute an important minority of the leukocytes in the atherosclerotic lesion. Candidate antigens for stimulating this adaptive immune response include modified lipoproteins, heat shock proteins, beta$_2$ glycoprotein Ib, and infectious agents. The antigen-presenting cells (macrophages, dendritic cells, or endothelial cells) allow the antigen to interact with T cells in a manner that triggers their activation. The activated T cells then can secrete copious quantities of cytokines that can modulate atherogenesis.

The T helper cells (transporting CD4) fall into two general categories. Cells of the T helper (Th) 1 subtype elaborate proinflammatory cytokines such as interferon gamma, lymphotoxin, CD40 ligand, and tumor necrosis factor-alpha. This panel of Th1 cytokines can, in turn, activate vascular wall cells and orchestrate alterations in plaque biology that can lead to plaque destabilization and heightened thrombogenicity. On the other hand, T helper cells slanted toward the production of Th2 cytokines such as interleukin 10 can inhibit inflammation in the context of atherogenesis (29).

Cytolytic T cells (bearing CD8) can express *fas* ligand and other cytotoxic factors that can promote cytolysis and apoptosis of target cells including smooth muscle and endothelial cells, and macrophages. Regulatory T cells (Treg) can elaborate transforming growth factor (TGF)-b as well as interleukin (IL)-10. Treg lymphocytes bear the markers CD4 and CD25. Both TGF–β and IL-10 can exert antiinflammatory effects (30).

## Smooth muscle cell migration

Smooth muscle cells (SMCs) in the normal arterial tunica media differ considerably from those in the intima of an evolving *(figure 3. 7)* atheroma (31).

While some SMCs are likely to arrive in the arterial intima early in life, others that accumulate in advancing atheroma seem to arise from cells that have migrated from the underlying media into the intima. The chemoattractants for SMCs are likely to include molecules such as platelet-derived growth factor (PDGF), a potent SMC chemoattractant secreted by activated macrophages and overexpressed in human atherosclerosis. These SMCs in the

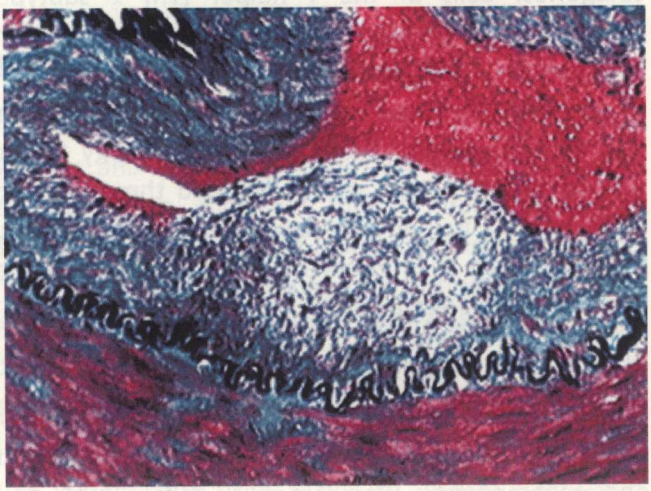


*Figure 3.7.* **Trichrome elastic tissue stain, which stains smooth muscle red, fibrous tissue blue, black elastica interna and elastic tissue black. The clarity of the lesion suggests the presence of free fats and/or foamy histiocytes** *(Image Contrib. by:T.V. Rajan).*

atherosclerotic intima can also multiply by cell division. Estimated rates of division of SMCs in the human atherosclerotic lesion are of the order of less than 1 percent. However, even such indolent replication might produce considerable SMC accumulation over the decades of lesion evolution. Instead of expressing primarily isoforms of smooth muscle myosin characteristic of adult SMCs, those in the intima have higher levels of the embryonic isoform of smooth muscle myosin (31). SMCs in the intima appear to recapitulate an embryonic phenotype. These intimal SMCs in atheroma appear morphologically distinct as well. They contain more rough endoplasmic reticulum and fewer contractile fibers than do normal medial SMCs. Although replication of SMCs in the steady state appears infrequent in mature human atheroma, bursts of SMC replication may occur during the life history of a given atheromatous lesion.

Some SMCs in advanced human atheroma exhibit fragmentation of their nuclear DNA characteristic of programmed cell death, or apoptosis (32). In addition to soluble cytokines that may trigger programmed cell death, the T cells in atheroma may participate in eliminating some SMCs. In particular, certain T cell populations known to accumulate in plaques can express *fas* ligand on their surface. *Fas* ligand can engage *fas* on the surface of SMCs, and in conjunction with soluble proinflammatory cytokines, can lead to death of the SMC (33). SMC accumulation in the growing atherosclerotic plaque probably results from a tug-of-war between cell replication and cell death. Contemporary cell and molecular biological research has identified candidates for mediating both the replication and desintegretion of SMCs, a concept that originated from the careful morphological observations of Virchow nearly 150 years ago. Referring to the SMCs in the intima, Virchow noted that early atherogenesis involves a "multiplication of their nuclei." However, he recognized that cells in lesions can "hurry on to their own destruction" because of death of SMCs.

Extracellular matrix rather than cells themselves makes up much of the volume of an advanced atherosclerotic plaque. The major extracellular matrix macromolecules that accumulate in atheroma include interstitial collagens (types I and III) and proteoglycans such as versican, biglycan, aggrecan, and decorin (34). Elastin fibers may also accumulate in atherosclerotic plaques. The vascular SMC produces these matrix molecules in disease, just as it does during development and maintenance of the normal artery. Stimuli for excessive collagen production by SMCs include platelet-derived growth factor and TGF–β, a constituent of platelet granules, and a product of many cell types found in lesions including regulatory T cells (Treg).

The biosynthesis of the extracellular matrix molecules is balanced by breakdown catalyzed in part by catabolic enzymes known as matrix metalloproteinases (MMPs). Dissolution of extracellular matrix macromolecules undoubtedly plays a role in migration of SMCs as they penetrate into the intima from the media through a dense extracellular matrix, traversing the elastin-rich internal elastic lamina. In injured arteries, overexpression of such proteinase inhibitors, known as tissue inhibitors of metalloproteinases (TIMPs) can delay smooth muscle accumulation in the intima of injured arteries (35). Extracellular matrix dissolution is also likely to play a role in arterial remodeling that accompanies lesion growth. During the first part of the life history of an atheromatous lesion, growth of the plaque is outward, rather than inward in a way that would lead to luminal stenosis. This outward growth of the intima leads to an increase in caliber of the entire artery. This so-called *positive* remodeling or *compensatory enlargement* must involve turnover of extracellular matrix molecules to accommodate the circumferential growth of the artery. Luminal stenosis tends to occur only after the plaque burden exceeds about 40 percent of the cross-sectional area of the artery.

Endothelial migration and replication occur as plaques develop a microcirculation, characterized by plexi of newly formed vessels. Such plaque neovessels usually require special stains for visualization. However, histological examination with appropriate markers for endothelial cells reveals a rich neovascularization in evolving plaques. These microvessels are likely to form in response to angiogenic peptides overexpressed in atheroma. These angiogenesis factors include acidic and basic fibroblast growth factors, vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and oncostatin M (36). In the advanced human atherosclerotic plaque, microvascular endothelium displays the mononuclear-selective adhesion molecules such as VCAM-1 much more prominently than does the macrovascular endothelium overlying the plaque. The microvascularization of plaques may also allow growth of the plaque overcoming diffusion limitations on oxygen and nutrient supply, in analogy with the concept of tumor angiogenic factors and growth of malignant lesions. Consistent with this view, administration of inhibitors of angiogenesis to mice with experimentally induced atherosclerosis limits lesion expansion. Finally, the plaque microvessels may be friable and prone to rupture similar to the neovessels in the diabetic retina (37). Hemorrhage and thrombosis in situ could promote a local round of SMC proliferation and matrix accumulation in the area immediately adjacent to the microvascular disruption. This design illustrates a special case of one of the "crises" described earlier in the evolution of the atheromatous plaque. Attempts to augment myocardial perfusion by enhancing new vessel growth by transfer of angiogenic proteins, or their genes, might have adverse effects on lesion growth or clinical complications of atheroma by these mechanisms.

Understanding of the mechanism of mineralization during evolution of atherosclerotic plaques has advanced. Some subpopulations of SMCs may foster calcification by enhanced secretion of cytokines such as bone morphogenetic proteins, homologues of TGF–β.

Atheromatous plaques may also contain proteins with gamma carboxylated glutamic acid residues specialized in sequestering calcium and thus promoting mineralization (38).

High Mobility Group 1 protein (HMGB1) is a chromatin component that, when leaked out by necrotic cells, triggers inflammation. HMGB1, secreted by SMC challenged with cholesterol is expressed within the human atherosclerotic plaque and promotes SMC migration and proliferation. HMGB1 appears as a key player in neointimal hyperplasia of atherosclerotic plaques and restenosis after angioplasty (39).

## Arterial stenosis

The discontinuous growth of coronary artery stenoses during the chronic asymptomatic or stable phase, with periods of rapid progression is supported by human angiographic studies (*figure* 3.8).

Eventually the stenoses may progress to a degree that impedes blood flow through the artery. Lesions that produce stenoses of greater than 60 percent can cause flow limitations under conditions of increased demand. This type of athero-occlusive disease commonly produces chronic stable angina pectoris or intermittent claudication on increased demand. Thus, the symptomatic phase of atherosclerosis usually occurs many decades after lesion initiation.

In many cases of myocardial infarction, however, no history of stable angina heralds the acute event. Several kinds of clinical observation suggest that many myocardial infarctions result not from high-grade stenoses, but from lesions that do not limit flow. In a compilation of four such serial angiographic studies, only approximately 15 percent of acute myocardial infarctions arise from lesions with degrees of stenosis greater than 60 percent

on an antecedent angiogram. Instead of progressive growth of the intimal lesion to a critical stenosis, we now recognize that thrombosis, complicating a not necessarily occlusive plaque, most often causes episodes of unstable angina or acute myocardial infarction (38).

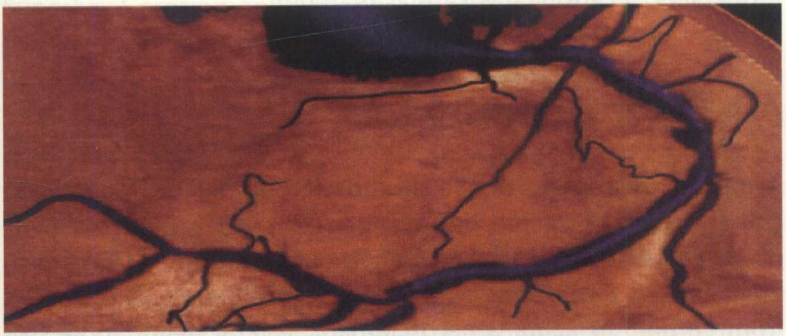


*Figure 3.8.* **Coronary Angiography of Stenotic Coronary Artery** (*pathweb.uchc.edu*).

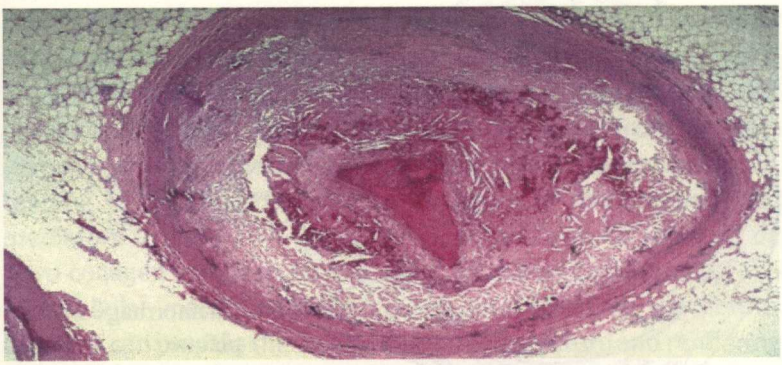


*Figure 3.9.* **Coronary thrombosis is seen microscopically occluding the remaining small lumen of this coronary artery. Such an acute coronary thrombosis is often the antecedent to acute myocardial infarction** (*Image Contrib. by:T.V. Rajan*).

The high-grade stenoses are more likely to cause acute myocardial infarction than nonocclusive lesions. However, because the noncritical stenoses by far outnumber the tight focal lesions in a given coronary tree, the lesser stenoses cause more infarctions, even though high-grade stenoses have a greater individual probability of causing myocardial infarction.

## Atherothrombosis

A physical disruption of the atherosclerotic plaque (*figure 3.9*) commonly causes acute thrombosis. This evolution in our view of the pathogenesis of the acute coronary syndromes places new emphasis on thrombosis as the critical mechanism of transition from chronic to acute atherosclerosis. Several major modes of plaque disruption provoke most coronary thrombi (40).

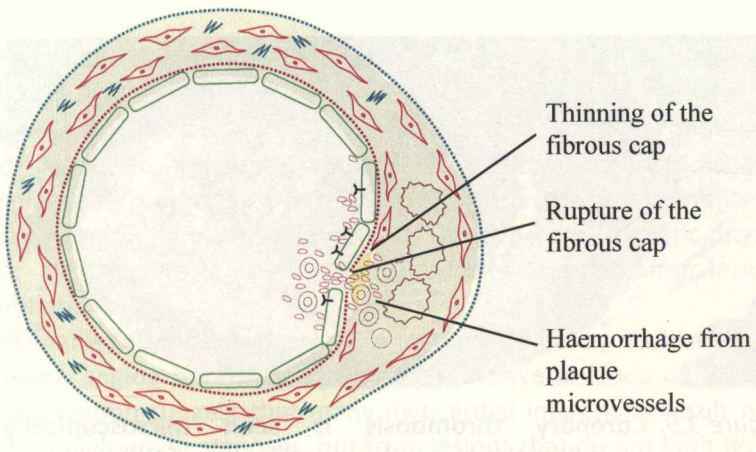



*Figure 3.10.* **The unstable atherosclerotic plaque**
(Koenig W., *Eur Heart J Suppl* 1999).

The first mechanism, accounting for nearly two-thirds of acute myocardial infarctions, involves a fracture of the fibrous cap of the plaque (*figure 3.10*). Another mode involves a superficial erosion of the intima, accounting for up to one-quarter of acute myocardial infarctions in highly selected referral cases from medical examiners on individuals who have succumbed to sudden cardiac death. Superficial erosion appears more frequently in women than in men as a mechanism of sudden cardiac death.

## Plaque rupture

Lipid-rich atherosclerotic plaques, or "vulnerable plaques" are prone to rupture, causing local intravascular thrombosis, with subsequent grave clinical consequences. The rupture of the plaque's fibrous cap probably reflects an imbalance between the forces that affect the plaque's cap and the mechanical strength of the fibrous cap (*figure 3.10*). Interstitial forms of collagen provide most of the biomechanical resistance to disruption to the fibrous cap. Factors that decrease collagen synthesis by SMCs can impair their ability to repair and maintain the plaque's fibrous cap. The T cell–derived cytokine gamma interferon potently inhibits SMC collagen synthesis. Certain mediators released from platelet granules during activation can increase SMC collagen synthesis, tending to reinforce the fibrous structure of the plaque. Such mediators include TGF–β and PDGF. In addition to reduced *de novo* collagen synthesis by SMCs and increased catabolism of the extracellular matrix macromolecules, they comprise the fibrous cap, that can provide the weakening of this structure and rendering it susceptible to rupture, and hence thrombosis. The same matrix-degrading enzymes thought to contribute to smooth muscle migration and arterial remodeling may contribute to weakening of the fibrous cap as well. Macrophages in advanced human atheroma overexpress matrix metalloproteinases and elastolytic

cathepsins that can break down the collagen and elastin of the arterial extracellular matrix. Thus, the strength of the plaque's fibrous cap is under dynamic regulation, linking the inflammatory response in the intima with the molecular determinants of plaque stability and hence, thrombotic complications of atheroma. The thinning of the plaque's fibrous cap, a result of reduced collagen synthesis and increased degradation, explains why pathological studies have shown that the thin fibrous cap characterizes atherosclerotic plaques that have ruptured and caused fatal myocardial infarction.

One trait of the so-called *vulnerable atherosclerotic plaque* defined by pathological analysis is a relative lack of SMCs. Inflammatory mediators (both soluble and associated with the surface of T-lymphocytes) can provoke programmed cell death of SMCs. "Dropout" of SMCs from regions of local inflammation within plaques probably contributes to the relative lack of SMCs at places where plaques rupture (41).

Because these cells are the source of the newly synthesized collagen needed to repair and maintain the matrix of the fibrous cap, the lack of SMCs may contribute to weakening of the fibrous cap and the propensity of that plaque to rupture.

From a strictly biomechanical viewpoint, a large lipid pool can concentrate biomechanical forces on the shoulder regions of plaques, common sites of rupture of the fibrous cap. From a metabolic standpoint, the activated macrophage characteristic of the plaque's core region produces the cytokines and the matrix-degrading enzymes thought to regulate aspects of matrix catabolism and SMC apoptosis in turn. Apoptotic macrophages as well as SMCs can generate particular tissue factor, a potential instigator of microvascular thrombosis after spontaneous or iatrogenic plaque disruption. The success of lipid-lowering therapy

in reducing the incidence of acute myocardial infarction or unstable angina in patients at risk may result from a reduced accumulation of lipid and decrease in inflammation and plaque thrombogenicity. Recent animal studies and monitoring of peripheral markers of inflammation in humans support this concept (42).

## Superficial erosion of plaques

In humans, superficial erosion appears more likely to cause fatal acute myocardial infarction in women and in individuals with hypertriglyceridemia and diabetes mellitus. Apoptosis of endothelial cells could go to degradation of endothelial cells in areas of superficial erosion. Likewise, matrix metalloproteinases such as certain gelatinases specialized in degrading the nonfibrillar collagen found in the basement membrane, might also sever the lesion of the endothelial cell and promote their desquamation (43). Repetitive cycles of plaque disruption, thrombosis *in situ* and healing probably contribute to lesion evolution and plaque growth. Such episodes of thrombosis and healing constitute one type of "crisis" in the history of a plaque that may cause a burst of SMC proliferation, migration, and matrix synthesis. Thrombin, generated at sites of mural thrombosis, potently stimulates SMC proliferation. The late stage or "burned out" fibrous and calcific atheroma may represent a late stage of a plaque previously lipid-rich and vulnerable but now rendered fibrous and hypocellular because of a wound-healing response mediated by the products of thrombosis.

## Plaque vulnerability

Inflammation plays a key role in the pathogenesis of CAD at every stage from initiation to progression and rupture of the

atherosclerotic plaque. Studies at autopsy of atherosclerotic plaques that caused fatal thrombosis brought the notion of vulnerable high-risk plaque to the fore. This stimulated many investigators to seek ways of identifying and treating such high risk atherosclerotic lesions. Current evidence, however, suggests that more than one such high risk plaque often resides in a given coronary tree (44). Careful analysis of angiograms of individuals with acute coronary syndromes has demonstrated evidence for plaque ulceration or thrombosis in more than one lesion in many cases. Individuals with multiple unstable lesions by angiographic criteria tend to have worse outcomes during follow-up. Angioscopic studies have also shown multiple sites of intracoronary thrombosis in patients with acute coronary syndromes. Systematic studies by intravascular ultrasound of the coronary arterial system in individuals with acute coronary syndromes have revealed that more than 80 percent of such individuals have more than one disrupted atherosclerotic plaque.

Several concordant lines of evidence support the systemic and diffuse nature of inflammation in individuals with acute coronary syndromes. Maseri's group demonstrated a transmyocardial gradient in the inflammatory marker myeloperoxidase when sampling from the great cardiac vein (draining the left coronary territory) in individuals with both left and right coronary artery lesions. Multiple studies have shown that various systemic markers of inflammation such as C-reactive protein increase in patients at risk for acute coronary syndromes (45). Observational studies have consistently demonstrated that higher plasma levels of CRP are associated with higher risk of CAD and measurement of CRP has been advocated as a means of improving risk prediction (46). There is considerable interest in whether CRP has a causal role in CAD or whether CRP is merely a marker of underlying atherosclerosis. This is so even in the absence of biochemical evidence of myocardial injury that might elicit a secondary acute

phase response. Thus, a combination of imaging studies and investigations using inflammatory markers support the diffuse and systemic nature of instability of atheromas in individuals with or at risk for acute coronary syndromes (47).

## Restenosis

The widespread introduction of stents has raised the problem of restenosis. The restenosis after percutaneous arterial intervention represents a special case of arteriosclerosis. Study of this very well standardized preparation promoted precise understanding of the kinetics of intimal thickening after this type of injury.

Although SMC proliferation appears prominent in the simple experimental models of intimal thickening, observations on human specimens showed relatively low rates of SMC proliferation and called into question therapeutic targeting of this process. Intravascular ultrasound studies in humans, and considerable evidence from animal experimentation, suggested that a substantial proportion of the loss of luminal caliber after balloon angioplasty resulted from a constriction of the vessel from the adventitial side, the so-called *negative remodeling*. These observations renewed interest in adventitial inflammation with scar formation and wound contraction as a mechanism of arterial constriction following balloon angioplasty (48). The process of in-stent stenosis, in contrast with restenosis after balloon angioplasty, depends uniquely on intimal thickening, as opposed to negative remodeling. The stent provides a firm scaffold that prevents constriction from the adventitia. Histological analyses reveal that a great deal of the volume of the in-stent restenotic lesion is made up of myxomatous tissue, comprising occasional stellate SMCs embedded in a loose and highly hydrated extracellular matrix.

# Infection

A large number of epidemiological studies supports a role for certain bacteria, notably Chlamydia pneumoniae, and certain viruses, notably cytomegalovirus (CMV), in the etiology of atherosclerosis (49,50). The serological data have incited a number of *in vivo* and *in vitro* experiments that lend varying degrees of support to this concept. The evidence for infection with Ch. pneumoniae may serve as a marker for tobacco use, a known risk factor for atherosclerotic events. Additionally, a strong bias favors publication of positive studies as opposed to negative studies. It is difficult to sort out coincidence from causality when the majority of the population studied has evidence of both infection and atherosclerosis. Although proof that bacteria or viruses can cause atherosclerosis remains elusive, it is plausible that infections may potentiate the action of traditional risk factors, such as hypercholesterolemia. Cells within the plaque itself may be a site for infection, which could spur their activation and accelerate the inflammatory pathways that we currently believe operate within the atherosclerotic intima. Specific microbial products, such as lipopolysaccharides, heat shock proteins, or other virulence factors, might act locally at the level of the artery wall to potentiate atherosclerosis in infected lesions.

Asssembling data show that atherosclerosis is an inflammatory disease; therefore, a great deal of attention has recently been focused on the possibility that infectious agents play a role in the etiology of CAD. Certain infectious agents have been implicated based on their isolation from the atheromatous plaques or on the presence of positive serology findings for organisms such as Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus, and cytomegalovirus.

Even though prospective studies have fallen short of providing definitive evidence, Ch. pneumoniae appears to exhibit the strongest association. Ch. pneumoniae has been isolated from autopsy and artectomy specimens and in both early and well-developed lesions. When studied by means of immunologic cytochemistry and tissue staining, the association has been found in 70-100% of cases. Possible mechanisms by which infectious agents exert their effect may include local effects on the endothelium, SMCs, or macrophages or systemic effects by generating cytokines, stimulating monocytes, and promoting hypercoagulability.

Some of the completed studies have shown variable results. In the Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infarction with Chlamydia (ACADEMIC) trial (51), markers of inflammation improved at 6 months in the subjects with positive serologic evidence of chlamydial infection, but no difference in clinical events was observed. In another trial, the Randomization Trial of Roxithromycin in Non–Q-Wave Coronary Syndromes (ROXIS), a reduction in CRP level was observed at 1 month and was associated with a significant decrease in triple clinical endpoint. The effect, however, dissipated by 3-6 months (52).

Several multicenter trials have evaluated the effect of antibiotic therapy on recurrent cardiac events when used as secondary prevention. The CLARIFY study, ANTIBIO study, ACADEMIC trial, Azithromycin in Acute Coronary Syndrome (AZACS) study, and the South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA) trial all returned negative results in terms of any significant benefit from antibiotic therapy. However, these trials were not powered to detect the difference in the rate of composite events to begin with, while 3 of the recently presented trials were powered to detect such a difference (53-56).

## References:


1. Weissberg P.L. *Atherogenesis: Current understanding of the causes of atheroma*.Heart, 2000,83:247-52.
2. Ross R, Fuster V. *The pathogenesis of atherosclerosis. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and Coronary Artery Disease*. Philadelphia, Pa: Lippincott-Raven; 1996:441-62.
3. *Impact of the PROVE IT-TIMI 22/REVERSAL trials on trends in intensive vs. moderate statin therapy in Ontario*, Canada,Austin P, Mamdani M. *Impact of the PROVE IT-TIMI 22/REVERSAL trials on trends in intensive vs. moderate statin therapy in Ontario*, Canada. Circulation. 2005; 112 (9): 1296-1300.
4. Libby P, Theroux P. *Pathophysiology of coronary artery disease. Circulation*. 2005;111:3481-3488.pmid:15983262.
5. Shunichi Toshima; Akira Hasegawa; Masahiko Kurabayashi; Hiroyuki Itabe; Tatsuya Takano; Jinpei Sugano; Kyoko Shimamura; Junji Kimura; Ichiro Michishita; Toru Suzuki; Ryozo Nagai. Circulating Oxidized Low Density Lipoprotein Levels A Biochemical Risk Marker for Coronary Heart Disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2000;20:2243.
6. Williams KJ, Tabas I: Lipoprotein retention and clues for atheroma regression. *Arterioscler Thromb Vasc Biol* 2005; 25:1536.
7. Griendling KK: Novel NAD(P)H oxidases in the cardiovascular system. Heart 2004; 90:491.
8. Koenig W. Atherosclerosis involves more than just lipids: focus on inflammation. Eur Heart J Suppl 1999;1(Suppl T);T19-26.
9. Werner N, Kosiol S, Schiegl T, et al: Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353:999.
10. Aird WC: Mechanisms of endothelial cell heterogeneity in health and disease. Circ Res 2006; 98:159.
11. Blankenberg S, Rupprecht HJ, Bickel C, et al. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation 2001;104:1336-42.

12. Ley K: The role of selectins in inflammation and disease. Trends Mol Med 2003; 9:263.

13. Cybulsky MI, Won D, Haidari M: Leukocyte recruitment to atherosclerotic lesions. Can J Cardiol 2004; 20(Suppl B):24B.

14. Tacke F, Alvarez D, Kaplan TJ, et al: Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest 2007; 117:185.

15. Werner N, Kosiol S, Schiegl T, et al: Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353:999.

16. Tacke F, Alvarez D, Kaplan TJ, et al: Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest 2007; 117:185.

17. Charo IF, Ransohoff RM: The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 2006; 354:610.

18. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulatio n. Sep 1 1995;92(5):1355-74.

19. Parmar KM, Larman HB, Dai G, et al: Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. J Clin Invest 2006; 116:49.

20. Libby P, Aikawa M, Jain MK: Vascular endothelium and atherosclerosis. Handb Exp Pharmacol 2006; 285.

21. Walter N. Durán, Jerome W. Breslin, Fabiola A. Sánchez. The NO cascade, eNOS location, and microvascular permeability. Oxford Journals Medicine Cardiovascular Research Volume87, Issue2 Pp. 254-261,7, 2010.

22. Sen Banerjee S, Lin Z, Atkins GB, et al: KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. J Exp Med 2004; 199:1305.

23. Yamawaki H, Pan S, Lee RT, et al: Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. J Clin Invest 2005; 115:733.

24. Aird WC: Mechanisms of endothelial cell heterogeneity in health and disease. Circ Res 2006; 98:159.

25. Miller YI, Chang MK, Binder CJ, et al: Oxidized low density lipoprotein and innate immune receptors. Curr Opin Lipidol 2003; 14:437.

26. Hansson GK: Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352:1685.

27. Hansson GK, Libby P, Schonbeck U, et al: Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res 2002; 91:281.

28. Pinderski LJ, Fischbein MP, Subbanagounder G, et al: Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient Mice by altering lymphocyte and macrophage phenotypes. Circ Res 2002; 90:1064.

29. Littlewood TD, Bennett MR: Apoptotic cell death in atherosclerosis. Curr Opin Lipidol 2003; 14:469.

30. Hansson GK, Robertson AK: TGF-beta in atherosclerosis. Arterioscler Thromb Vasc Biol 2004; 24:e137.

31. Manabe I, Nagai R: Regulation of smooth muscle phenotype. Curr Atheroscler Rep 2003; 5:214.

32. Geng YJ, Libby P: Progression of atheroma: A struggle between death and procreation. Arterioscler Thromb Vasc Biol 2002; 22:1370.

33. Clarke MC, Figg N, Maguire JJ, et al: Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. Nat Med 2006; 12:1075.

34. Wight TN, Merrilees MJ: Proteoglycans in atherosclerosis and restenosis: key roles for versican. Circ Res 2004; 94:1158.

35. Dollery CM, Libby P: Atherosclerosis and proteinase activation. Cardiovasc Res 2006; 69:625.

36. Moulton KS: Angiogenesis in atherosclerosis: Gathering evidence beyond speculation. Curr Opin Lipidol 2006; 17:548.

37. Kolodgie FD, Gold HK, Burke AP, et al: Intraplaque Hemorrhage and progression of coronary atheroma. N Engl J Med 2003; 349:2316.

38. Doherty TM, Asotra K, Fitzpatrick LA, et al: Calcification in atherosclerosis: Bone biology and chronic inflammation at the arterial crossroads. Proc Natl Acad Sci U S A 2003; 100:11201.

39. Tiziana Bonaldi, Fabio Talamo, Paola Scaffidi, Denise Ferrera, Annalisa Porto, Angela Bachi, Anna Rubartelli, Alessandra Agresti, and Marco E. Bianchi. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J. 2003 October 15; 22(20): 5551–5560.

40. Virmani R, Burke AP, Farb A, et al: Pathology of the vulnerable plaque. J Am Coll Cardiol 2006; 47:C13.

41. Geng YJ, Libby P: Progression of atheroma: A struggle between death and procreation. Arterioscler Thromb Vasc Biol 2002; 22:1370.

42. Libby P, Aikawa M: Stabilization of atherosclerotic plaques: New mechanisms and clinical targets. Nat Med 2002; 8:1257.

43. Sugiyama S, Kugiyama K, Aikawa M, et al: Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression: Involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. Arterioscler Thromb Vasc Biol 2004; 24:1309.

44. Libby P: Act local, act global: Inflammation and the multiplicity of "vulnerable" coronary plaques. J Am Coll Cardiol 2005; 45:1600.

45. Verma S, Szmitko PE, Ridker PM. C-reactive protein comes of age. Nat Clin Pract Cardiovasc Med. 2005;2(1):29–36.

46. Shah T, Casas JP, Cooper JA, et al. Critical appraisal of CRP measurement for the prediction of coronary heart disease events: new data and systematic review of 31 prospective cohorts. Int J Epidemiol. 2009;38(1):217–231.

47. Libby P, Ridker PM: Inflammation and atherothrombosis: From population biology and bench research to clinical practice. J Am Coll Cardiol 2006; 48:A33.

48. Libby P, Simon D. I., Rogers C: Inflammation and arterial injury. In: Topol EJ, ed. Textbook of Interventional Cardiology, 4th ed. Philadelphia: Elsevier Science; 2003:381.

49. Kalayoglu MV, Libby P, Byrne GI: Chlamydia pneumoniae as an emerging risk factor in cardiovascular disease. JAMA 2002; 288:2724.

50. Anestiadi V., Zota I., Groppa S., Melnic E, Foca E., Zota E . Some aspects in pathogenesis of atherosclerosis. BULETIN OF THE ACADEMYOF SCIENSES OF MOLDOVA, 2005,2(2),37-42.

51. J. Thomas Grayston. Secondary Prevention Antibiotic Treatment Trials for Coronary Artery Disease. Circulation. 2000;102:1742.

52. Gurfinkel E, Bozovich G, Daroca A, Beck E, Mautner B, Gurfinkel E, Bozovich G, Beck E, Testa E, Livellara B, Mautner B. Randomised trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS pilot study Treatment with the antibiotic roxithromycin in patients with acute non-Q-wave coronary syndromes Eur Heart J 1999;20:121-7.

53. Sinisalo J, Mattila K, Valtonen V, et al, Clarithromycin in Acute Coronary Syndrome Patients in Finland (CLARIFY) Study Group. Effect of 3 months of antimicrobial treatment with clarithromycin in acute non-Q-wave coronary syndrome. Circulation. 2002;105:1555-1560. pmid:11927522.

54. Stone AFM, Mendall MA, Kaski JC, et al. Effect of treatment for Chlamydia pneumoniae and Helicobacter pylori on markers of inflammation and cardiac events in patients with acute coronary syndromes: South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA). Circulation. 2002;106:1219-1223. pmid:12208796.

55. Zahn R, Schneider S, Frilling B, et al. Antibiotic Therapy After Acute Myocardial Infarction (ANTIBIO): a prospective randomized trial. Circulation. 2003;107:1253-1259.pmid:12628944.

56. Cercek B, Shah PK, Noc M, et al. Effect of short-term treatment with azithromycin on recurrent ischaemic events in patients with acute coronary syndrome in the Azithromycin in Acute Coronary Syndrome (AZACS) trial: a randomized controlled trial. Lancet. 2003;361:809-813. pmid:12642046.

*We are coming to understand health not as the absence of disease, but rather as the process by which individuals maintain their sense of coherence (i.e. sense that life is comprehensible, manageable, and meaningful) and ability to function in the face of changes in themselves and their relationships with their environment*

**Aaron Antonovsky (1987), *Unraveling The Mystery of Health*.**

# Chapter 4. Disease-causing genes for coronary artery disease and myocardial infarction

## Classification

Classification of genes that are associated with complex human diseases such as coronary artery disease (CAD) and myocardial infarction (MI) (1).

Three major categories there exist:

- disease-causing genes
- susceptibility genes
- disease-linked genes.

Disease-causing genes are the genes that are directly responsible for the pathogenesis of disease when mutated. In this case, the mutations are clearly defined or well established as the primary cause of the disease.

Susceptibility genes are the genes that increase or decrease the risk of developing disease and may or may not cause the disease in the context of other genetic and environmental factors. Mutations or single-nucleotide polymorphisms (SNPs) in these genes are present in both normal and diseased populations, but the frequencies differ in the two populations. For individuals, susceptibility genes have less predictive value for the development and prognosis of the disease.
Disease-linked genes are the genes that are connected to the disease by molecular biologic microarray, or proteomic analyses, but their relation to the disease as a cause or a consequence is not established. Some disease-linked genes may serve as biomarkers for the disease.

## Epidemiological methods for studying genes and environmental factors in coronary artery disease

The recent exciting developments in genomic research have been catalyzed by the generation of a reference sequence of the human genome (2) and the identification of millions of sequence variants (3), combinations of which make each human genetically unique. Additionally, correlation patterns among these variants in individuals of diverse continental ancestry have been comprehensively catalogued as part of the International HapMap Project (4). To identify sequence variants in humans that influence disease susceptibility, two general methods have historically been employed by genetic researchers:

- linkage analysis
- association analysis

*Linkage analysis tests* for the joint transmission of chromosomal segments and disease within families. Linkage is the method of choice to identify rare variants with a large impact on disease risk that aggregate in families. The diseases caused by such variants show obvious inheritance patterns and are typically called Mendelian diseases, after Gregor Mendel who described the general patterns of inheritance (5). Mendelian diseases in cardiovascular medicine include the congenital long-QT syndrome, hypertrophic cardiomyopathy and familial hypercholesterolemia.

*Association analysis* tests for differences in allele frequencies of variants between cases and controls, is the method of choice to identify functional variants that are common in the general population, termed polymorphisms. These polymorphisms typically have a modest impact on risk for common diseases, such as CAD and myocardial infarction. These diseases are called complex diseases because they arise from multiple genetic and environmental causes and, although often aggregating in families, do not show distinct inheritance patterns. Historically, association studies have been able to examine polymorphisms in only the candidate genes known or proposed to play a role in the pathophysiology of a disease.

## Genome-wide association analyses

More recently, it has become possible to examine large numbers of polymorphisms, in the order of 100 000-1 000 000, throughout the genome using highly-parallel genotyping arrays (6). These genome-wide association (GWA) analyses systematically examine variation throughout the human genome regardless of putative biologic function. Owing to the fact that common polymorphisms

are correlated, a smaller number of polymorphisms can be chosen to serve as proxies for the majority of common sequence variations. The GWA study is hypothesis generating in the sense that it can identify polymorphisms near genes without a recognized pathophysiological link to disease. The method has recently been successfully used to identify common variants in previously unsuspected genes associated with diseases including myocardial infarction (7-9), type 2 diabetes (10-12), as well as medication side-effects such as statin-induced myopathy (13) and excessive anticoagulation from warfarin (14).

## DNA sequence variants and linkage disequilibrium

Variation in the genetic sequence between any two individuals constitute approximately 0.1 percent of the genome and can take different forms, including substitutions, deletions, insertions, duplications, or inversions. Regional and global (15,16) resequencing studies have found smaller sequence variants to be the most common, with single-base substitutions, also termed single nucleotide polymorphisms (SNPs), predominating. Large-scale SNP discovery projects have identified more than 10 million SNPs in human populations, available in such online catalogs (www.ncbi.nlm.nih.gov/projects/SNP) as dbSNP (17). Although individually less frequent in the genome, other sequence variants called copy number polymorphisms (CNPs) account for a larger proportion of the total number of bases that differ between individuals. Typically, these variants stretch over just a few nucleotides, but may be long enough so that whole genes may be duplicated or absent on a given chromosome. Hundreds of CNPs have recently been identified in large-scale discovery projects (18), and a number of CNPs have been linked to common diseases (19). Common variants are highly correlated in blocks across stretches of DNA spanning tens of kilobases, a phenomenon termed

linkage (LD) disequilibrium (20). This extensive correlation among neighboring variants results from the few generations since the last common human ancestor relative to the rates of mutation and recombination and the restriction of the majority of recombination events to focal hotspots (21). In European- and Asian-derived populations these blocks are typically longer than in African-derived populations, in which genetic diversity is somewhat higher owing to the relatively small number of Africans who founded the modern European and Asian populations, approximately 50 000 years ago.

## Candidate gene association studies

Historically, limitations in genotyping throughput and cost have restricted association studies to the examination of only a few variants in a narrow genetic region typically focused on a specific gene, termed candidate gene (CG) studies. For example, one of the most extensively studied genetic polymorphisms in cardiovascular disease involves a common 287 base pair sequence in the gene coding for angiotensin I-converting enzyme (ACE) which is either present or absent (insertion or deletion, termed "in/del") and determines about 50% of interindividual variability of plasma ACE levels (22). The ACE gene is clearly a strong biological candidate given its well- known role in the activation of vasoactive hormones and the important clinical role of ACE inhibitors in the treatment of hypertension and heart failure. The ACE polymorphism has been examined for association with many diseases including myocardial infarction (23-26), ventricular hypertrophy, stroke, and hypertension (27,28), as well as diabetic nephropathy and Alzheimer's disease. This insertion/deletion polymorphism illustrates several properties of candidate gene association studies. First, although examined for association with multiple diseases, only findings with diabetic nephropathy and Alzheimer's

disease can be considered relatively conclusive with inconsistent findings for other diseases (29), a problem that is well known to have plagued candidate gene association studies (30). The study of the genetic basis of complex traits was set back by the failure of association findings reported in the 1990s and early 2000s to be replicated, which arose from use of inappropriately permissive P-value thresholds and small sample sizes in replication samples (31). Second, although the association of the ACE insertion/deletion polymorphism with changes in circulating ACE concentration is well established, the basic mechanism linking the polymorphism to changes in gene expression is uncertain. Unlike the situation for DNA sequence that encodes amino acid sequence, we have a limited understanding of the determinants of gene expression. The ACE in/del may be in LD with the underlying causal variant or prove to be causal by an as yet unknown mechanism. Several other polymorphisms have also been identified in the ACE gene and examined for association with different diseases, most of them strongly correlated with the insertion/deletion. Long-range LD thus represents both an advantage (association of ungenotyped causal variants is detectable indirectly through genotyping of correlated variants) and a disadvantage (correlation among neighboring variants makes identification of the specific functional variant difficult because of the number of proxies to sort through). More recently, taking advantage of the catalogue of SNPs and LD patterns in HapMap, candidate gene association studies have moved from the examination of a few variants to systematic studies of all common variations across a gene locus. By genotyping "tagging SNPs" chosen to represent (tag) polymorphisms to which they are correlated, costs are reduced without loss of coverage (32). Ultimately, discovery of the full spectrum of sequence variants, including variants with minor allele frequency 5% at a locus, requires resequencing of the gene.

## Utility of candidate gene and genome-wide approaches

Even with the development of global GWA approaches, candidate gene efforts remain an integral tool in the genetics of common traits. GWA studies may be viewed as hypothesis-generating screening tools, whereas candidate gene association studies are useful for more focused examination of genes recognized to have a role in human physiology whether through prior basic investigation or discoveries from GWA approaches. A genome-wide association study examines the large fraction of common variation throughout the human genome. First proposed in 1996 following the failure of linkage analysis to identify genetic determinants of common diseases, association methods are more powerful to detect the modest effect sizes of common variants which represent a blind spot for linkage approaches (33).

One disadvantage of genome-wide studies is the higher costs of genotyping. However, if one considers the costs on a per SNP basis of low- throughput genotyping platforms that can determine 1–50 SNPs per array, genome-wide arrays can produce a genotype at 100- to 1 000-fold lower cost. Another disadvantage of GWA studies is the loss of power from the strict significance thresholds required to filter out spurious associations resulting from the large numbers of tests performed. The solution is to maximize the sample size to ensure adequate power to achieve P-value thresholds as low as $5 \cdot 10^{-8}$, or similar thresholds proposed in the literature. The $5 \cdot 10^{-8}$ threshold has been proposed based on Bonferroni adjustment for the effective 1 000 000 independent tests in the human genome of European and Asian ancestry individuals (34). African ancestry DNA sequence includes a larger number of independent tests owing to its greater diversity as described above.

Association studies can be performed in :

- **case–control,**
- **cohort,**
- **family- based**

study samples using different analytic methods.

A case-control study ascertains cases after they have occurred and matches on relevant characteristics that might otherwise confound a study. A cohort study prospectively enrolls subjects irrespective of clinical characteristics and examines the distribution of characteristics at a single time point (cross-sectional) or follows for future development of incident cases (longitudinal).

Family-based studies can enroll pedigrees (multiple generations) or siblings and can ascertain on an individual case (probands) or sample from the general population. A case–control or family-based case ascertainment may be better suited for diseases with relatively low population frequency as the number of cases in cohort studies may be insufficient for adequate power to detect the modest effect sizes typically seen in common variants (35). Even in case–control and family-based designs, however, there are often difficulties in achieving the sample sizes necessary for GWA studies, which typically exceed 1 000 cases and 1 000 controls as this sample size detects only the few strong variants. This problem is most often solved by investigators forming multicenter consortia. Recently, GWA studies have been performed in several large cohort studies available, including the Framingham Heart Study (36,37) and Women's Health Study (60), and have primarily focused on quantitative traits rather than case status. The pooling of several studies offers distinct advantages of the cohort design for the possibility to examine, in adequately powered samples, the population predictive value of genetic variants, gene-environment and gene-gene interactions.

The potential to elucidate the precise genetic architecture of common diseases and traits offers four primary opportunities for the medical sciences: (1) identification of novel markers of disease risk at the population level, (2) identification of novel genes and pathways involved in human pathophysiology, (3) inference of causality to epidemiological observations, and (4) the identification of drug targets for which the *in vivo* relevance in humans has already been demonstrated.

As discussed above, GWA studies have led to identification of several novel pathways in disease pathophysiology and established a causal role for biomarkers which has not been possible with non-genetic epidemiological associations, but the initial incentive for genetic association studies was the prospect of identifying novel risk markers for utilization in prediction of common diseases. A gain in predictive power may come from models incorporating interactions among polymorphisms (epistasis) or between polymorphisms and environmental factors, but to date few such interactions have been demonstrated (38).

## Recent Progress: The Haplotype Map Project

The primary goal of the International Haplotype Map (HapMap) Project (39) was to create a public resource of common SNPs to capture most of the common human genome sequence variability. A second objective was to characterize the LD structure of the genome on the basis of the analysis of these SNPs. Because of the strong LD displayed by most regions of the genome, the combination of alleles at neighboring SNPs, called haplotypes, generates much less diversity than would be expected if they were uncorrelated. Recent studies have shown that the human genome is organized into a succession of distinct haplotype blocks that are ancestrally conserved (40). By resequencing the genome of 270

individuals from populations with African, Asian, and European ancestry, the HapMap Project has identified a set of SNPs that tag most of the common haplotypes in the human genome (41). This resource is used to search for polymorphisms associated with susceptibility to common diseases. For this purpose, genotyping arrays built with tag SNPs that encompass the whole genome or specific regions of interest, are used.

In 2001, the complete sequence of the human genome was published, and with it came a powerful ability to identify genes, particularly for complex traits. Since that time, the trend has been a tremendous acceleration in the discovery of genes for complex traits, and this trend is likely to continue. The HapMap is an ongoing effort to identify all of the major haplotypes (specific combinations of genetic differences in blocks on chromosomes) throughout the genome, and this is expected to greatly simplify association studies. Also, the ability to rapidly and efficiently survey gene expression for most genes will help to identify disease gene pathways (42).

## References:

1.Wang Q, Bond M, Elston RC, Tian X. Molecular genetics. In: Topol EJ, editor. *Textbook of Cardiovascular Medicine.* edn 2. Philadelphia: Lippincott Williams & Wilkins; 2001, electronic chapter 97.

2. Lander ES, Linton LM, Birren B et al. Initial sequencing and analysis of the human genome. Nature 2001; 409: 860–921.

3. Sachidanandam R, Weissman D, Schmidt SC et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 2001; 409: 928–933.

4. Altshuler D, Brooks LD, Chakravarti A et al. A haplotype map of the human genome. Nature 2005; 437: 1299–1320.

5. Mendel G. Experiments in plant hybridization. Journal of the Brno Natural History Society 1866; 4: 3–47.

6. Gresham D, Dunham MJ, Botstein D. Comparing whole genomes using DNA microarrays. Nature Reviews 2008; 9: 291–302.

7. Samani NJ, Erdmann J, Hall AS et al. Genomewide association analysis of coronary artery disease. New England Journal of Medicine 2007; 357: 443–453.

8. McPherson R, Pertsemlidis A, Kavaslar N et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007; 316: 1488–1491.

9. Helgadottir A, Thorleifsson G, Manolescu A et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007; 316: 1491–1493.

10. Saxena R, Voight BF, Lyssenko V et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007; 3b16: 1331–1336.

11. Zeggini E, Weedon MN, Lindgren CM et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007; 316: 1336–1341.

12. Sladek R, Rocheleau G, Rung J et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007; 445: 881–885.

13. Link E, Parish S, Armitage J et al. SLCO1B1 variants and statin-induced myopathy: a genomewide study. New England Journal of Medicine 2008; 359: 789–799.

14. Schwarz UI, Ritchie MD, Bradford Y et al. Genetic determinants of response to warfarin during initial anticoagulation. New England Journal of Medicine 2008; 358: 999–1008.

15. Levy S, Sutton G, Ng PC et al. The diploid genome sequence of an individual human. PLoS Biology 2007; 5: e254.

16. Wheeler DA, Srinivasan M, Egholm M et al. The complete genome of an individual by massively parallel DNA sequencing. Nature 2008; 452: 872–876.

17. Hinds DA, Stuve LL, Nilsen GB et al. Whole-genome patterns of common DNA varition in three human populations. Science 2005; 307: 1072–1079.

18. Kidd JM, Cooper GM, Donahue WF et al. Mapping and sequencing of structural variation from eight human genomes. Nature 2008; 453: 56–64.

19. McCarroll SA, Altshuler DM. Nature Genetics 2008; 39: S37–S42.

20. Slatkin M. Linkage disequilibrium: understanding the evolutionary past and mapping the medical future. Nature Reviews 2008; 9: 477–485.

21. McVean GA, Myers SR, Hunt S et al. The fine-scale structure of recombination rate variation in the human genome. Science 2004; 304: 581–584.

22. Rigat B, Hubert C, Alhenc-Gelas F et al. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. The Journal of Clinical Investigation 1990; 86: 1343–1346.

23. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol. 2000; 20: 484–492.

24. Istrati V, Manea D, Iachim A. şi al. O variantă alelică a genei enzimei de conversie a angiotensinei (genotipulDD) în infarctul miocardic. Anale ştiinţifice. Probleme clinico-terapeutice în Medicina Internă, Zilele Universităţii consacrate Anului Ştefan cel Mare şi Sfânt, 2004, 2,V:219-220.

25. Ichim A., Istrati V., Manea D. şi al. Asocierea variantelor alelice ale genelor ACE şi eNOS cu infarctul miocardic în populaţia Republicii Moldova. Anale ştiinţifice. Probleme actuale în Medicina Internă, 3,VIII:87-92,2007.

26. Keavney B, McKenzie C, Parish S, et al. Large-scale test of hypothesised associations between the angiotensin-converting-enzyme insertion/ deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls: International Studies of Infarct Survival (ISIS) Collaborators. Lancet. 2000; 355: 434–442.

27. Curocichin Gh. *Complexul dereglărilor metabolice la pacienţii hipertensivi: caracteristica clinico-genetică.* Teza de doctor habilitat în medicină, 2009.

28. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A: **Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis.** *Arterioscler Thromb Vasc Biol* 2003 , **23:**1269-1275.

**29.** Sayed-Tabatabaei FA, Oostra BA, Isaacs A et al. ACE polymorphisms. Circulation Research 2006; 98: 1123–1133.

30. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genetics in Medicine 2002; 4: 45–61.

31. Lohmueller KE, Pearce CL, Pike M et al. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nature Genetics 2003; 33: 177–182.

32. de Bakker PI, Yelensky R, Pe'er I et al. Efficiency and power in genetic association studies. Nature Genetics 2005; 37: 1217–1223.

33. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996; 273: 1516–1517.

34. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol 2008; 32: 381–5.

35. Manolio TA, Bailey-Wilson JE, Collins FS. Genes, environment and the value of prospective cohort studies. Nature Reviews 2006; 7: 812–820.

36. Cupples LA, Arruda HT, Benjamin EJ et al. The Framingham Heart Study 100K SNP genome-wide association study resource: overview of 17 phenotype working group reports. BMC Medical Genetics 2007; 8 Suppl 1: S1.

37. Ridker PM, Chasman DI, Zee RY et al. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25 000 initially healthy American women. Clinical Chemistry 2008; 54: 249–255.

38. Lasky-Su J, Lyon HN, Emilsson V et al. On the replication of genetic associations: timing can be everything! American Journal of Human Genetics 2008; 82: 849–858.

39. The International HapMap Consortium. A haplotype map of the human genome. Nature. 2005; 437: 1299–1320 (http://www.hapmap.org).

40. Conrad DF, Jakobsson M, Coop G, Wen X, Wall JD, Rosenberg NA, Pritchard JK. A worldwide survey of haplotype variation and linkage disequilibrium in the human genome. Nat Genet. 2006; 38: 1251–1260.

41. Zeggini E, Rayner W, Morris AP, Hattersley AT, Walker M, Hitman GA, Deloukas P, Cardon LR, McCarthy MI. An evaluation of HapMap sample size and tagging SNP performance in large-scale empirical and simulated data sets. Nat Genet. 2005; 37: 1320–1322.

42. Schadt EE, Monks SA, Drake TA, et al. Genetics of gene expression surveyed in maize, mouse and man. Nature. 2003; 422: 297–302.

*The thousand mysteries around us would*
*not trouble but interest us,*
*if only we had cheerful, healthy hearts.*
Friedrich Wilhelm Nietzsche

# Chapter 5. Genetic basis of coronary artery disease

## Inheritance patterns

There is no question that CAD runs in families. We know first-degree relatives of people who develop CAD at any early age are at a much higher risk of developing CAD than the general population. Researches have identified more than 250 genes that play a role in CAD. Although researches are a long way from confirming whether even half of those genes are actually involved, CAD often results from the blended effects of genes. The genetics of CAD are extremely complicated, with many different genes influencing a person's risk. In most cases CAD is not inherited in a clearly dominant or recessive manner. A person may have mutations in some genes that increase the risk and mutations in genes that decrease the risk. Genetic predisposition to CAD is usually polygenic and rarely (does) CAD results from a single gene mutation, rather disease develops as a consequence of two-way interactions between the initial conditions (coded in the genotype) and exposures to environmental agents over time (1).

The preponderant part of CAD occurs on the ambience of polygenic susceptibility. It is the nature of polygenic disease that any single variant will only provide a small or modest contribution to risk. At the molecular level, atherosclerosis is a time-dependent, multistep process involving the interaction of many different key biochemical pathways. These include lipoprotein and glucose metabolism, blood pressure, coagulation and inflammation *(table 5.1)*. Gene variants in any of these metabolic pathways may lead to altered production or function of key proteins and hence upset the delicate balance of homeostasis. Intermediate phenotypes such as hypertension, diabetes and obesity (all polygenic traits) will interact to modulate risk. Environmental risks such as smoking, sedentary lifestyle and high-calorie diets are not simply additive with genetic risk.

*Table 5.1*

**Pathways in which mutations or polymorphisms have been shown to modify coronary artery disease risk** (adapted from Funke and Assmann: 1999, *International Task Force for Prevention of Coronary Heart Disease*)

- lipoprotein metabolism
- blood pressure
- glucose metabolism
- hemostasis
- inflammation response genes
- oxidation mediators
- adhesion mediators
- gene regulatory factors

A gene-environmental interaction is very important and it is believed to be present in subjects with a genetic predisposition, who also adopt a high-risk environmental risk, but have a major effect in a particular environment. Gene-gene interactions are also likely to be important, although relatively little is understood about these in the context of CAD (1).

Atherosclerosis includes numerous cell types and organs and a number of different physiological processes. Many genes shown here have been examined in population-association studies, but only a fraction have been convincingly confirmed as playing role in CAD or genetic susceptibility to its risk factors *(figure 5.1)*. This complexity is clearly illustrated by studies of rodent models; experiments with transgenic and gene-targeted mice have revealed > 100 genes that can influence the development of atherosclerotic lesions.

Pathological studies suggest that the development of thrombus-mediated acute coronary events depends principally on the composition and vulnerability of a plaque rather than the severity of stenosis (*figure 5.2*).

Vulnerable plaques generally have thin fibrous caps and increased numbers of inflammatory cells. Maintenance of the fibrous cap reflects matrix production and degradation, and products of inflammatory cells are likely to influence both processes. For example, T cells produce interferon (IFN-a), which inhibits the production of matrix by smooth muscle cells (SMCs), and macrophages produce various proteases that degrade extracellular matrix, including interstitial collagenase, gelatinases and stromolysin. Rupture frequently occurs at the lesion edges, which are rich in foam cells, suggesting that factors contributing to inflammation may also influence thrombosis. In this regard, it is notable that the incidence of myocardial infarction and stroke increases during acute infections (3).

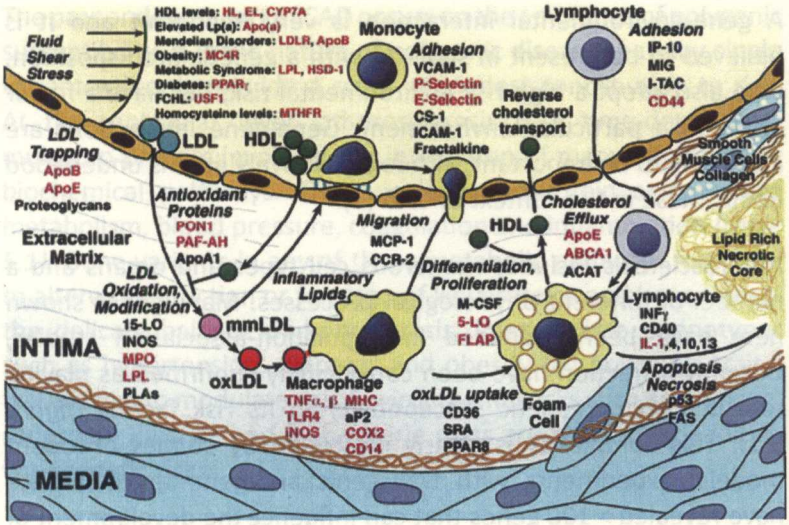



*Figure 5.1.* **Candidate genes for genetic susceptibility to CAD and some pathways thought to be involved in development of atherosclerotic lesions.**

Early stages of lesion formation. Genes with preliminary evidence of association with CAD are shown in red. 5-HT2A receptor indicates serotonin 2A receptor; ACE, angiotensin-converting enzyme; ADD1, adducin 1; AGE, advanced glycosylation end product; aP2, fatty acid–binding protein; BMPs, bone morphogen proteins; C3, complement component 3; CCR2, chemokine receptor 2; CLPN10, calpain 10; COX2, cyclooxygenase-2; CRP, C-reactive protein; CYP7A, cholesterol 7α-reductase; EL, endothelial lipase; GPIIIA, glycoprotein IIIA; HL, hepatic lipase; HO-1, heme oxygenase-1; HSD-1, 11ß-hydroxysteroid e reductase-1; IL, interleukin; INF, interferon; MC4R, melanocortin-4; MCP-1, monocyte chemoattractant protein-1; MGP, matrix gla protein; MHC, major histocompatibility complex; MMPs, metalloproteinases; MPO, myeloperoxidase; NOS, nitric oxide synthase; RAGE, receptor for AGE; SAA, serum amyloid A; SLC 2A1, solute carrier family 2, glucose transporter, membrane 1; sPLA2, secretory phospholipase; TIMPs, tissue inhibitors of metalloproteinasese; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule-1; and VEGF, vascular endothelial growth factor. (*Image Contrib. by Lusis AJ. Nature. 2000*).

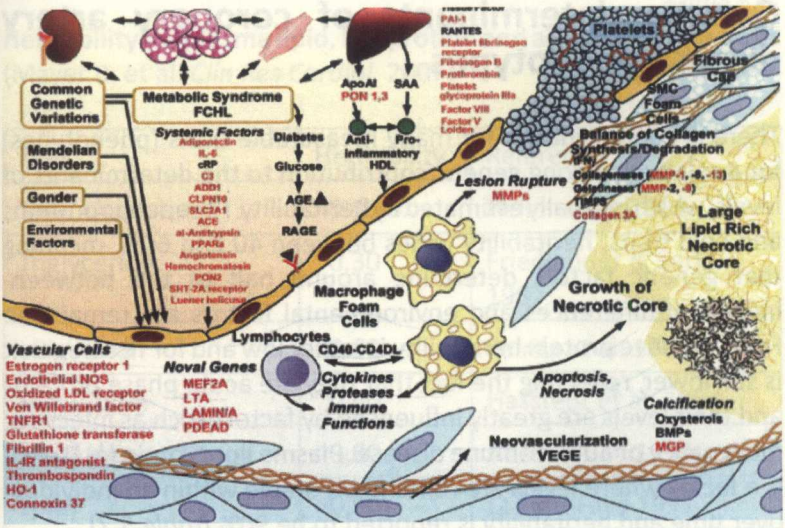



*Figure 5.2.* **Candidate genes for genetic susceptibility to CAD and some pathways thought to be involved in rupture of atherosclerotic lesions.**

Later stages of lesion; Genes with preliminary evidence of association with CAD are shown in red. 5-HT2A receptor indicates serotonin 2A receptor; ACE, angiotensin-converting enzyme; ADD1, adducin 1; AGE, advanced glycosylation end product; aP2, fatty acid–binding protein; BMPs, bone morphogen proteins; C3, complement component 3; CCR2, chemokine receptor 2; CLPN10, calpain 10; COX2, cyclooxygenase-2; CRP, C-reactive protein; CYP7A, cholesterol 7α-reductase; EL, endothelial lipase; GPIIIA, glycoprotein IIIA; HL, hepatic lipase; HO-1, heme oxygenase-1; HSD-1, 11ß-hydroxysteroid e reductase-1; IL, interleukin; INF, interferon; MC4R, melanocortin-4; MCP-1, monocyte chemoattractant protein-1; MGP, matrix gla protein; MHC, major histocompatibility complex; MMPs, metalloproteinases; MPO, myeloperoxidase; NOS, nitric oxide synthase; RAGE, receptor for AGE; SAA, serum amyloid A; SLC 2A1, solute carrier family 2, glucose transporter, membrane 1; sPLA2, secretory phospholipase; TIMPs, tissue inhibitors of metalloproteinasese; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule-1; and VEGF, vascular endothelial growth factor (*Image Contrib. by Lusis AJ. Nature. 2000).*

# Genetic determinants of coronary artery disease phenotypes

There is good evidence for many measurable traits (phenotypes) for a relatively strong genetic contribution to the determinants of levels, which is usually estimated by heritability. For apolipoproteins and lipid traits, heritability varies between 40 and 60%, meaning that genetic factors determine around half of the between-individual differences and environmental factors are remainder. For C-reactive protein heritability is rather low and for fibrinogen it is still lower, reflecting the fact that they are acute phase proteins and their levels are greatly influenced by factors such as infection, malignancy or auto-immune disease. Plasma lipoprotein (a) [Lp(a)] is a factor where levels are remarkably stable within an individual over time and heritability is reported to be 90% *(table 5.2).*

Variability at the locus coding for the apolipoprotein A gene itself accounts for almost all of the variance of plasma Lp(a) in normal population studies (4). The relevance of this is that a recent meta-analysis reported that levels of Lp(a) in the top tertile was associated with a 1,6-fold greater risk of CAD, an effect which is of similar magnitude as smoking and thus the apoA gene would appear to be a major genetic factor for CAD. In support of this, a common SNP in the Lp(a) gene has a replicated effect on CAD risk (hazard ratio=1,62). Measuring Lp(a) is a relevant genetic factor to add in clinical management of high risk patients (5). Although there is no clear mendelian pattern of segregation for MI or CAD, there is substantial evidence for a familial component to these diseases, particularly those with an early age of onset (5). A seminal Twin study showed strong relative risks for MI among identical twins and less strong but significant associations among nonidentical twins, with the highest risks observed with a very early age of onset for MI in the affected twin (6). Prospective studies report relative risk estimates for MI and CAD ranging from 1.2 to >3.0 for offsprings who reported that their parents had had the disease (7).

*Table 5. 2*

**Heritability for plasma lipid, lipoprotein and apolipoprotein traits** (Mayer B. et al. *Clin Res Cardiol.* 2007)

| Trait | Heritability | Reference |
|---|---|---|
| Total cholesterol | ~0.50 | Heller et al. 1993 |
| Triglyceride | ~0.30 | Friedlander & Kark, 1987 |
| LDL/ApoB | ~0.60 | Pairitz et al. 1988 |
| HDL/ApoAI | ~0.60 | Hasstedt et al. 1984 |
| Small dense LDL | ~0.60 | Austin et al. 1993 |
| Lp(a) | >0.90 | Lamon-Fava 1991 |
| Fibrinogen | 0.3–0.5 | de Lange et al. 2001 |
| Plasminogen activator inhibitor-1 (PAI-1) | 6.60 | de Lange et al. 2001 |
| Platelet aggregates | 0.44–0.62 | O'Donnell et al. 2001 |
| Homocysteine | ~0.55 | Berg et al. 1992 |
| SICAM | 0.24 | Keaney et al. 2004 |

More recently, the fully multivariable-adjusted risk of offspring for all atherosclerotic CAD associated with premature-onset parental disease was placed at 1.7 for women and 2.0 for men using validated parental and offspring outcomes and similar magnitudes of multivariable-adjusted risk were noted for association with sibling CAD (8). Subclinical measures of atherosclerotic CAD, assessed in the carotid artery by ultrasound and the coronary and aortic arteries with computed tomography or magnetic resonance imaging have augmented the evidence for the role of genetics in CAD.

Substantial heritability (≈35% to 60%) is reported for carotid intima-media thickness (IMT) (9), coronary artery calcification (10) and abdominal aortic calcification. Moreover, a positive parental history of CAD is associated with a significantly increased burden of atherosclerosis determined by carotid intima media thickness or coronary artery calcification (11,12,13). Collectively, a substantial body of evidence suggests the role of genetics in atherosclerosis.

## Candidate gene for coronary artery disease

A large number of candidate gene association studies have been conducted for subclinical measures of MI and CAD. However, in general, relatively few gene polymorphism associations have been consistently replicated for CAD (14). Reasons for inconsistencies may include genetic heterogeneity, varying patterns of linkage disequilibrium (LD) in differing population groups, lack of statistical power or an excessive false-positive rate, confounded by other genetic or environmental factors, or phenotypic heterogeneity. CAD is caused by many factors and some factors may be operating in some forms of CAD but not others. These studies have been summarized in recent reviews (15) and risk ratio findings have generally been quite modest in association studies and meta-analyses often have been required to achieve statistical significance.

Meta-analyses have been reported for polymorphisms studied in a very large number of independent studies, including variants of the angiotensin-1 converting enzyme (ACE), (Agerholm-Larsen B., 2000), plasminogen activator inhibitor 1 (PAI1 Boekholdt SM, 2001), MTHFR genes (Klerk M, Verhoef P, 2002) and apolipoprotein E (Song Y, 2004). In general, results have not been consistent across studies and the overall magnitude of association for these common polymorphisms is modest. Table 5.3 summarizes recent

meta-analyses and overviews of association studies of candidate gene variants for atherosclerotic cardiovascular disease.

*Table 5.3*

**Gene variants of artery disease risk**

| Gene/Polyimorphism | Risk Genotype | No studies (cases) | Size of effect ( CI95%) |
|---|---|---|---|
| APOB Gln4154Lys (Q4154L) | LL | 14 (1796) | 1.73(1.19–2.50) |
| NOS3 Glu298Asp (E298D) | DD | 14 (6036) | 1.31(1.13–1.51) |
| APOE, E2,E3,E4 | E4 | 48 (15,492) | 1.30(1.18–1.43) |
| F5 (FactorV-Leiden)R506Q | Q+ | 20 (5313) | 1.10(0.88–1.36) |
| ACE Insertion/Deletion | DD | 43 (14,292) | 1.22(1.11–1.35) |
| F2 (Prothrombin) G20210A | A+ | 19 (4944) | 1.21(0.99–1.58) |
| SERPINE1 (PAI-1) 5G/4G | 4G4G | 7 (2813) | 1.20(1.04–1.39) |
| AGT Angiotensinogen Met235Thr (M235T) | TT | 21 (4001) | 1.19(1.10–1.30) |
| APOB Signal peptide Ins/ Del | DD | 22 (6007) | 1.19(1.05–1.35) |
| MTHFR C677T | TT | 40 (11,162) | 1.14(1.01–1.28) |
| ITGB3 (GPIIb-IIIa) | A2+ | 34 (6173) | 1.13(1.02–1.26) |
| PON1 Paraoxonase-1 Q192R | R192 | 44 (10,106) | 1.12(1.07–1.16) |
| LPL Lipoprotein lipase Ser/Ter (S447X) | X+ | 4 (2252) | 0.80 (0.7–1.0) |
| CETP TaqIB | B1B2 | 7 (7681) | 0.78(0.66–0.93) |

Recently, high-throughput genotyping has been used in case-control studies that have typed from 62 to >13 000 genes. Genetic variants in the lymphotoxin-alpha (LTA), PAI1, gap junction protein, α-4 (GJA4, also known as connexin 37), matrix metalloproteinase-3 (MMP3, also known as stromelysin-1) and arachidonate 5-lipoxygenase (ALOX5) genes, as well as several thrombospondin (THBS) genes were associated with MI (20,21). These studies represent the first of a number of studies under way that are designed to comprehensively screen single-nucleotide polymorphisms (SNPs) in coding regions or nearby regions of all known human genes.

A number of candidate gene association studies have been conducted for subclinical atherosclerosis phenotypes, particularly intima media thickness and coronary artery calcification. The association data for dozens of gene variants with carotid IMT have recently been exhaustively reviewed (22). Of the many studies conducted, only 1 variant that was previously linked to clinical disease, the 5A/6A polymorphism of the MMP3 gene, showed consistently positive associations with carotid IMT, although in a small number of studies. The paraoxonase 1 (PON1) leu55met variant is weakly associated in subgroups only.

There are several existing general population-based prospective studies in which DNA has been stored; these include the "offspring" arm of the Framingham Study in the USA (23), the PROCAM Study in Germany (24), the Atherosclerosis Risk in the Communities (ARIC) Study (25) and the Second Northwick Park Heart Study (NPHS-II) in the UK (26). All of these studies have published genetic associations with CAD and risk traits. Focusing on NPHS-II study (*table 5.4*), some associations have been identified. To date more than 30 gene loci have been explored as possible CAD risk genes and, as shown in *tables 5.4,5.5*, statistically significant associations with CAD risk have been identified for 14 genes or gene clusters. These include those encoding apolipoproteins,

enzymes of lipid metabolism and vascular biology, nuclear factors, receptors, cytokines and intracellular and extracellular proteins (27). The data highlight the importance of the chosen system in determining CAD risk factors, and imply that, to some extent at least, disturbances of these systems could be causal for CAD and not simply a consequence of the disease. They also identified other potential candidate genes for study, coding for other proteins that may be involved in the upstream or downstream consequences of the rate-limiting step controlled by the gene (e.g. the association of the IL6 gene with CAD "nominates" new candidate genes encoding IL-6 binding proteins and the IL-6 receptor).

*Table 5.4*

**Genetic association on coronary artery disease in Second Northwick Park Heart Study**

| Gene | Variant 1 | Genetic model | Main genetic RR(95%CI) | Candidate system |
|---|---|---|---|---|
| LPL | D9N | N9 vs D9 | 2.33 (1.08–5.03) | Lipid levels Plaque foam cell development |
| APOE | E3/E2/E4 | E4 vs E3 | 0.82 (0.59–1.15) | Levels of atherogenic lipoprotein |
| IL6 | −174 G > C | GC vs CC | 1.55 (1.06–2.22) | Atherosclerotic inflammatory process |
| MMP3 | 5A > 6A | 6A vs 5A | 1.91 (1.11–3.28) | Plaque matrix deposition and plaque receptor |
| PECAM | R670G | RR vs GG | 1.07 (0.75–1.94) | Monocyte ingress into plaque |
| PPARA | Intron 7G > C | CC vs GG | 1.83 (0.96–3.51) | Co-ordinate control of lipid and energy metabolism in adipose and muscle tissue |

Several of the genes examined to date in NPHS-II were chosen because knowledge of their biology raised the possibility that genotypic effects might be modified by certain environmental exposures.

*Table 5.5*

**Results of selected genetic association studies on coronary artery disease in Second Northwick Park Heart Study**

| Gene | Variant1 | Genetic model | Main genetic RR (95%CI) | Candidate System |
|---|---|---|---|---|
| PPARA | L162V | VV + VL vs LL | 0.75 (0.45–1.26) | |
| APOA4 | T347S | SS vs TT | 2.04 (1.02–4.05) | Oxidative stress in plaque |
| UCP2 | –866G > A | AA:GA vs GG | 1.86 (1.33–2.59) | Oxidative stress in plaque |
| AGT1R | 1166A > C | CC:AC vs AA | 1.65 (1.05–2.59) | Cardiac growth in response to Hypertension |
| AGT2R6 | 1657A > G | A vs G | 1.0 (0.75–1.34) | |
| BDKRB1 | –699G > C | GG vs CC+ GC | 1.0 (0.66–1.51) | |
| BDKRB2 | +9/–9bp | +9+9 vs –9–9 | 0.75 (0.32–1.75) | |
| THBD | A455V/1208 DelTT | Hetero vs Homo | 1.35 (0.96–1.89) | Lipid driven control of thrombus Growth |

Miller et al. 2002 in the NPHS-II study have estimated the risk of CAD from family history of CAD (FHCAD) in 2 827 healthy European middle-aged men and explored the extent to which this can be explained by classical and genetic risk factors. The risk for those with a positive family history who were also current smokers was 3.01 compared to non-smokers without FHCAD, which was greater than the risk posed by smoking or FHCAD alone (1.96 and 2.05, respectively) compared to non-smokers without FHCAD, but not significantly different from a multiplicative model (p-value for interaction 0.33). Allele frequencies for 13 candidate gene variants were not significantly different between those with and without FHCAD. In those with FHCAD, smokers who carried the apoE4 allele (e4+) had a hazard ratio of 5.66 compared to non-smokers who had no FHCAD and were not apoE4+, with a significant interaction between smoking and apoE4 in those with FHCAD (p= 0.001). These data demonstrated the complex interaction between genetic and environmental factors in determining CAD risk.

## Endothelial nitric oxide synthase (eNOS) gene polymorphism in coronary artery disease

Using data from 10 399 CAD cases, Casas et al. found that homozygosity for the Asp298 and intron-4a alleles but not the -786C allele of the eNOS gene was associated with a small but significant increase in the risk of CAD (Odds Ratio (OR) 1.31; 95% CI, 1.13 to 1.51; OR, 1.34; 95% CI, 1.03 to 1.75; and OR, 1.06; 95% CI, 0.89 to 1.25, respectively).

The role for Asp298 and intron-4a alleles is very similar in magnitude to those reported for apolipoprotein E, angiotensin-converting enzyme, and methylenetetrahydrofolate reductase polymorphisms (28-30) and suggests that the genetic contribution to CAD is through small to moderate effects of many genes.

Therefore, it seems unlikely that these polymorphisms individually will make a useful contribution to risk prediction in asymptomatic individuals, but whether the combined genotype analysis integrated with orthodox assessment of cardiovascular risk will enhance the prediction of CAD requires additional analysis (31). The Glu298Asp polymorphism is the only coding region variant identified in eNOS, and mechanistic studies indicate a functional effect of this substitution. Associations have been described between the Glu298Asp polymorphism and NO synthesis or endothelial function, (32) and a mechanism by which eNOS Asp298 might reduce NO bioavailability has also been reported. eNOS Asp298 is subject to selective proteolytic cleavage in endothelial cells and vascular tissues. Because the cleaved fragments would be expected to lack NO synthase activity, this could account for reduced vascular NO generation in subjects homozygous for this variant. Individuals homozygous for the Asp298 allele have also been shown to exhibit a reduced blood pressure fall after exercise training (33) and to have lower basal blood flow and reduced vasodilatation to adenosine in their coronary arteries (34). In addition, they have an enhanced systemic pressor response to phenylephrine and a reduced flow-mediated dilatation of the brachial artery (35). These findings suggest that subjects homozygous for the Asp298 allele generate low NO *in vivo* and may be more susceptible to endothelial dysfunction, which might account for the increased risk of CAD. Conflicting associations between the intron-4 variant and NO pathway activity have also been described. Some reports indicate that carriers of this variant have lower NO plasma levels and decreased protein expression (36), but this is not supported by all studies (37).

A functional effect for the -786T>C polymorphism has also been proposed from *in vitro* reporter gene assays. Lower eNOS mRNA and serum nitrite/nitrate levels have been found in individuals with the -786C variant (38) but not all studies (39).

## Endothelial nitric oxide synthase gene polymorphism in Moldovan patients with acute myocardial infarction

A case-control study of 100 patients with acute myocardial infarction and 50 healthy gender- and age-matched control subjects was performed in Moldova (40). The 4a/4b variable-number tandem repeat (VNTR) minisatelite marker of endothelial nitric oxide synthase genetic polymorphism was analyzed by basic PCR with allele specific primers for 4a/4b VNTR minisatelite *(figure 5.3)*.

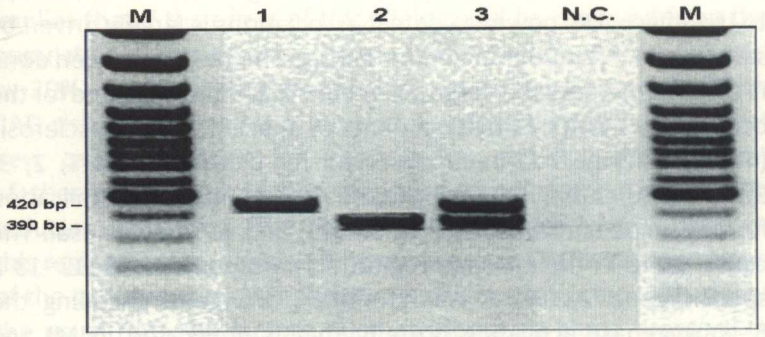



*Figure 5.3.* **Electrophoretic results of PCR products with allele specific primers for 4a/4b VNTR minisatelite marker in Moldovan patients with acute myocardial infarction.**

M – marker (100 pb, Ferments, Lithuania); 1 – specific amplicon sizes for 4b and 4a homozygotes, respectivly; 3 – heterozygotes; N.C. – negative control

The carrier of the intron- 4a/4b and 4b/4b polymorphism of the endothelial nitric oxide synthase gene was associated with atherosclerotic coronary lesion (≥75%) in 86% of cases and increased risk of myocardial infarction. The endothelial nitric oxide synthase allele frequency in patients with acute myocardial

infarction was distributed: 4a/4a-10,0%, 4b/4b-45,0%, 4a/4b-35,0%. These results imply that patients who are carriers for the intron- 4a/4b and 4b/4b polymorphism of the endothelial nitric oxide synthase gene may be genetically predisposed to myocardial infarction, however, these mutations were not related to the severity of coronary atherosclerosis.

## Novel genes for coronary artery disease

Recent genome-wide association studies of MI and other forms of CAD have led to the discovery of at least 13 genetic loci. In addition to the effect size, power to detect associations is largely driven by sample size. A number of genetic linkage analyses have been done in at least 7 moderate to large collections of families selected for the occurrence of MI or measurements of subclinical atherosclerosis (41-44). Linkage has been observed for chromosomes 1, 2, 3, 9,13, 14, 16, and X, although there has been no strong replication for any single chromosomal region (45) and very few causal. The causal gene in their linkage region on chromosome 13q12–13 a specific gene, ALOX5AP was identified, the gene encoding the 5'-lipoxygenase-activating protein, that is implicated in MI and stroke (46). Although the evidence for statistical significance for linkage in this study has not been strong to date, the association of genetic variants in the ALOX5AP locus with MI or stroke has been replicated in some but not all follow-up studies (47,48). The Iceland study represents one of the first-reported linkage findings that has successfully led to the identification of a strong candidate gene for an MI or stroke.

Recently, three genome-wide association studies (GWAS), one led by the Icelandic company DeCode, one led by a Canadian-based group (McPherson, R. et al.) and Samani et al. (50,56) from the UK Wellcome Trust Case-Control Consortium reported six other loci as harboring SNPs associated with CAD, all identifying

a single region on chromosome 9p21.3 associated with CAD or myocardial infarction risk. Because of the size of the studies (a total number>10 000 cases and 20 000 controls) the level of statistical certainty was large ($p<10^{-20}$) and the replication in a number of independent studies means that this finding cannot be in any doubt. Interestingly, SNPs in this region have also been associated with type2 diabetes and with risk of abdominal aortic aneurism, suggesting a unifying underlying mechanism for these three diseases. Because of the high frequency of the risk allele (50% of subjects are carriers, with 23% having two copies) and because of the size of the effect, this SNP has been estimated to have a population attributable risk of 20% for myocardial infarction. This implies that if the effect of this allele could be removed from the population the incidence of myocardial infarction would be reduced by 20%. The identification of this locus as being associated with CAD risk thus identifies a novel and potentially highly important new causal pathway for investigation and the development of therapeutic approaches that will complement current risk-reducing modalities. The mechanism by which DNA variants in this region of chromosome 9 increase risk of CAD is unclear. None of the genotypes used in the studies were associated with any of the usual CAD risk factors, such as increased blood pressure or lipid levels. The chromosomal 9 region has relatively few identified genes, but the most likely candidates are a cluster consisting of CDKN2A-ARF-CDKN2B, which are all involved in cell-cycle control. This locus is often deleted in malignant tumours, but also plays a role in cell proliferation, senescence and apoptosis, all features implicated in atherogenesis. Although no sequence changes that directly alter the function of these proteins were identified by the recent publications, it is possible that DNA changes which alter the level of expression of these key proteins may be important. This would then influence the ability of endothelial smooth muscle cells in the vascular system to continue proliferation and as a consequence, lead to senescence and apoptosis, causing plaque progression or rupture.

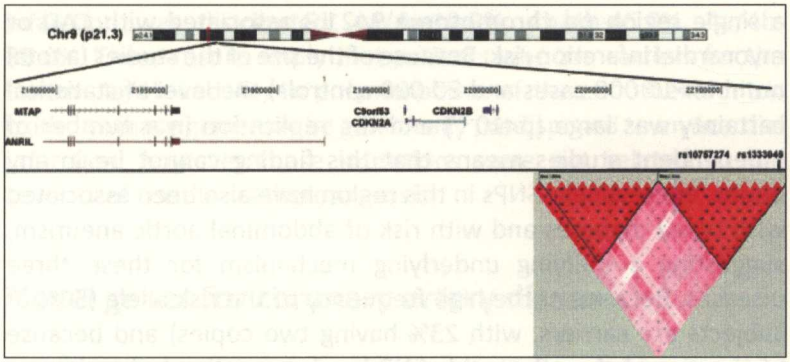



*Figure 5.4.* **Cartoon of the chr9p21.3 locus showing the nearest genes (MTAP, ANRIL, CDKN2A and CDKN2B) and the location of the 2 GWAS-identified SNPs, rs10757274 and rs1333049, reported in the meta-analyses, with a Haploview linkage disequilibrium plot of the region around the 2 SNPs** (Samani NJ. Et al., *Arterioscler ThrombVascBiol.*, 2009).

The original GWASs identified 2 "lead" SNPs, rs10757274 and rs1333049, and many subsequent studies have genotyped only 1 of these, which makes a direct comparison problematic. These 2 SNPs are in strong linkage disequilibrium (estimated $r^2$ from data available in the report by Kathiresan et al. (49), and both SNPs are strongly associated with CAD in white people, with similar effect sizes in case-control and prospective studies *(figure 5.4).*

Genome-wide association studies have identified several other CAD loci and their chromosome locations, the frequency of the risk allele and the reported size of the risk effect are shown in *table 5.6.* Although the CELSR2/SORT1/PSRC1 locus is associated with

LDL-C levels, (51) whose variation in SORT1 appeared to be the most likely candidate (52), other loci showed no association with measured phenotypes, and it appeared that their mechanism of risk did not operate by influencing known conventional risk factors (CRF) traits such as lipids or blood pressure.

As expected for any biomarker of modest effect, not all studies demonstrated a statistically significant effect owing to issues of small sample size and power and the play of chance, although effects may also have been modified by different study characteristics, such as the prevalence of smoking or other "environmental" CAD risk factors. However, there is no significant evidence for heterogeneity of effect for either SNP, with very similar overall per-allele CAD risk effects of 1.29 (95% CI 1.19 to 1.40) for rs10757274 and 1.29 (95% CI 1.24 to 1.35) for rs1333049. It is possible that the use of both or additional SNPs at this locus (*table 5.6)* may refine and improve the identification of the "risk haplotype" (50). The risk-effect sizes found in these GWASs are of the same magnitude (1.2 to 1.6) as those seen in the meta-analyses of candidate SNPs (53). As for the candidate-gene SNPs, replication of effects and meta-analyses of published SNP data are vital to validate the findings, even though the original GWAS reports included replication studies (54). Achievement of the numbers required to confirm or refute these modest effects is possible with the establishment of international consortia. The near future, will bring genotype data from >200 000 individuals genotyped with the Human CVD BeadChip, including many of the CAD GWAS hits, and this provides a source of replication (55). The GWAS-identified loci reported in *table 5.6* only represent the locus nearest to the risk-associated SNP(s). One of the current challenges in molecular genetics is to identify these functional variants.

*Table 5.6*

**Chromosomal localizations of genes associated with myocardial infarction**

| Chromosome Localization /Gene | Risk Genotype SNIP | Risk allele frequency | Effect (Conf. Ind.95%) | P |
|---|---|---|---|---|
| 1p13.3 CELSR2/ PCSR1/SORT1 | rs646776–T* rs599839-A | 0.81 0.28 | 1.19 (1.13–1.26) 1.13 (1.08–1.19) | $7.9x10^{-12}$ $1.4x10^{-7}$ |
| 1q41 MIA3 | rs17465637-C* rs3008621-G | 0.72 0.16 | 1.14 (1.10–1.19) 1.10 (1.04–1.17) | $1.4x10^{-9}$ $1.0x10^{-3}$ |
| 2q36 | rs2943634-C | 0.34 | 1.05 (1.0–1.1) | 0.03 |
| 3q22.3 MRAS | rs9818870-T | 0.20 | 1.15 (1.11–1.19) | $7.4x10^{-13}$ |
| 6q25 MTHFD1L | rs6922269-A* rs6922269-A | 0.26 0.26 | 1.09 (1.0–1014) 1.05 (1.0–1.1) | $2.3x10^{-5}$ 0.02 |
| 10q11 CXCL12 | rs1746048-C* rs501120-T | 0.84 0.13 | 1.17 (1.11–1.24) 1.11 (1.05–1.18) | $7.4x10^{-9}$ $4.3x10^{-4}$ |
| 12q24 HNF1A/ C12orf43 | rs2259816-A | 0.36 | 1.08 (1.05–1.11) | $4.8x10^{-7}$ |
| 15q22 SMAD3 | rs17228212-T* rs17228212-C | 0.73 0.73 | 1.05 (1.01–1.09) 1.00 (0.95–1.04) | 0.02 0.9 |

The chromosome 9p21 risk SNPs lie in a gene-poor region (*figure 5.4*), and the nearest genes (CDKN2A-ARF-CDKN2B) are >100-kilobases (kb) upstream from the risk SNPs (56). CDKN2A/CDKN2B are involved in cell cycle control, and thus, alterations in their expression could be postulated to lead to senescence and apoptosis, both of which are processes involved in plaque progression and rupture. However, a more detailed analysis of the region between these genes and the risk SNPs has suggested an alternative candidate. In the PROCARDIS study (Precocious Coronary Artery Disease), susceptibility to coronary artery disease was encoded by

2 common haplotypes spanning the 53-kb region that overlaps with ANRIL, a gene encoding an antisense noncoding RNA (57,58). This is a member of a gene family involved in transcriptional control that overlaps and regulates CDKN2B and is expressed in atheromatous human vessels in vascular endothelial cells, monocyte-derived macrophages, and coronary smooth muscle cells, all of which are involved in atherosclerosis. A recent paper (using a mouse model with 58 kb of chr 9p21 deleted) has presented data suggesting that ANRIL expression is not the most likely mechanism, and identified a *cis*-acting element influencing the expression of CDKN2A/2B and thus cell apoptosis. This chr9p21 region is clearly a disease "*hot spot*," being associated with risk of heart failure, type 2 diabetes mellitus, abdominal aortic aneurysms, stroke (59). The fact that common variation in this gene region is involved in such a wide range of diseases suggests that this locus encodes 1 or more key players in cell homeostatic processes that are involved in this set of complex multifactorial diseases and raises the possibility that influencing the expression at this locus may have important therapeutic consequences.

A total of 3 247 patients with acute coronary syndrome (ACS) enrolled in the Global Registry of Acute Coronary Events (GenGRACE) in three distinct populations (UK, Belgium and Poland) were prospectively followed for 6 months and genotyped for rs1333049, in addition to 3 004 and 2 467 healthy controls from the UK and Belgium. After having confirmed that the at-risk C allele of rs1333049 was associated with ACS in the UK and Belgian populations, Buysschaert I. et al. (2010) found that the rs1333049 at-risk C allele was significantly and independently associated with recurrent MI (age- and gender-adjusted hazard ratio (HR) 1.48, CI= 1.00-2.19, P= 0.048; and multivariable-adjusted HR 1.47, CI= 0.99-2.18; P= 0.053) and with recurrent MI or cardiac death (age- and gender-adjusted HR 1.58, CI= 1.00-2.48; P= 0.045; and multivariable adjusted HR 1.49, CI= 1.03-1.98; P= 0.028) within 6 months after ACS. Inclusion of rs1333049 into the GRACE risk score

significantly improved classification for recurrent MI or cardiac death (P= 0.040), as calculated by the integrated discrimination improvement method. In this large observational study, the 9p21 variant was independently associated with adverse cardiac outcome after ACS (60).

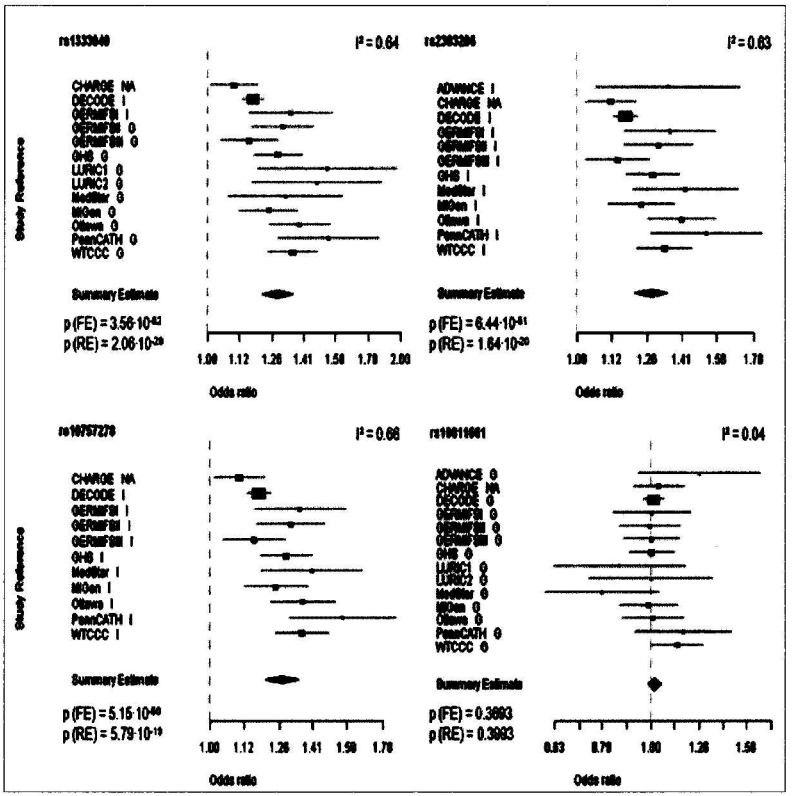



*Figure 5.5.* **Forest plots for SNPs on chromosome 9p21** (61).

Random effects (RE) models were calculated for SNPs rs1333049 (risk allele=C), rs2383206 (risk allele=G), and rs10757278 (risk allele=G); the fixed effects (FE) model was calculated for SNP rs10811661 (risk allele=C). Heterogeneity between the studies is indicated by I2, and for every study, it is indicated whether the respective SNP was genotyped (G) or imputed (I) or a mixture of genotyped and imputed (NA).

The Coronary ARtery DIsease Genome-wide Replication And Meta-analysis (CARDIoGRAM) consortium was formed to maximize the chance of finding novel susceptibility loci (*figure 5.5*) for CAD and MI (61). CARDIoGRAM combined data from all published and several unpublished GWAS in individuals with European ancestry; included >22 000 cases with CAD, MI, or both and >60 000 controls and unified samples from the Atherosclerotic Disease VAscular functioN and genetiC Epidemiology study, CADomics, Cohorts for Heart and Aging Research in Genomic Epidemiology, deCODE, the German Myocardial Infarction Family Studies I, II, and III, Ludwigshafen Risk and Cardiovascular Heath Study/AtheroRemo, MedStar, Myocardial Infarction Genetics Consortium, Ottawa Heart Genomics Study, PennCat, and the Wellcome Trust Case Control Consortium. Genotyping was carried out on Affymetrix or Illumina platforms followed by imputation of genotypes in most studies. On average, 2.2 million single nucleotide polymorphisms were generated per study. The result from each study was combined using meta-analysis. It has been found that risk variants at 9p21 and rs1333049 confer a 29% increase in risk for MI per copy ($P=2\times10^{-20}$).

The recent report of two genomewide association studies was designed to analyse the hypothesis that genetic factors predisposing to MI in patients with coronary atherosclerosis are distinct from those that associate with the presence of coronary atherosclerosis. They used coronary angiography to ascertain whether an individual had coronary artery disease, which allows discrimination of risk alleles for plaque rupture and MI from those for coronary atherosclerosis. For the first study, Reilly and colleagues compared 12 393 individuals with coronary artery disease with 7 383 without any stenosis on angiography (controls) and identified a new locus, ADAMTS7, to be a common genetic risk factor for coronary atherosclerosis, with a 19% increased risk for carriers.

In the newest study (2011), the researchers compared 5 783 patients with coronary artery disease and MI (cases) with 3 644 individuals with coronary artery disease but free from MI (controls) to identify variants associated with coronary thrombosis but not atherosclerosis. They found that blood group ABO single nucleotide polymorphisms were associated with MI in the context of coronary artery disease. The ABO association was attributable to the glycotransferase-deficient enzyme that encodes the ABO blood group O phenotype previously proposed to protect against MI. They consider that discovery of ABO as the top locus for myocardial infarction in patients with angiographic CAD is notable, in view of decades of work suggesting a relation between ABO blood groups and both thrombosis and coronary heart disease (62).

## Genetic loci sssociated with C-Reactive Protein levels

The twin and family studies have estimated that genetic factors could contribute up to 35–50% of the variation of CRP (63,64). Several population-based association studies have shown that common genetic variants at the CRP locus are significantly associated with plasma CRP levels (65). Furthermore, the CRP locus has been shown to associate with CAD risk, providing additional support for a causal role of CRP in CAD. Dr. Ridker et al. (66) showed that data from the Women's Genome Health Study revealed distinct patterns of gene polymorphisms affecting levels of CRP, fibrinogen and soluble ICAM-1 (Intercellular adhesion molecule-1). They confirmed the associations of common genetic variants in the LEPR, IL6R, CRP, and HNF1A loci and APOE-CI-CII cluster with CRP levels *(table 5.7)*. The variety of loci implicated for CRP levels indicates its role as a marker of inflammation and metabolic dysfunction (67,68.)

*Table 5.7*

**Candidate genes linked to mediators of inflammation**

| Phenotype | Gene symbol | OMIM Nr | Gene Map Locus |
|---|---|---|---|
| C-reactive protein | CRP | 123260 | 1q21-q23 |
| Five-lypoxigenase-activation protein | ALOX5AP | 603700 | 13q12 |
| Intercelular adhesion molecule 1 | ICAM1 | 147840 | 19p13.3-p13.2 |
| Interleukin-6 | IL6 | 147620 | 7p21 |
| Membrane cofactor protein 1 | CCL | 158105 | 17q11.2-q12 |
| P-Selectin | SELP | 173610 | 1q23-q25 |

The genome-wide association study conducted by Elliott P. et al. (2009) included a total of more than 28 000 cases with CAD (69) and found no association of variants in the CRP locus and CAD, arguing against a causal role for CRP in atherosclerosis. The data from The Rotterdam Study by Pai JK, (2005), Kardys I, Casas JP. (2006) do not support that the common variation in the C-reactive protein gene has a large effect on the occurrence of coronary heart disease (70-72).

To generate a data set of sufficient power to confirm or refute a modest but potentially important effect of CRP on fatal and nonfatal CAD events, the CRP Coronary Heart Disease Genetics Collaboration (CCGC) coordinated a large-scale Mendelian randomisation meta-analysis of individual participant data from 47 epidemiological studies in 15 countries that included 46 557 CAD cases and 119 524

controls (73). Information was available on four CRP gene tagging single nucleotide polymorphisms (rs3093077, rs1205, rs1130864, rs1800947), concentration of C reactive protein, and levels of other risk factors (74). CRP variants were each associated with up to 30% per allele difference in concentration of C reactive protein and were unrelated to other risk factors. Risk ratios for coronary heart disease per additional copy of an allele associated with raised C reactive protein were 0.93 (95% confidence interval 0.87 to 1.00) for rs3093077; 1.00 (0.98 to 1.02) for rs1205; 0.98 (0.96 to 1.00) for rs1130864; and 0.99 (0.94 to 1.03) for rs1800947. In a combined analysis, the risk ratio for coronary heart disease was 1.00 (0.90 to 1.13) per 1 SD higher genetically raised natural log (ln) concentration of C reactive protein. The genetic findings were discordant with the risk ratio observed for coronary heart disease of 1.33 (1.23 to 1.43) per 1 standard deviation higher circulating ln concentration of C reactive protein in prospective studies (P=0.001 for difference). The authors concluded that C reactive protein concentration itself was unlikely to be even a modest causal factor in coronary heart disease.

## Loci associated with disorders of coagulation and thrombosis

A meta-analysis of prospective observational and case-control studies, showed fibrinogen level to be predictive of CAD risk, this reported to be 1.8 (95% CI, 1.6-2.0) for the top to the bottom third of the fibrinogen distribution. However, the existing atherosclerosis may increase fibrinogen levels and reverse causation will lead fibrinogen to predict future CAD events. There is also substantial confounding, with higher fibrinogen levels being seen in several population subgroups known to have increased CAD risk, for example, cigarette smokers, people with less favorable socioeconomic backgrounds, nondrinkers, and people who engage in less leisure time activity.

Observational studies demonstrated that circulating fibrinogen levels are associated with increased coronary heart disease risk. Smith GD. et al. (2005) performed a meta-analysis of case-control and prospective studies of the G-455→A and C-148→T ß-fibrinogen promoter region variants, in relation to CAD risk. The 19 studies included 12 393 cases and 21 649 controls. Fibrinogen levels were robustly related to the genetic variants (mean increase per allele, 0.117 g/L; 95% CI, 0.091–0.142 g/L). However, the genetic variants were unrelated to CAD risk (odds ratio per allele, 0.976; 95% CI, 0.916–1.040). The predicted causal odds ratio for a 1 g/L higher plasma fibrinogen level, given the genetic variant–fibrinogen and genetic variant– CAD associations, was 0.81 (95% CI, 0.46–1.40). The predicted causal effect of fibrinogen on CAD was different from the odds ratio of 1.8 (95% CI, 1.6–2.0) for an increase of 1 g/L derived from a meta-analysis of observational studies. This evidence suggested that lowering the fibrinogen level might not reduce CAD risk. In their meta-analysis of studies that relate genetic variants associated with differences in fibrinogen level to coronary heart disease risk, Smith GD. et al. found no association. These data suggest that fibrinogen is not a cause of increased coronary heart disease risk (75).

*Table 5.8*

**Candidate Genes associated with Disorders of Coagulation and Thrombosis and coronary artery disease**

| Phenotype | Gene symbol | Nr OMIM | Gene Map locus |
|---|---|---|---|
| Dysfibrinogenemia, α type | FGA | 134820 | 4q28 |
| Factor VII level | F7 | 227500 | 13q34 |
| Glycoprotein IIIa | ITGB3 | 173470 | 17q221.32 |
| Plasminogen activator inhibitor deficiency | PAI1 | 173360 | 7q21.3-q22 |

The annotations p and q refer to band patterns in chromosomes detected cytochemically that mark specific regions.

During the last decade several genes involved in the atherosclerotic process and their polymorphisms have been suspected to increase the thrombotic predisposition and to influence the risk for acute coronary syndromes. Among these genes, polymorphisms of those involved in platelet function have been extensively studied. Indeed, platelets play a pivotal role in atherothrombosis (76) and their function is strongly related to the interactions of the glycoprotein IIb/IIIa receptor (GP IIb/IIIa) and the von Willebrand factor, as well as fibrinogen, leading to platelets aggregation (77,78). GP IIIa is a high polymorphic protein with platelet antigen 1 (PlA1) and 2 (PlA2) as the most common allelic isoforms. In the PlA2 allele, cytosine is substituted for thymidine in exon 2, which is phenotypically translated in the substitution of proline for leucine at position 33 of the mature GP IIIa. A study *in vitro* demonstrates that the PlA2 variant enhances the binding of the GPIIb/IIIa receptor to fibrinogen and therefore increases the platelet aggregation induced by agonists (79).

The clinical impact of PlA2 polymorphism has been investigated in several diseases, in which thrombus formation is a key pathogenetic factor, but the definition of the specific role of such polymorphisms on thrombotic coronary and cerebrovascular complications has been challenging. Weiss et al. (80) observed a strong association between the PlA2 polymorphism of the GP IIIa gene and acute coronary thrombosis, and this association was strongest in patients who had had coronary events before the age of 60 years, suggesting this polymorphism as an inherited risk factor for coronary thrombosis. These findings were further expanded on peripheral artery disease by Mikkelson (79) who reported an association between PlA2 variant and the progression of atherosclerosis in the abdominal aorta. Similarly, in the Copenhagen City Heart Study, a

prospective study with 9 149 subjects, there was a three-fold and four-fold risk of ischemic cardiovascular disease and myocardial infarction in men <40 years homozygous for PlA2 polymorphism (81). On the other hand, several studies failed to confirm this association; indeed a metanalysis of 23 of such negative studies, showed the lack of association between the PlA2 allele and the risk of myocardial infarction and this negative result persisted even after subgroup analyses (82). Since the original report from Weiss et al.(80) indicating PlA2 polymorphism as a risk factor for myocardial infarction or unstable angina, several studies have investigated this polymorphism in the effort to discover a novel thrombogenic risk factor but to date results have been inconclusive and often controversial (81).

In the CAD population Gennaro Galasso (83) observed that the presence of PlA2 patients was associated with a 3-times higher risk of death or 2.8 increased risk of acute myocardial infarction. Noteworthy, patients harboring the PlA2 allele presented a higher risk of needing new revascularization at follow up, with 50% of patients undergoing new PCI for the occurrence of a new myocardial infarction. This is consistent with data by Kastrati et al.(84), reporting a higher risk of restenosis after coronary stent placement in these patients. Moreover, in an independent population of hypertensive patients with cerebrovascular events, the presence of the PlA2 allele was associated with a 4.1 higher risk to develop stroke rather than transient ischemic attack. Thus, these data suggest that, in a high risk, clinical scenario represented by patients with a greater prevalence of atherothrombotic risk factors, the presence of PlA2 is associated with a more aggressive disease leading to a higher incidence of major ischemic events and to an unfavorable outcome.

In a subgroup analysis of the Physicians' health study (PHS) by Ridker et al. (85) which prospectively studied the risk associated

with the PlA2 polymorphism for myocardial infarction, stroke or venous thrombosis and reported no associations between PlA2 polymorphism and the relative risk to develop any cardiac or cerebro-vascular event. Indeed, as already remarked by previous studies (79) the PHS was conducted in a very healthy population, with an event rate 4 times less than general population, and therefore with a very low risk of cardiovascular events. Many studies suggested the PlA2 polymorphism to be associated with a more aggressive atherothrombotic disease and adversely affecting the prognosis in these high risk patients. Indeed, in patients with severe CAD undergoing PCI the presence of PlA2 allele was associated with increased risk of death, myocardial infarction and new myocardial revascularization. Moreover, high risk hypertension patients harbouring the PlA2 allele show an increased risk to develop severe cerebral damage (83).

## Genetic determinants of restenosis

Restenosis is still the major limitation of percutaneous coronary interventions (PCI), resulting from injury of the vessel wall caused by balloon dilation and stent placement. The vascular damage is characterized by irritation of endothelial and subendothelial structures and injury of medial regions with rupture of the internal elastic lamina. This damage causes segmental thrombus formation and subsequent invasion of macrophages and polymorphonuclear leukocytes, followed by expression and release of numerous growth factors and cytokines from blood cells, leading to proliferation of smooth muscle cells (87,88). Vascular inflammation thus plays an important role in this complex multifactorial process (89). The GENetic DEterminants of Restenosis (GENDER) project analyzed 3 104 consecutive patients after successful PCI and forty-eight polymorphisms in 34 genes involved in the inflammatory process. The 16Gly variant of the $ß_2$-adrenergic receptor gave an increased risk of target vessel revascularization (TVR). After multivariable

analysis, Pascalle S. Monraats et al. identified the rare alleles of the CD 14 gene (-260T/T), colony-stimulating factor 2 gene (117Thr/Thr), and eotaxin gene (-1328A/A) were associated with decreased risk of TVR. However, through the use of multiple testing corrections with permutation analysis, the probability of finding 4 significant markers by chance was 12% (90). Although neointimal formation is more pronounced after stenting and remodeling is prominent after plain balloon angioplasty, stenting did not change the associations between the 4 genes and restenosis.

The first gene- the -260 T/T genotype of CD14 was found to be protective against restenosis after PCI. These data are in conflict with findings of two previous studies that investigated the role of CD14 in the development of restenosis, one being a prospective study by Zee RY et al. (91) in 779 patients and the other being a prospective study by Shamada et al. (92) in 129 patients. They found the -260 T/T genotype to be a risk factor for restenosis.

The second gene that was found to be associated with target vessel revascularization is the $ß_2$-adrenergic receptor gene (ADRB2), located on chromosome 5q31-q32. ADRB2s are cell-surface receptors that on binding to norepinephrine activate cellular adenylylcyclase through coupling to G-proteins. The ADRB2 gene has a role in the inflammatory response, because adrenoceptors are present on human platelets, and ADRB2 stimulation activates platelet nitric oxide synthase (93). The nitric oxide synthase catalyzes the formation of NO, which has an inhibitory role on leukocyte adhesion, platelet adhesion and aggregation, smooth muscle cell proliferation, and synthesis of matrix proteins, and it promotes endothelial survival and proliferation (94). In addition, ADRB2 has an effect on the immune system because lymphocytes express ADRB2s (95). The polymorphism in the ADRB2 gene that after adjusted analyses showed to be a risk factor for restenosis was the 16A/G polymorphism that results in an amino acid

change of glycine to arginine at position 16 (Arg16Gly). Patients with homozygosity for the 16Gly variant had a higher risk of TVR compared with patients with the 16Arg variant (11.3% versus 9.1%, respectively). *In vivo* and *in vitro* studies have suggested that this Arg16Gly variant may differently affect functional responses to adrenergic stimulation, thereby possibly modulating cardiovascular and metabolic phenotypes. It has been reported that the 16Gly variant of ADRB2 is associated with faster agonist-induced downregulation of the receptor, as compared to the 16Arg variant (96). The higher risk of TVR may be related to less vasodilatation as a result of the downregulation of the receptor containing 16Gly, as compared to the receptor containing 16Arg. Moreover, downregulation of the ADRB2 could result in impaired inhibition of platelet aggregation (93).

The polymorphism in the colony-stimulating factor 2 gene (CSF2) that is significantly associated with restenosis is the 117T/C polymorphism, which results in an isoleucin for threonin substitution on position 117. The Thr117 variant showed a protective association with TVR. The functional effect of this CSF2, also known as granulocyte-macrophage colony- stimulating factor, polymorphism still has to be investigated.

Another polymorphism associated with restenosis is eotaxin (CCL11), a CC chemokine that is localized on chromosome 17. The promoter variant of this gene (1328A/A) demonstrated a protective association with TVR. Economou et al. (96) reported eotaxin to be elevated in plasma of patients with advanced atherosclerosis. The plasma level of eotaxin in their study rose in the first day after PCI and declined to baseline in the following 3 months. In what way polymorphism determines the expression level on a protein basis is as yet unknown.

The inconsistence of findings in literature can be mostly attributed to differences in the design as well as to the choice of the control group and the endpoint of the studies. Moreover, studies differ in the variation of environmental factors and ethnicity, present biases in the selection of patients and controls and often aim to different clinical endpoints. Since atherosclerosis is a multifactorial disease, it would be too simplistic to explain interindividual variations based on genetic inheritance alone. Indeed several challenges exist in identifying the genetic determinants of such a complex disease including genetic heterogeneity, gene-gene and gene-environment interactions. Furthermore, interaction of multiple genes that are in linkage disequilibrium and simultaneous studies of several genes may reveal associations that at present seem to be weak. Moreover, such approaches are costly, and impose the use of large populations and heavy statistic modeling in order to carefully peruse small impact of single gene variants on multifactorial disease. There is still room for candidate gene association study that can be performed in relatively small populations that are carefully characterized and selected for homogeneity (86).

In conclusion, considerable progress in genetic research is being made, especially by GWAS analyses in combination with gene-environment interaction research. This technique, where per patient in general > 500 000 genetic markers are checked, does lead to new clues, but this GWAS technique does not answer all questions. In common complex diseases like CAD, genetic information can often explain <10% of the disease, but in twins, for example, around 30-50% of these CAD traits seem heritable.

GWAS technique is leading to a better understanding of CAD and may provide new therapeutic targets. Making and applying appropriate predictive models (partially) based on GWAS results is, however, currently still challenging.

## References:


1. Priori S. G., Napolitano C., Humphries S. E. and Skipworth J. Genetics of Cardiovascular Diseases in:A. JOHN CAMM, THOMAS F. LUSCHER, PATRICK W. SERRUYSESC Textbook of Cardiovascular Medicine, 2(1), 2009.
2. Funke H., Assmann G. International Task Force for Prevention of Coronary Heart Disease Curr. Opin. Lipidol. 1999,10, 285-91.
3. Lusis AJ. Atherosclerosis. Nature. 2000; 407: 233–241.
4. Mayer B, Erdman J, Schunkert H. Genetics and heritability of coronary artery disease and myocardial infarction. Clin Res Cardiol. 2007; 96: 1-7.
5. Borge G. Nordestgaard, M. John Chapman, Kausik Ray, Jan Borén, Felicita Andreotti, Gerald F. Watts, Henry Ginsberg, Pierre Amarenco, Alberico Catapano, Olivier S. Descamps, Edward Fisher, Petri T. Kovanen, Jan Albert Kuivenhoven, Philippe Lesnik, Luis Masana, Zeljko Reiner, Marja-Riitta Taskinen, Lale Tokgözoglu, Anne Tybjærg-Hansen. Lipoprotein(a) as a cardiovascular risk factor: current status.Eur Heart J (2010) doi: 10.1093/eurheartj/ehq386 First published online: October 21, 2010
6. Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med. 1994; 330: 1041–1046.
7. Sesso HD, Lee IM, Gaziano JM, Rexrode KM, Glynn RJ, Buring JE. Maternal and paternal history of myocardial infarction and risk of cardiovascular disease in men and women. Circulation. 2001; 104: 393-398.
8. Murabito JM, Pencina MJ, Nam BH, D'Agostino RB Sr, Wang TJ, Lloyd-Jones D, Wilson PW, O'Donnell CJ. Sibling cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults. JAMA. 2005; 294: 3117–3123.
9. Fox CS, Polak JF, Chazaro I, Cupples A, Wolf PA, D'Agostino RA, O'Donnell CJ. The Framingham Heart Study. Genetic and environmental contributions to atherosclerosis phenotypes in men and women: heritability of carotid intima-media thickness in the Framingham Heart Study. Stroke. 2003; 34: 397-401.

10. Peyser PA, Bielak LF, Chu JS, Turner ST, Ellsworth DL, Boerwinkle E, Sheedy PF 2nd. Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults. Circulation. 2002; 106: 304-308.

11. Wang TJ, Nam BH, D'Agostino RB, Wolf PA, Lloyd-Jones DM, MacRae CA, Wilson PW, Polak JF, O'Donnell CJ. Carotid intima-media thickness is associated with premature parental coronary heart disease: the Framingham Heart Study. Circulation. 2003; 108: 572-576.

12. Fornage M, Lopez DS, Roseman JM, Siscovick DS, Wong ND, Boerwinkle E. Parental history of stroke and myocardial infarction predicts coronary artery calcification: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Eur J Cardiovasc Prev Rehabil. 2004; 11: 421-426.

13. Nasir K, Michos ED, Rumberger JA, Braunstein JB, Post WS, Budoff MJ, Blumenthal RS. Coronary artery calcification and family history of premature coronary heart disease: sibling history is more strongly associated than parental history. Circulation. 2004; 110: 2150-2156.

14. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med. 2002; 4: 45-61

15. Lusis AJ, Fogelman AM, Fonarow GC. Genetic basis of atherosclerosis, part I: new genes and pathways. Circulation. 2004; 110: 1868–1873.

16. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med. 2004; 141: 137-147.

17. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR, Peters RJ. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. Circulation. 2001; 104: 3063–3068.

18. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol. 2000; 20: 484–492.

19. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA. 2002; 288: 2023-2031.

20. Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, Sato H, Hori M, Nakamura Y, Tanaka T. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet. 2002; 32: 650-654;

21. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M, Yokota M. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med. 2002; 347: 1916–1923.

22. Manolio TA, Boerwinkle E, O'Donnell CJ, Wilson AF. Genetics of ultrasonographic carotid atherosclerosis. Arterioscler Thromb Vasc Biol. 2004; 24: 1567-1577.

23. Anderson, K. M. & Wilson, P. W. et al. (1987) Longitudinal and secular trends in lipoprotein cholesterol measurements in a general population sample. The Framingham Offspring Study. Atherosclerosis 68, 59-66.

24. Assmann, G. & Cullen, P. et al. (2002) Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. Circulation 105, 310-315.

25. Pereira, M. A. & Schreiner, P. J. et al. (2000) The Family Risk Score for coronary heart disease: associations with lipids, lipoproteins, and body habitus in a middle-aged bi-racial cohort: the ARIC Study. Ann Epidemiol 10, 239-245.

26. Miller, G. J. & Bauer, K. A. et al. (2002) Does inflammatory proteolytic activity contribute to the increased factor IX activation peptide in men at high risk of coronary heart disease? A preliminary study. Thromb Haemost 87, 415-420.

27. Benjamin, E. J. & Larson, M. G. et al. (2004) Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. Circulation 109, 613-619.

28. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ. 2002; 325: 1–7,

29. Keavney B, McKenzie C, Parish S, et al. Large-scale test of hypothesised associations between the angiotensin-converting-enzyme insertion/deletion polymorphism and myocardial infarction in about 5000 cases and

6000 controls: International Studies of Infarct Survival (ISIS) Collaborators. Lancet. 2000; 355: 434–442.

30. Keavney B, Parish S, Palmer A, et al. Large-scale evidence that the cardiotoxicity of smoking is not significantly modified by the apolipoprotein E epsilon2/epsilon3/epsilon4 genotype. Lancet. 2003; 361: 396–398.

31. Yang Q, Khoury MJ, Botto L, et al. Improving the prediction of complex diseases by testing for multiple disease-susceptibility genes. Am J Hum Genet. 2003; 72: 636–649.

32. Leeson CP, Hingorani AD, Mullen MJ, et al. Glu298Asp endothelial nitric oxide synthase gene polymorphism interacts with environmental and dietary factors to influence endothelial function. Circ Res. 2002; 90: 1153–1158.

33. Rankinen T, Rice T, Perusse L, et al. NOS3 Glu298Asp genotype and blood pressure response to endurance training: the HERITAGE Family Study. Hypertension. 2000; 36: 885–889.

34. Naber CK, Baumgart D, Altmann C, et al. eNOS 894T allele and coronary blood flow at rest and during adenosine-induced hyperemia. Am J Physiol Heart Circ Physiol. 2001; 281: H1908–H1912.

35. Naber CK, Baumgart D, Altmann C, et al. eNOS 894T allele and coronary blood flow at rest and during adenosine-induced hyperemia. Am J Physiol Heart Circ Physiol. 2001; 281: H1908–H1912

36. Wang XL, Sim AS, Wang MX, et al. Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. FEBS Lett. 2000; 471: 45–50.

37. Jeerooburkhan N, Jones LC, Bujac S, et al. Genetic and environmental determinants of plasma nitrogen oxides and risk of ischemic heart disease. Hypertension. 2001; 38: 1054–1061.

38. Miyamoto Y, Saito Y, Nakayama M, et al. Replication protein A1 reduces transcription of the endothelial nitric oxide synthase gene containing a -786T→C mutation associated with coronary spastic angina. Hum Mol Genet. 2000; 9: 2629–2637.

39. Lusis AJ, Fogelman AM, Fonarow GC. Genetic basis of atherosclerosis, part II: clinical implications. Circulation. 2004; 110: 2066–2071.

40. Capros N., Istrati V., Popescu V., Butovschaia C., Josan D., Popovici I., Bumacov L. Endothelial nitric oxide synthase gene polymorphism in patients with acute myocardial infarction. European Heart Journal (2010) 31(Abstract Supplement, IF2.1), P3183,538-539. ESC Congress, Stockholm.

41. Broeckel U, Hengstenberg C, Mayer B, Holmer S, Martin LJ, Comuzzie AG, Blangero J, Nurnberg P, Reis A, Riegger GA, Jacob HJ, Schunkert H. A comprehensive linkage analysis for myocardial infarction and its related risk factors. Nat Genet. 2002; 30: 210–214.

42. Hauser ER, Crossman DC, Granger CB, A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. Am J Hum Genet. 2004; 75: 436–447.

43. Helgadottir A, Manolescu A, Thorleifsson G, The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet. 2004; 36: 233–239.

44. Samani NJ, Burton P, Mangino M, Ball SG, Balmforth AJ, Barrett J, Bishop T, Hall A, for the BHF Family Heart Study Research Group. A genomewide linkage study of 1,933 families affected by premature coronary artery disease: the British Heart Foundation (BHF) Family Heart Study. Am J Hum Genet. 2005; 77: 1011–1020.

45. Altmuller J, Palmer LJ, Fischer G, Scherb H, Wjst M. Genomewide scans of complex human diseases: true linkage is hard to find. Am J Hum Genet. 2001; 69: 936–950.

46. Helgadottir A, Manolescu A, Thorleifsson G, The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet. 2004; 36: 233–239.

47. Zee RY, Cheng S, Hegener HH, Erlich HA, Ridker PM. Genetic variants of arachidonate 5-lipoxygenase-activating protein, and risk of incident myocardial infarction and ischemic stroke: a nested case-control approach. Stroke. 2006; 37: 2007–2011.

48. Kajimoto K, Shioji K, Ishida C, Iwanaga Y, Kokubo Y, Tomoike H, Miyazaki S, Nonogi H, Goto Y, Iwai N. Validation of the association between the gene encoding 5-lipoxygenase-activating protein and myocardial infarction in a Japanese population. Circ J. 2005; 69: 1029–1034.

49. Kathiresan S, Voight BF, Purcell S, et al,Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009; 41: 334–341.

50. Samani NJ, Deloukas P, Erdmann J, Hengstenberg C, Kuulasmaa K, McGinnis R, Schunkert H, Soranzo N, Thompson J, Tiret L, Ziegler A. Large scale association analysis of novel genetic loci for coronary artery disease. Arterioscler Thromb Vasc Biol. 2009; 29: 774–780.

51. Sandhu MS, Waterworth DM, Debenham SLet al, LDL-cholesterol concentrations: a genome-wide association study. Lancet. 2008; 371: 483–491.

52. Linsel-Nitschke P, Heeren J, Aherrahrou Z, et al. ,)Genetic variation at chromosome 1p13.3 affects sortilin mRNA expression, cellular LDL-uptake and serum LDL levels which translates to the risk of coronary artery disease. Atherosclerosis. 2010; 208: 183–189.

53. Casas JP, Cooper J, Miller GJ, Hingorani AD, Humphries SE. Investigating the genetic determinants of cardiovascular disease using candidate genes and meta-analysis of association studies. Ann Hum Genet. 2006; 70: 145–169.

54. Karvanen J, Silander K, Kee F, Tiret L, Salomaa V, Kuulasmaa K, Wiklund PG, Virtamo J, Saarela O, Perret C, Perola M, Peltonen L, Cambien F, Erdmann J, Samani NJ, Schunkert H, Evans A. The impact of newly identified loci on coronary heart disease, stroke and total mortality in the MORGAM prospective cohorts. Genet Epidemiol. 2009; 33: 237–246.

55. Keating BJ, Tischfield S, Murray SS, Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One. 2008; 3: e3583.

56. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH, Cohen JC. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007; 316: 1488–1491, The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447: 661–678.

57. Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, Clarke R, Collins R, Franzosi MG, Tognoni G, Seedorf U, Rust S, Eriksson P, Hamsten A,

Farrall M, Watkins H. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet. 2008; 17: 806–814,

58. Pasmant E, Laurendeau I, Heron D, Vidaud M, Vidaud D, Bieche I. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. Cancer Res. 2007; 67: 3963–3969.

59. Helgadottir A, Thorleifsson G, Magnusson KP, The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008; 40: 217–224.

60. Buysschaert Ian, Carruthers Kathryn F., Dunbar Donald R.et al A variant at chromosome 9p21 is associated with recurrent myocardial infarction and cardiac death after acute coronary syndrome: The GRACE Genetics Study Eur Heart J (2010) 31 (9): 1132-1141.

61. Preuss Michael, König Inke R., Thompson John R. et al.,Design of the Coronary ARtery DIsease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) StudyA Genome-Wide Association Meta-analysis Involving More Than 22 000 Cases and 60 000 Controls.Circulation: Cardiovascular Genetics. 2010; 3: 475-483.

62. Reilly MP, Li M, Jing H, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. Lancet 2011. 2011; 377(9761):200-203.

63. Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, Liu K, Williams OD, Iribarren C, Lewis EC, Fornage M, Boerwinkle E, Gross M, Jaquish C, Nickerson DA, Myers RM, Siscovick DS, Reiner AP: Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. Am J Hum Genet 77:64-77, 2005.

64. Miller DT, Zee RY, Suk Danik J, Kozlowski P, Chasman DI, Lazarus R, Cook NR, Ridker PM, Kwiatkowski DJ: Association of common CRP gene variants with CRP levels and cardiovascular events. Ann Intern Med 69:623–638, 2005.

65. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, Sharma P, Lowe GD, World MJ, Humphries SE, Hingorani AD: Human CRP gene

polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol 23:2063-2069, 2003.

66. Ridker PM, Pare G, Parker A, et al., Reiner AP, Barber MJ, Guan Y, et al. Polymorphisms of the HNF1A gene encoding hepatocyte nuclear factor-1 alpha are associated with C-reactive protein. Am J Hum Genet. 2008;82(5):1193-1201.

67. Miller DT, Zee RY, Suk Danik J, Kozlowski P, Chasman DI, Lazarus R, Cook NR, Ridker PM, Kwiatkowski DJ. Association of common CRP gene variants with CRP levels and cardiovascular events. Ann Hum Genet. 2005 Nov;69(Pt 6):623-38.

68. Suk Danik J, Chasman DI, Cannon CP, Miller DT, Zee RY, Kozlowski P, Kwiatkowski DJ, Ridker PM.Influence of Genetic Variation in the C-Reactive Protein Gene on the Inflammatory Response During and After Acute Coronary Ischemia Ann Hum Genet. 2006 Nov;70(Pt 6):705-16.

69. Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Erdmann J, Braund P, Engert JC, Bennett D, Coin L, Ashby D, Tzoulaki I, Brown IJ, Mt-Isa S, McCarthy MI, Peltonen L, Freimer NB, Farrall M, Ruokonen A, Hamsten A, Lim N, Froguel P, Waterworth DM, Vollenweider P, Waeber G, Jarvelin MR, Mooser V, Scott J, Hall AS, Schunkert H, Anand SS, Collins R, Samani NJ, Watkins H, Kooner JS. Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. JAMA. 2009 Jul 1;302(1):37-48.

70. Kardys I, de Maat MP, Uitterlinden AG, Hofman A, Witteman JC.C-reactive protein gene haplotypes and risk of coronary heart disease: the Rotterdam Study. Eur Heart J. 2006 Jun;27(11):1331-7.

71. Casas JP, Shah T, Cooper J, et al, Lawlor DA, Harbord RM, Timpson NJ, et al. The association of C-reactive protein and CRP genotype with coronary heart disease: findings from five studies with 4,610 cases amongst 18,637 participants. PLoS ONE. 2008;3(8):e3011.

72. Pai JK, Mukamal KJ, Rexrode KM, Rimm EB, Lange LA, Carlson CS, Hindorff LA, et al, Miller DT, Zee RY, Suk Danik J, et al, Kardys I, de Maat MPM, Uitterlinden AG, Hofman A, Witteman JC, Chen J, Zhao J, Huang J, Su S, Qiang B, Gu D. -717A>G polymorphism of human C-reactive protein gene

associated with coronary heart disease in ethnic Han Chinese: the Beijing atherosclerosis study. J Mol Med. 2005;83(1):72–78).

73. CRP CHD Genetics Collaboration. Collaborative pooled analysis of data on C-reactive protein gene variants and coronary disease: judging causality by Mendelian randomisation. Eur J Epidemiol 2008;23:531-540.

74. Wensley F, Gao P, Burgess S.et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC). BMJ. 2011 Feb 15;342:d548.

75. George Davey Smith; Roger Harbord; Julie Milton; Shah Ebrahim; Jonathan A.C. Sterne.Does Elevated Plasma Fibrinogen Increase the Risk of Coronary Heart Disease? Evidence from a Meta-Analysis of Genetic Association Studies Arteriosclerosis, Thrombosis, and Vascular Biology. 2005;25:2228.

76. Davi G, Patrono C: Platelet activation and atherothrombosis. The New England journal of medicine 2007: 357(24):2482-2494.

77. Lefkovits J, Plow EF, Topol EJ: Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. The New England journal of medicine 1995, 332(23):1553-1559.

78. Atherosclerosis T, Vascular Biology Italian Study Group: No evidence of association between prothrombotic gene polymorphisms and the development of acute myocardial infarction at a young age. Circulation 2003, 107(8):1117-1122.

79. Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, Hamlington J, Barnard MR, Kickler T, Christie DJ, et al.: Platelet GP IIIa PI(A) polymorphisms display different sensitivities to agonists. Circulation 2000, 101(9):1013-1018.

80. Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G, Goldschmidt-Clermont PJ: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. The New England journal of medicine 1996, 334(17):1090-1094.

81. Bojesen SE, Juul K, Schnohr P, Tybjaerg-Hansen A, Nordestgaard BG: Platelet glycoprotein IIb/IIIa PI(A2)/PI(A2) homozygosity associated with risk of ischemic cardiovascular disease and myocardial infarction in young

men: the Copenhagen City Heart Study. Journal of the American College of Cardiology 2003, 42(4):661-667.

82. Zhu MM, Weedon J, Clark LT: Meta-analysis of the association of platelet glycoprotein IIIa PlA1/A2 polymorphism with myocardial infarction. The American journal of cardiology 2000, 86(9):1000-1005. A1008.

83. The GPIIIA PlA2 polymorphism is associated with an increased risk of cardiovascular adverse events. Gennaro Galasso, Gaetano Santulli, Federico Piscione, Roberta De Rosa, Valentina Trimarco, Raffaele Piccolo, Salvatore Cassese, Guido Iaccarino, Bruno Trimarco and Massimo Chiariello.BMC Cardiovascular Disorders 2010, 10:41.

84 Kastrati A, Schomig A, Seyfarth M, Koch W, Elezi S, Bottiger C, Mehilli J, Schomig K, von Beckerath N: PlA polymorphism of platelet glycoprotein IIIa and risk of restenosis after coronary stent placement. Circulation 1999 , 99(8):1005-1010.

85. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K: PlA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. Lancet 1997, 349(9049):385-388.

86. Kullo IJ, Ding K: Mechanisms of disease: The genetic basis of coronary heart disease. Nature clinical practice 2007, 4(10):558-569.

87. Agema WR, Jukema JW, Pimstone SN, Kastelein JJ. Genetic aspects of restenosis after percutaneous coronary interventions: towards more tailored therapy. Eur Heart J. 2001; 22: 2058-2074.

88. Bhargava B, Karthikeyan G, Abizaid AS, Mehran R. New approaches to preventing restenosis. BMJ. 2003; 327: 274-279.

89. Hojo Y, Ikeda U, Katsuki T, Mizuno O, Fukazawa H, Fujikawa H, Shimada K. Chemokine expression in coronary circulation after coronary angioplasty as a prognostic factor for restenosis. Atherosclerosis. 2001; 156: 165-170.

90. Pascalle S. Monraats, Nuno M.M. Pires, Willem R.P. Agema Circulation. Genetic Inflammatory Factors Predict Restenosis After Percutaneous Coronary Interventions 2005;112:2417-2425.

91. Zee RY, Hoh J, Cheng S, Reynolds R, Grow MA, Silbergleit A, Walker K, Steiner L, Zangenberg G, Fernandez-Ortiz A, Macaya C, Pintor E, Fernandez-Cruz A, Ott J, Lindpainter K. Multi-locus interactions predict risk for post-

PTCA restenosis: an approach to the genetic analysis of common complex disease. Pharmacogenomics J. 2002; 2: 197-201.

92. Shimada K, Miyauchi K, Mokuno H, Watanabe Y, Iwama Y, Shigekiyo M, Matsumoto M, Okazaki S, Tanimoto K, Kurata T, Sato H, Daida H. Promoter polymorphism in the CD14 gene and concentration of soluble CD14 in patients with in-stent restenosis after elective coronary stenting. Int J Cardiol. 2004; 94: 87-92.

93. Queen LR, Xu B, Horinouchi K, Fisher I, Ferro A. Beta(2)-adrenoceptors activate nitric oxide synthase in human platelets. Circ Res. 2000; 87: 39-44.

94. Chen AF, Ren J, Miao CY. Nitric oxide synthase gene therapy for cardiovascular disease. Jpn J Pharmacol. 2002; 89: 327-336.

95. Wahle M, Stachetzki U, Krause A, Pierer M, Hantzschel H, Baerwald CG. Regulation of beta2-adrenergic receptors on CD4 and CD8 positive lymphocytes by cytokines in vitro. Cytokine. 2001; 16: 205-209.

96. Hoit BD, Suresh DP, Craft L, Walsh RA, Liggett SB. Beta2-adrenergic receptor polymorphisms at amino acid 16 differentially influence agonist-stimulated blood pressure and peripheral blood flow in normal individuals. Am Heart J. 2000; 139: 537-542.

*Let thy food be thy medicine, and thy medicine be thy food.*
Hippocrates (460-370 B.C.)

# Chapter 6. Dyslipidemias

Lipid metabolism can be disturbed in different ways, leading to changes in plasma lipoprotein function and/or levels. This by itself and through interaction with other cardiovascular (CV) risk factors may affect the development of atherosclerosis. Therefore, dyslipidaemias cover a broad spectrum of lipid abnormalities, some of which are of great importance in CAD prevention. Consistent evidence worldwide has demonstrated an association between lipid level and lipoprotein on one hand and cardiovascular disease incidence on the other hand (1).

Whereas high concentrations of low density lipoprotein (LDL) cholesterol are associated with increased risk of CAD, high concentrations of high density lipoproteins (HDL) cholesterol are associated with decreased risk of CAD. Specifically, it has been estimated that each 1% decrease in LDL cholesterol concentrations reduces the risk of coronary heart disease by 1% (2,3) and each 1% increase in HDL cholesterol concentrations reduces the risk of coronary heart disease by 2% (4).

# Lipid metabolism

Serum cholesterol and triglyceride levels are primarily determined by a series of metabolic pathways, ligands and receptors that operate in the small intestine, liver, adipose tissue and skeletal muscle *(figure 6.1)*. Dietary lipids initially enter the circulation in the form of chylomicron (Cm) particles, where they may provide peripheral tissues with an important source of energy through the β-oxidation of fatty acids. In the post-prandial period, a proportion of these dietary lipids re-enter the circulation in the form of very low density lipoprotein (VLDL) particles (5), which are assembled in the liver. VLDL also transports non-dietary lipids formed from the catabolism of dietary carbohydrate, takes part in the recycling of cellular membranes and the esterification of free fatty acids that may derive from adipose tissue. The small intestine and liver additionally secrete nascent high density lipoproteins (HDL), which return excess cholesterol from diverse sources to the liver for excretion from the body in bile. These sources include macrophages/foam cells and the products of triglyceride-rich lipoprotein catabolism, namely Cm remnant particles, intermediate density lipoproteins and LDL. The increased transfer of cholesteryl esters from HDL to intermediate density lipoproteins and from LDL to VLDL, in return for triglyceride contributes to the formation of highly, atherogenic, small-dense LDL particles (6).

Although the term hyperlipidemia has long been used in clinical practice, the term dyslipoproteinemia more appropriately reflects the disorders of the lipid and lipoprotein transport pathways associated with arterial diseases. Dyslipidemia encompasses disorders often encountered in clinical practice such as low HDL cholesterol level and elevated triglyceride level but an average total plasma cholesterol level. Certain rare lipoprotein disorders can cause overt clinical manifestations, but most common dyslipoproteinemias themselves only rarely cause symptoms or produce clinical signs that are evident on physical examination. Rather, they require laboratory tests for

detection. Dyslipoproteinemias constitute a major risk factor for atherosclerosis and coronary artery disease, and their proper recognition and management can reduce cardiovascular and total mortality rates (7).

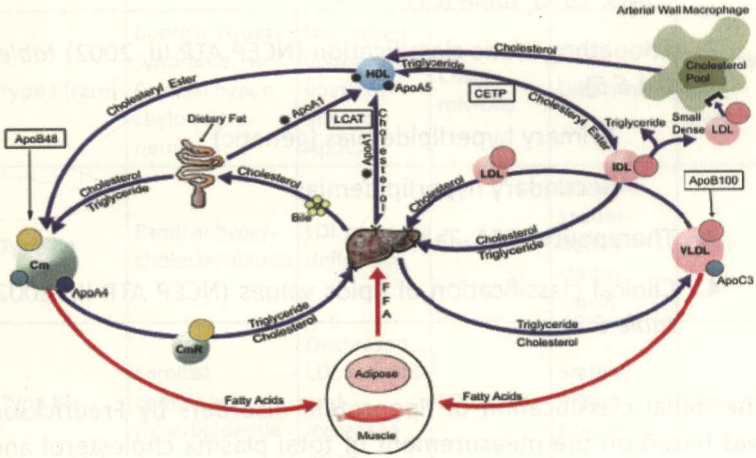



*Figure 6.1.* **Simplified view of cholesterol and triglyceride transport.** Apolipoprotein (apo)B is the obligatory protein component of Cm, VLDL, LDL, intermediate density lipoproteins (IDL) and remains with these particles throughout their catabolism. The lower molecular weight apolipoproteins, A1, C3 and A4 readily exchange between lipoproteins of different classes. CETP=cholesterol ester transfer protein; CmR=chylomicron remnant particle; FFA=non-esterified fatty acids; LCAT=lecithin-cholesterol acyltransferase; receptors for lipoprotein and lipid uptake; asterisk=ApoA1. (C.C. Shoulders, HMG, 13,1,R149-R160, 2004).

# Classification of dyslipidemias

Dyslipidemias are classified on the different aspects:

1. Fredrickson phenotypic classification of hyperlipidemias (OMS, 1970, *table 6.1)*
2. Etiopathogenetic classification (NCEP ATP III, 2002) *tables 6.4,6.5*):

   Primary hyperlipidemias (genetic)

   Secundary hyperlipidemias
3. Therapeutic (ESA, Table 6.2)
4. Clinical classification of lipids values (NCEP ATP III, 2002, *table 6.3*).

The initial classification of lipoprotein disorders by Fredrickson was based on the measurement of total plasma cholesterol and triglycerides and analyzed lipoprotein patterns after separation by electrophoresis.

This classification recognized elevations of chylomicrons (type I), VLDL or pre-beta lipoproteins (type IV), "broad beta" disease (or type III hyperlipoproteinemia), beta lipoproteins (LDL) (type II), and elevations of both chylomicrons and VLDL (type V). In addition, the combined elevation of pre-beta lipoproteins (VLDL) and beta (LDL) lipoproteins was recognized as type IIb hyperlipoproteinemia. The World Health Organization, the European Atherosclerosis Society and, more recently, the National Cholesterol Education Program have classified lipoprotein disorders on the basis of arbitrary cut points (8).

*Table 6.1*

## Fredrickson classification of Hyperlipidemias

| Hyperlipo-proteinemia | Synonyms | Defect | Increased lipo-protein | Treatment | Serum appea-rance |
|---|---|---|---|---|---|
| **Type I (rare)** | Buerger-Gruetz syndrome, or Familial hyper-chylo-micro-nemia | Decreased lipoprotein lipase or altered ApoC2 | Chylo-microns | Diet control | Creamy top layer |
| **Type IIa** | Familial hyper-cholesterolemia | LDL receptor deficiency | LDL | Bile acid seques-trants, statins, niacin | Clear |
| **Type IIb** | Familial combined hyperlipidemia | Decreased LDL receptor and increased ApoB | LDL, VLDL | Statins, niacin, fibrate | Clear |
| **Type III (rare)** | Familial dysbeta-lipoproteinemia | Defect in Apo E synthesis | LDL | Fibrates, statins | Turbid |
| **Type IV** | Familial hyper-lipidemia | Increased VLDL production and decreased elimination | VLDL | Fibrates, niacin, statins | Turbid |
| **Type V (rare)** | Endogenous hyper-triglyceridemia | Increased VLDL production and decreased lipoprotein lipase | VLDL and Chylo-microns | Niacin, fibrate | Creamy top layer, turbid bottom |

*Table 6.2.*

**Clinical classification of lipids values** (NCEP ATP III, 2002), [9]

| Lipidic values [mmol/l (mg/dl)] | Value |
|---|---|
| **Total colesterol** | |
| < 5,0 (< 190) | Optimal |
| < 4,5 (< 175), optional < 4,0 (< 155) | Optimal normal |
| < 5,2 (< 200) | Near optimal/ above optimal |
| 5,2–6,2 (200-239) | Borderline high |
| ≥ 6,2 (≥ 240) | High |
| ≥ 8 (≥ 320) | Very high |
| **LDL-colesterol** | |
| < 3,0 (< 115) | Optimal |
| < 2,5 (< 100), optional < 2,0 (< 80) | Optimal |
| < 3,4 (< 130) | Near optimal/above optimal |
| 3,4-4,1 (130-159) | Borderline high |
| 4,1-4,9 (160-189) | High |
| ≥ 4,9 (≥ 190) | Very high |
| ≥ 6 (≥ 240) | Severe high |
| **Trigliceride** | |
| < 1,7 (< 150) | Normal |
| 1,7-2,2 (150-199) | Borderline high |
| 2,3-5,6 (200-499) | High |
| ≥ 5,6 (≥ 500) | Very high |
| **HDL-colesterol** | |
| < 1,0 (< 40), male | Low |
| < 1,3 (< 50), female | Low |
| ≥ 1,6 (≥ 60) | High |

*Table 6.3.*

**European Atherosclerosis Society classification of dyslipidemia** (10)

| | |
|---|---|
| A | Hypercholesterolemia |
| B | Mixt hyperlipidemia |
| C | Hypertrigliceridemia |

A practical classification characterizes the lipoprotein disorder by the absolute plasma levels of lipids (cholesterol and triglycerides) and lipoprotein cholesterol levels (LDL and HDL cholesterol) and considers clinical manifestations of hyperlipoproteinemia in the context of biochemical characterization.

Elevation of total cholesterol (TC) and low-density lipoprotein-cholesterol has received most attention, particularly because it can be modified by lifestyle changes and drug therapies. The evidence showing that reducing TC and LDL-C can prevent CAD is strong and compelling, based on results from multiple randomized controlled trials (RCTs). Total cholesterol and LDL-C levels continue therefore to constitute the primary targets of therapy. Besides an elevation of TC and LDL-C levels, several other types of dyslipidaemias appear to predispose to premature CAD. A particular pattern, termed the atherogenic lipid triad, is more common than others, and consists of the co-existence of increased very low density lipoprotein remnants manifested as mildly elevated triglycerides, increased small dense low-density lipoprotein particles, and reduced high density lipoprotein-cholesterol levels. However, clinical trial evidence is limited on the effectiveness and safety of intervening in this pattern to reduce CV risk; therefore, this pattern or its components must be regarded as optional targets of CAD prevention **(11)**. The measurement of apolipoproteins AI and B may add little substantial information to that provided by the conventional lipid profile. Taken as a single measurement, the apo B level provides information on the number of potentially atherogenic particles and can be used as a goal of lipid-lowering therapy. Dyslipidaemias may also have a different meaning in certain subgroups of patients which may relate to genetic predisposition and/or co-morbidities. This requires particular attention complementary to the management of the total CV risk **(12)**.

# Genetic Lipoprotein Disorders

Classification of genetic lipoprotein disorders usually requires a biochemical phenotype in addition to a clinical phenotype. With the exception of familial hypercholesterolemia, monogenic disorders tend to be infrequent or rare. Disorders considered heritable on careful family study may be difficult to characterize unambiguously because of age, gender, penetrance, and gene-gene and environmental interactions. Most common lipoprotein disorders encountered clinically result from the interaction of increasing age, lack of physical exercise, weight gain, and a suboptimal diet with individual genetic make-up.

Genetic lipoprotein disorders can affect LDL, lipoprotein(a), remnant lipoproteins, triglyceride-rich lipoproteins such as chylomicrons and VLDL or HDL (*table 6.4,6.5*). Within each of these, genetic disorders can cause an excess or a deficiency of a specific class of lipoprotein.

Acquired hyperlipidemias (also called secondary dyslipoproteinemias) may mimic primary forms of hyperlipidemia and can have similar consequences. They may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome.

*Table 6.4*

**Genetic Lipoprotein Disorders**

| Disorders | Gene |
|---|---|
| **LDL Particles** | |
| Familial hypercholesterolemia | LDL-R |

| | |
|---|---|
| Familial defective apo B-100 | Apo B |
| Autosomal dominant hypercholesterolemia | PCSK9 |
| Autosomal recessive hypercholesterolemia | ARH |
| Abetalipoproteinemia | MTP |
| Hypobetalipoproteinemia | Apo B |
| Familial sitosterolemia | ABCG5/ABCG8 |
| Familial lipoprotein(a) hyperlipoproteinemia | Apo (a) |
| **Remnant Lipoproteins** | |
| Dysbetalipoproteinemia type III | Apo E |
| Hepatic lipase deficiency | HL |
| **Triglyceride-Rich Lipoproteins** | |
| Lipoprotein lipase deficiency | LPL |
| Apo CII deficiency | Apo CII |
| Apo AV | Apo AV |
| Familial hypertriglyceridemia | Polygenic |
| Familial combined hyperlipidemia | Polygenic |
| **HDL Particles** | |
| Apo AI deficiency | Apo AI |
| Tangier disease/ Familial HDL deficiency | ABCA1 |
| Familial LCAT deficiency syndromes | LCAT |
| LCAT | CETP |
| Niemann-Pick disease types A and B | SMPD1 |
| Niemann-Pick disease type C | NPC1 |

*Table* 6. 5

**Unclassified familial forms of dyslipidemia**

| |
|---|
| • Hypo-alpha lipoproteinemia |
| • Hypo-beta lipoproteinemia (prevalence 0.01-0.1%) |

## Acquired (secondary) forms of dyslipidemia

*Table 6.6*

**Causes of acquired hyperlipidemia**

| |
|---|
| Diabetes mellitus |
| Use of drugs (diuretics, beta blockers, estrogens) |
| Hypothyroidism |
| Renal failure |
| Nephrotic syndrome |
| Alcohol |
| Some rare endocrine disorders and metabolic disorders |

# Type I hyperlipidemia

## Familial chylomicronemia

Type I hyperlipidemia, also known as familial chylomicronemia, is a rare genetic disorder inherited as an autosomal recessive trait. It occurs because of lipoprotein lipase (LPL) or apo C-II deficiency and almost always presents in infancy and early childhood. The classic triad is eruptive xanthomas, lipemia retinalis, and pancreatitis. The patients have fasting HTG and the degree of chylomicronemia depends upon the amount of fat intake (13). This rare disorder of severe hypertriglyceridemia associates with elevations in fasting plasma triglycerides greater than 11.3 mmol/liter (>1 000 mg/dl). Interestingly, severe hypertriglyceridemia can also be associated with xerostomia, xerophthalmia, and behavioral abnormalities. The hypertriglyceridemia results from a markedly reduced or absent LPL activity or, more rarely, the absence of its activator, apo CII. These defects lead to a lack of hydrolysis of chylomicrons and VLDL and their accumulation in plasma, especially after meals. Extreme elevations of plasma triglycerides (>113 mmol/liter; >10 000 mg/dl) can result. Plasma from a patient with high triglycerides is milky white, and a clear band of chylomicrons can be seen on top of the plasma after it stands overnight in a refrigerator. Populations with a founder effect can have a high prevalence of LPL mutations. At least 60 LPL mutations can cause LPL deficiency. LPL188, LPLasn291ser, and LPL207 are frequently associated with hyperchylomicronemia. Heterozygotes for the disorder tend to have an increase in fasting plasma triglycerides and smaller, denser LDL particles. Many patients with complete LPL deficiency present in childhood fail to thrive and have recurrent bouts of pancreatitis. To subestimate the importance of LPL, the LPL-deficient mouse leads to a perinatal lethal phenotype (14).

# Type II Hyperlipidemia

## Familial hypercholesterolemia

Only a few patients have known monogenic defects, the most common of which is familial hypercholesterolemia (FH), occurring in 1 in 500 patients. FH is an autosomal co-dominant disorder caused by mutations that directly affect the rate at which LDL-C is cleared from the blood. Carl Miller, a physician at Oslo County Hospital, Norway, first described the disorder about 70 years ago. He noted that the triad of elevated cholesterol, tendon xanthomas, and early heart disease segregated together in families, providing strong evidence for an association between blood lipids and atherosclerosis. Joseph Goldstein and Michael Brown further examined the disease in the early 1970s, and over the last 3 decades, their landmark studies have fostered many insights in the control of cholesterol levels and the etiology and treatment of CAD. Along the way, they discovered receptor-mediated endocytosis and novel mechanisms for transcriptional regulation by lipids. Their studies revealed that FH is the result of mutations that destroy the ability of the LDL receptor to mediate the binding, internalization, or degradation of LDL (*figure* 6.2).

*F*Individuals carrying 2 mutant copies have extremely high levels of cholesterol (>600 mg/dL), whereas heterozygotes with 1 mutant copy have levels of ≈400 mg/dL. Caused by mutations in LDL receptor (LDLR), FH causes elevated serum LDL-C concentrations. In FH homozygotes, LDL-C levels are generally unresponsive to dietary and pharmacological intervention; patients often develop clinically significant CAD before 30 years of age. FH heterozygotes have elevated LDL-C, albeit lower than homozygotes, and generally are more influenced by environment and responsive to drug treatment.

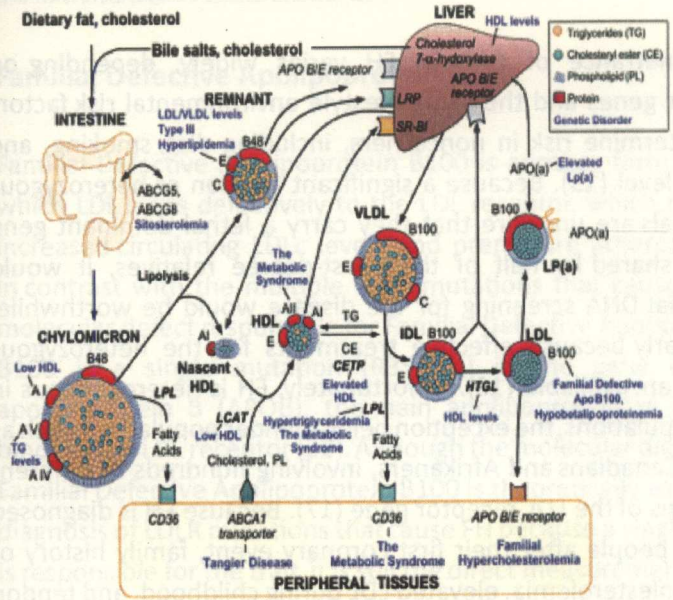



*figure 6.2.* **Candidate genes in lipoprotein metabolism.** Lipids are transported through the circulation as complexes with various apolipoproteins that package lipids and act as cofactors for enzymes or ligands for uptake by cellular receptors. Dietary lipids are absorbed in intestine, packaged into chylomicrons, and secreted into lymph. On entering circulation, triglycerides are hydrolyzed through action of LPL and the resulting remnants taken up by interaction of apoE with LDL receptor (apoB, E receptor) or LRP. During lipolysis, surface phospholipids and chylomicron proteins slough off to give rise to HDL precursors. Liver cells package triglycerides and cholesterol esters into VLDL particles. LPL acts on them to produce intermediate-density lipoproteins (IDL), which can be taken up by B, E-receptor, or further lipolyzed, partly through hepatic lipase (HL) action, to produce LDL. LDL retains a single major protein, apoB100, and is removed from circulation by the apoB, E receptor. Because of slow kinetics of LDL uptake, LDL particles constitute major cholesterol-carrying particle in most individuals. LDL can complex with apo(a) protein to form Lp(a) particles, which appear particularly atherogenic. HDLs are formed largely in the circulation from apoAI and AII secreted by liver and intestine and from chylomicrons' surface and VLDL during their lipolysis. HDL precursors take up cholesterol from various tissues through interaction with ABCA1 transporter, and cholesterol is esterified by lecithin:cholesterol acyl transferase (LCAT) action. Lipids can be transferred between lipoproteins through the actions of cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP).

The penetrance of CAD in FH varies widely, depending on modifier genes and the same lifestyle environmental risk factors that determine risk in noncarriers, including diet, smoking, and activity level (15). Because a significant fraction of heterozygous individuals are unaware that they carry a lethal dominant gene that is shared by half of their first-degree relatives, it would seem that DNA screening for the disease would be worthwhile, particularly because effective treatments for the heterozygous disease are available (16). Unfortunately, FH is heterogeneous in most populations, the exception being founder populations such as French Canadians and Afrikaners, involving hundreds of different mutations of the LDL receptor gene (17). Because FH is diagnosed in most people after their first coronary event, family history of hypercholesterolemia, elevated LDL during childhood, and tendon xanthomas over the extensor tendons (metacarpophalangeal joints and patellar, triceps and Achilles tendons) and xanthelasmas are important clues to FH and should trigger screening of family members. In the most severe cases, people with FH may actually have heart attacks in childhood (18).

To date, there are more than 1 000 identified mutations of the LDLR gene. In some people with this disorder, the receptor is simply not produced. In others, it binds LDL poorly or not at all (19).

The most recently-identified gene for FH codes for protein convertase subtilisin/kexin type 9 (PCSK9), which is an enzyme involved in degrading the LDL-R protein in the lysosome of the cell and preventing it recycling (20). One common gain-of-function mutation (pD374Y) in the PCSK9 gene has been identified this occuring in the UK in 2% of FH patients and carriers of this mutation tend to have very severe CAD (21).

## Familial Defective Apolipoprotein B100

Familial Defective Apolipoprotein B100 is another form of FH in which LDL binds defectively to the LDL receptor, which results in increased circulating LDLc levels and premature atherosclerosis. In contrast with the multiple LDLR mutations that cause FH, the molecular defect responsible for Familial Defective Apolipoprotein B100 is a single mutation (R3500Q) in the gene encoding apolipoprotein B (APOB), the main apolipoprotein in LDL that binds to the LDL receptor (22). Although the molecular diagnosis of Familial Defective Apolipoprotein B100 is theoretically easier than diagnosis of LDLR mutations that cause FH because a single variant is responsible for the trait, it is still the direct measurement of LDLc that appears the most appropriate to evaluate the risk of CHD and monitor the drug response in Familial Defective Apolipoprotein B100 patients.

## Type III hyperlipoproteinemia

Type III hyperlipoproteinemia, also referred to as dysbetalipoproteinemia or broad beta disease, is a rare genetic lipoprotein disorder characterized by an accumulation in plasma of remnant lipoprotein particles. On lipoprotein agarose gel electrophoresis, a typical pattern of a broad band between the pre-beta (VLDL) and beta (LDL) lipoproteins is observed, hence the name broad beta disease. Patients with this disease clearly have increased cardiovascular risk. The clinical presentation consists of pathognomonic tuberous xanthomas and palmar striated xanthomas. The lipoprotein profile shows increased cholesterol

and triglyceride levels and reduced HDL cholesterol. Remnant lipoproteins (partly catabolized chylomicrons and VLDLs) store in plasma and accumulate cholesterol esters. The defect results from abnormal Apolipoprotein E (APOE), which does not bind to hepatic receptors that recognize APOE as a ligand. The ratio of VLDL cholesterol to triglycerides, normally less than 0.7 mmol/liter (<0.30 mg/dl), is elevated in patients with type III hyperlipoproteinemia because of cholesteryl ester enrichment of remnant particles. The diagnosis includes plasma ultracentrifugation for lipoprotein separation, lipoprotein electrophoresis, and APOE phenotyping or genotyping. Patients with type III hyperlipoproteinemia have the APOE 2/2 phenotype or genotype. Three common alleles for APOE exist: apo E2, E3, and E4. The apo E2 allele has markedly decreased binding to the apo B/E receptor.

In a normal population the prevalence of the apo E2/2 phenotype is approximately 0.7 to 1 percent. Type III hyperlipoproteinemia occurs in approximately 1 percent of subjects bearing the apo E2/2 phenotype. The reasons for the relative rarity of type III dyslipoproteinemia are not fully understood. Other rare mutations of the APOE gene can cause type III hyperlipoproteinemia (23). In general, type III dyslipoproteinemia responds well to dietary therapy; correction of other metabolic abnormalities (diabetes, obesity); and, in cases requiring drug therapy, fibric acid derivatives or statins are used. The importance of the APOE gene and protein is underscored by the widespread use of the APOE-deficient mouse, which develops experimental atherosclerosis.

## Familial hypertriglyceridemia

Familial hypertriglyceridemia is not associated with clinical signs such as corneal arcus, xanthoma, and xanthelasmas. Plasma triglycerides, VLDL cholesterol, and VLDL triglycerides are moderately to markedly elevated; LDL cholesterol level is usually low, as is HDL cholesterol. Total cholesterol is normal or elevated, depending on VLDL cholesterol levels. Fasting plasma concentrations of triglycerides are in the range of 2.3 to 5.7 mmol/liter (200 to 500 mg/dl). After a meal, plasma triglycerides may exceed 11.3 mmol/liter (1 000 mg/dl). The disorder is found in first-degree relatives, but phenotypic variability is related to gender, age, hormone use (especially estrogens), and diet. Alcohol intake potently stimulates hypertriglyceridemia in these subjects, as does caloric or carbohydrate intake. The relationship with coronary artery disease is not as strong as with familial combined hyperlipidemia and not seen in all studies. Depending on criteria used, the prevalence of familial hypertriglyceridemia ranges from 1 in 100 to 1 in 50. The disorder is highly heterogeneous and likely results from several genes, with a strong environmental influence. An unrelated disorder, familial glycerolemia, a chromosome X-linked genetic disorder, may mimic familial hypertriglyceridemia because most measurement techniques for triglycerides use the measurement of glycerol after enzymatic hydrolysis of triglycerides (24).

Hepatic overproduction of VLDL causes familial hypertriglyceridemia, the catabolism (uptake) of VLDL particles can be normal or reduced. Lipolysis by LPL appears not to be a limiting factor, although the triglyceride load, especially in the postprandial state, may limit processing of VLDL particles. The genetic basis of familial hypertriglyceridemia is unknown, and the whole-genome scan for linkage to LDL size and triglyceride levels in kindreds with familial hypertriglyceridemia has not yielded fruit this far (25).

## Hypobetalipoproteinemia and abetalipoproteinemia

Mutations within the APOB gene can lead to truncations of the mature apolipoprotein B 100 peptide. Many such mutations cause a syndrome characterized by reduced LDL and VLDL cholesterol, a condition referred to as hypobetalipoproteinemia this having little or no clinical manifestations and no known risk of cardiovascular disease. Apolipoprotein B truncated close to its amino terminus loses the ability to bind lipids, producing a syndrome similar to abetalipoproteinemia, a rare recessive lipoprotein disorder of infancy that causes mental retardation and growth abnormalities. Abetalipoproteinemia is caused by a mutation in gene coding for the microsomal triglyceride transfer protein (MTP) required for assembly of apolipoprotein B-containing lipoproteins in the liver and the intestine. The resulting lack of apolipoprotein B-containing lipoproteins in plasma causes a marked deficiency of fat-soluble vitamins (A, D, E, and K) that circulate in lipoproteins. In turn, this results in mental and developmental retardation in affected children.

## Sitosterolemia

A rare condition of increased intestinal absorption and decreased excretion of plant sterols (sitosterol and campesterol) can mimic severe familial hypercholesterolemia, with extensive xanthoma formation. Premature atherosclerosis, often apparent clinically well before adulthood, occurs frequently in patients with sitosterolemia. Diagnosis requires specialized analysis of plasma sterols demonstrating an elevation in sitosterol, campesterol, cholestanol, sitostanol, and campestanol. Interestingly, plasma cholesterol is normal or reduced, and triglycerides are normal. Positional cloning techniques have localized the defect to chromosome 2p21. Mutations in the adenosine triphosphate

binding cassette G5 and G8 genes (ABCG5 and ABCG8) have been found in patients with sitosterolemia. The gene products of ABCG5 and ABCG8 are half ABC transporters and are thought to form a heterodimer characteristic of the full ATP-binding cassette transporters (ABC transporters). The complex is located in the villous border of intestinal cells and actively pumps plant sterols back into the intestinal lumen. A defect in either of the genes renders the complex inactive, and absorption of plant sterols (rather than their elimination) ensues. ABCG5 and ABCG8 mutations leading to sitosterolemia are rare (26).

## Lipoprotein(a)

Numerous studies have linked elevated plasma levels of lipoprotein (a), an LDL-like moiety that circulates in the blood attached to apolipoprotein (a), with the development of coronary artery disease (27). The apo(a) moiety consists of a protein with a high degree of homology with plasminogen. The apo(a) gene appears to have arisen from the plasminogen gene by nonhomologous recombination. The apo(a) gene has multiple repeats of one of the kringle motifs (kringle IV), varying in number from 12 to more than 40 in each individual. Plasma lipoprotein(a) levels depend almost entirely on genetics and correlate inversely with the number of kringle repeats and, therefore, with the molecular weight of apo(a). Few environmental factors or medications modulate plasma lipoprotein(a) levels. The pathogenesis of lipoprotein(a) may result from an antifibrinolytic potential and ability to bind oxidized lipoproteins. The results of prospective studies have been discordant and have not proven the relationship between elevated plasma levels of lipoprotein (a) and coronary artery disease inconclusively (28). Niacin is known to reduce plasma levels of lipoprotein (a), although whether this truly is a modifiable risk factor remains unclear.

## Familial combined hyperlipidemia

Familial combined hyperlipidemia (FCHL) is a genetically complex lipid disorder that is diagnosed in families by combinations of increased cholesterol, triglycerides and APOB levels in patients and their first-degree relatives. Early CAD is also common in familial combined hyperlipidemia (FCH), a familial disorder characterized by elevated triglycerides and LDL that occurs in 1-2% of the population. A single gene involved in this disease has not been found, and the disease is likely polygenic. Typically, patients are identified after their first cardiac event but do not have tendon xanthomas. In the recent study were identified at 1p21-31 and 17p11-q21 for APOB, 12p13 for total serum cholesterol, and 4p15-16 for serum triglycerides by nonparametric linkage analyses of a full genome scan of 150 sibpairs in 22 familial combined hyperlipidemia sibships. Multiple quantitative trait locus (QTL) were observed at 4q34-35, 16p12-13, and 17p11-q21 (29).

## Reduced plasma levels of high-density lipoproteins

Reduced plasma levels of HDL cholesterol consistently correlate with the development or presence of CAD. Most cases of reduced HDL cholesterol result from elevated plasma triglycerides or apo B levels and often keep company with other features of the metabolic syndrome. Primary forms of reduced HDL cholesterol have been identified in cases of premature CAD and helped shed light on the complex metabolism of HDL particles. Genetic disorders of HDL can result from decreased production or abnormal maturation and increased catabolism. Genetic lipoprotein disorders leading to moderate to severe elevations in plasma triglycerides cause a reduction in HDL cholesterol levels. Familial hyperchylomicronemia, familial hypertriglyceridemia, and

familial combined hyperlipoproteinemia are all associated with reduced HDL cholesterol levels. In complex disorders of lipoprotein metabolism such as familial combined hyperlipidemia, the metabolic syndrome, and common forms of hypertriglyceridemia, several factors most likely correlate to low HDL cholesterol level. Plasma triglycerides and HDL cholesterol levels vary inversely. For several reasons, patients with elevated apolipoprotein B levels also have reduced HDL cholesterol levels. First, decreased lipolysis of triglyceride-rich lipoproteins (each VLDL contains one molecule of apo B) decrease the substrate (phospholipids) available for HDL maturation. Second, triglyceride enrichment of HDL increases their catabolic rate and hence reduces their plasma concentration. Third, exchange of lipids between HDL and triglyceride-rich lipoprotein is reduced, leading to a more rapid disappearance of HDL from plasma (30). The inverse relationship between HDL cholesterol levels and plasma triglycerides reflects the interdependency of the metabolism of triglyceride-rich lipoproteins and HDL particles.

## Apolipoprotein A1 gene defects

Apolipoprotein A1 (apo AI) is a protein that is packaged along with cholesterol in high density lipoprotein (HDL, the "good cholesterol"). Certain mutations in the APOA1 gene result in low apo A1 levels, low HDL levels, early heart attacks, and strokes. In general, overall HDL levels are a good indication of apo A1 levels, apo A1 is not usually measured separately (31). Scientists are especially interested in one particular mutation in APOA1 found in some residents of Milan, Italy. The "Milano" mutation results in very low levels of HDL; however, this mutant HDL is exceptionally efficient in removing plaque from arteries. Thus, although these people have very low levels of HDL, they also have a low incidence of CAD.

Primary defects affecting production of HDL particles consist predominantly of APOA1-CIII-AIV gene defects. More than 46 mutations affect the structure of APOA1 (32) leading to a marked reduction in HDL cholesterol levels. Not all of these defects are associated with premature cardiovascular disease. Clinical presentations can vary from extensive atypical xanthomatosis and corneal infiltration of lipids to no manifestations at all. Treatment of these APOA1 gene defects generally fails to raise HDL cholesterol levels. Other mutations of APOA1 lead to an increased catabolic rate of apo AI and may not be associated with cardiovascular disease. One such mutation, $APOA1_{Milano}$ (apo $AI_{Arg173Cys}$), appears to confer longevity despite low HDL levels.

## LCAT, CETP deficiency

Genetic defects in the HDL-processing enzymes give rise to interesting phenotypes. Deficiencies of lecithin-cholesterol acyltransferase (LCAT), the enzyme that catalyzes the formation of cholesteryl esters in plasma, cause corneal infiltration of neutral lipids and hematological abnormalities due to abnormal constitution of red blood cell membranes. LCAT deficiency can lead to an entity called "fish eye disease" because of the characteristic pattern of corneal infiltration observed in affected individuals (33).

Patients without cholesteryl ester transfer protein (CETP) have very elevated HDL cholesterol levels, enriched in cholesteryl esters. Because CETP assists the transfer of HDL cholesteryl esters into triglyceride-rich lipoproteins, a deficiency of this enzyme causes accumulation of cholesteryl esters within HDL particles. Cholesteryl ester transfer protein deficiency is not associated with premature CAD but may not afford protection against CAD (34).

## Tangier disease and familial HDL deficiency

A rare form of genetic HDL deficiency is Tangier disease, which has been diagnosed in ≈60 patients worldwide and is associated with an almost complete absence of HDL-C (35). This rare disorder of HDL deficiency was identified in a proband from the Chesapeake Bay island of Tangier in the United States. The proband, whose sister was also affected, had markedly enlarged yellow tonsils and nearly absent HDL cholesterol levels. The cellular defect in Tangier disease consists of a reduced cellular cholesterol efflux in skin fibroblasts and macrophages from affected subjects. Tangier disease is a very rare recessive deficit of high-density lipoprotein–cholesterol (HDL-C) metabolism caused by mutations in the ATP-binding cassette transporter 1 (ABCA1) gene. ABCA1 encodes a protein that regulates the cellular efflux of cholesterol and phospholipids to an apolipoprotein transporter. Several mutations responsible for Tangier disease have been identified, all of which result in a complete or partial loss of function that leads to an accumulation of cellular cholesterol, low plasma HDL-C levels, and increased risk of CAD. Apart from these very rare mutations, numerous coding variants of "intermediate" to low frequency in the ABCA1 gene may contribute to a significant fraction of the low HDL-C levels in the population. In the Dallas Heart Study, 20 of 128 individuals in the bottom 5% of the HDL-C distribution were carriers of nonsynonymous variants in the ABCA1 gene versus only 2 of 128 individuals in the top 5% of the HDL distribution. This finding was replicated in an independent study, and biochemical studies indicated that most of the variants associated with low HDL-C were functionally important (36). Common polymorphisms in the ABCA1 gene, including several nonsynonymous changes, have been identified by systematically resequencing the gene in a limited number of individuals, and some of these polymorphisms have been shown to be associated with plasma HDL-C or apoA1 in the population at large (37).

The genetic defect in Tangier disease and in familial HDL deficiency results from mutations at the ATP binding cassette A1 gene (ABCA1) that encodes the ABCA1 transporter. At least 100 mutations have been reported within ABCA1, causing Tangier disease (homozygous or compound heterozygous mutations) or familial HDL deficiency (heterozygous mutations). Although subjects with Tangier disease and familial HDL deficiency are at increased risk for CAD, their low levels of LDL cholesterol appear to have a protective effect. ABCA1 appears to shuttle from the late endosomal compartment to the plasma membrane and act as a membrane-bound transporter of phospholipids and cholesterol onto acceptor proteins such as apo AI and apo E. Hydroxysterols regulate ABCA1 via the LXR/RXR nuclear receptor pathway. ABCA1 undergoes phosphorylation via protein kinase A and acts as a receptor for apo AI.

## Niemann-Pick type C disease

Niemann-Pick type C disease is a disorder of lysosomal cholesterol transport. In patients with Niemann-Pick type C disease, mental retardation and neurological manifestations occur frequently. The cellular phenotype involves markedly decreased cholesterol esterification and cellular cholesterol transport defect to the Golgi apparatus. Unlike Tangier disease/familial HDL deficiency, the cellular defect in Niemann-Pick type C disease appears proximal to the transport of cholesterol to the plasma membrane. The gene for Niemann-Pick type C disease (NPC1) has been mapped to 18q21 and the gene codes for a 1278-amino acid protein, the role of which appears to be involved in cholesterol shuttling between the late endosomal pathway and the plasma membrane. The NPC1 gene product shares homology with the morphogen receptor patched and the SREBP cleavage activating protein (SCAP). Niemann-Pick type C cells lack NPC1 protein, and cholesterol sequestration within the late endosome compartment prevents up-regulation of ABCA1. These patients have impaired cellular cholesterol efflux

and HDL assembly. Niemann-Pick type I disease (subtypes A and B), caused by mutations at the sphingomyelin phosphodiesterase-1 (SMPD-1) gene, is associated with a low HDL cholesterol level. The SMPD-1 gene codes for a lysosomal (acidic) and secretory sphingomyelinase. The low HDL cholesterol level in Niemann-Pick A and B patients appears to result from a decrease in LCAT reaction because of abnormal HDL constituents (38).

## Secondary causes of hyperlipidemia and the metabolic syndrome

Several clinical disorders lead to alterations in lipoprotein status.

### Hormonal causes

Hypothyroidism, a not-infrequent cause of secondary lipoprotein disorders, often manifests with elevated LDL cholesterol, triglycerides, or both. An elevated level of thyroid-stimulating hormone is key to the diagnosis, and the lipoprotein abnormalities often revert to normal after correction of thyroid status. Rarely, hypothyroidism may uncover a genetic lipoprotein disorder such as type III hyperlipidemia. Estrogens can elevate plasma triglycerides and HDL cholesterol levels, probably because of increases in both hepatic VLDL and apo AI production. In postmenopausal women, estrogens may reduce LDL cholesterol by up to 15 percent. The use of estrogens for the treatment of lipoprotein disorders is no longer recommended because of the slight increase in cardiovascular risk with prolonged use of estrogens in the postmenopausal period (39). Rarely, pregnancy causes severe increases in plasma triglycerides, on a background of lipoprotein lipase deficiency. Such cases present a serious threat to mother and child and require referral to specialized centers. Male sex hormones and anabolic steroids can increase hepatic lipase activity and have been used in the treatment of hypertriglyceridemia in men. Growth hormone can reduce LDL cholesterol and augment HDL cholesterol but is not recommended in the treatment of lipoprotein disorders.

## Metabolic causes

The increased visceral fat (abdominal obesity), elevated blood pressure, and impaired glucose tolerance with increased plasma triglycerides and reduced HDL cholesterol level are the major components of the metabolic syndrome (40). The lack of internationally recognized uniform criteria for the metabolic syndrome among other issues casts doubt on the usefulness of this syndrome as a diagnostic entity (41). Overt diabetes, especially type II diabetes, frequently elevates plasma triglycerides and reduces HDL cholesterol. These abnormalities have prognostic implications in patients with type II diabetes. Poor control of diabetes, obesity, and moderate to severe hyperglycemia can yield severe hypertriglyceridemia with chylomicronemia and increased VLDL cholesterol levels. Subjects with type 1 diabetes can also have severe poorly controlled hypertriglyceridemia. Familial lipodystrophy (complete or partial) may be associated with increased VLDL secretion. Dunnigan lipodystrophy, a genetic disorder with features of the metabolic syndrome, is caused by mutations within the Lamin A/C gene and is associated with limb-girdle fat atrophy. Excess plasma triglycerides often accompany glycogen storage disorders.

## Renal causes

The clinical trials PLANET I,II related that in subjects with protein-losing nephropathies, a marked increase in secretion of hepatic lipoproteins could raise LDL cholesterol levels. Investigators in AURORA study showed the patients with chronic renal failure to have a pattern of hypertriglyceridemia with reduced HDL cholesterol. Patients with end-stage renal disease, including those on hemodialysis or chronic ambulatory peritoneal dialysis, had a poor prognosis and accelerated atherosclerosis and had to undergo aggressive treatment of lipoprotein disorders. This approach, however, has been recently challenged when a recent

trial of statins in diabetic patients on dialysis showed no reduction in cardiovascular endpoints. After organ transplantation, the immunosuppressive regimen (glucocorticoids and cyclosporine) typically elevates triglycerides and reduces HDL cholesterol levels. Because transplant patients generally have an increase in cardiovascular risk, a secondary hyperlipidemia may warrant treatment. Patients receiving the combination of statin plus cyclosporine merit careful dose titrations and monitoring for myopathy.

### Liver disease

Obstructive liver disease, especially primary biliary cirrhosis, may lead to the formation of an abnormal lipoprotein termed lipoprotein-x. This type of lipoprotein is found in cases of LCAT deficiency and consists of an LDL-like particle but with a marked reduction in cholesteryl esters. Extensive xanthoma formation on the face and palmar areas can result from accumulation of lipoprotein-x.

### Lifestyle

Factors contributing to obesity such as an imbalance between caloric intake and energy expenditure, lack of physical activity, and a diet rich in saturated fats and refined sugars influence in large part the lipid and lipoprotein lipid levels within a population.

### Medication

Several medications can alter lipoproteins. Thiazide diuretics can increase plasma triglyceride levels. Beta blockers, especially non-beta-1 selective, increase triglycerides and lower HDL cholesterol levels. Retinoic acid and estrogens can increase triglyceride levels, sometimes dramatically. Corticosteroids and immunosuppressive agents can increase plasma triglyceride levels and lower HDL

cholesterol levels. Estrogens can increase plasma HDL cholesterol significantly and often increase triglyceride concentrations.
In clinical practice, many dyslipoproteinemias, other than the genetic forms mentioned earlier, share an important environmental cause. Lifestyle changes (diet, exercise, reduction of abdominal obesity) should form the foundation for the treatment of most dyslipidemias. The effects of marked alterations in lifestyle, reduction in dietary fats, especially saturated fats, and exercise can improve cardiovascular prognosis.

## Candidate gene association studies for hypercholesterolemia and other dyslipidemias

The recent approach has dramatically expanded both the number of genetic polymorphisms typed within a gene and the number of genes typed. For example, Knoblauch and colleagues (42) recently used a multicandidate gene approach to explain variance in serum LDL-C and HDL-C. By genotyping 93 SNPs in 13 genes known to be important in lipid metabolism and constructing 230 SNP haplotypes (a linear array of genetic variants within a chromosomal region inherited as a block), genetic variance on LDL-C explained 26% of the total variance; the genetic variance on HDL-C explained 38%. Although the results of this study are limited to a "normal" population (subjects in families with known FH and those with known heart disease were excluded), the ability to explain a large part of the genetic variance of LDL-C and HDL-C on the basis of genetic variants in a small set of genes points to the possibility of using such genetic information to predict CV risk.

## Apolipoprotein E polymorphism

Many candidate gene association studies have been conducted for hypercholesterolemia-related phenotypes, and the data for associations of dozens of gene variants with lipid-related traits have been reviewed (43). Of the many studies conducted, the

most consistent evidence has been found for the Apolipoprotein E (APOE) gene. The common APOE allele is called E3 and there are two variants, E4 and E2 (allele frequency in Europeans roughly 0.15 and 0.07, respectively). The sequence changes in the gene alter two charged amino acids, which affect plasma clearance of the protein and the cholesterol-rich lipoproteins carrying them. The consequence of this is a strong and consistent impact on plasma lipid levels (E2 lowering and E4 raising), which translates into a modest E2 lower and E4 higher impact on CV risk. In a recent meta-analysis, E4 carriers, who represent >20% of the population, were shown to have a 40% higher risk of CAD compared with E3/E3 homozygotes, whereas the relationship between E2 and risk was less obvious (44).

Lehtinen S.et al. (45) in their study on patients with clinically proven coronary artery disease, observed increasing plasma total and LDL cholesterol according to the APOE phenotype in the order APOE3/2<E3/3<E3/4 and E4/4. The study suggested that the E4 allele affects plasma cholesterol and LDL cholesterol levels and the potential of developing severe coronary heart disease.

The Framingham Offspring Study and the Multiple Risk Factor Intervention Trial (MRFIT) study (46) observed a strong association of the E4 allele and coronary heart disease. The CARDIA study on African Americans and Whites in the United States suggested that apo E phenotype could be a risk factor for cardiovascular disease in both the populations, and association of CAD patients with E4 allele occurred more frequently as compared to the controls. Certain studies have linked the E4 allele with a greater risk for coronary artery disease and myocardial infarction. In a case-control study (47), the frequency of homozygotes for the E4 allele in men aged less than 40 years with clinical coronary angioplasty was considerably higher than in healthy subjects. It was observed that men with the E4 allele had significantly lower coronary event-free survival rates than the carriers of other APOE alleles. In a five year longitudinal study involving elderly Finnish men (48), the E4 allele frequency was significantly higher in men with fatal

myocardial infarction than the survivors. A meta-analysis of nine case-control studies (49) showed that the E4 genotype was more frequent among patients with ischaemic cerebrovascular disease as compared to non-ischaemic subjects. In a case-control study conducted by Kumar P. et al. (2003), in north Indian patients with premature myocardial infarction (50), a significant association of APOE gene polymorphism with coronary heart disease in Asian Indians was observed. In a study on an unrelated heterogeneous group of Indian subjects (51), a higher frequency of APOE3 allele was observed similar to the reports on the Mala community of southern India. Within the subjects with angiographically verified CAD, the total cholesterol levels were significantly elevated in APO E4 carriers by 16 per cent as compared to APOE3/3 carriers (52). Lenzen HJ. et al (53) reported that 60 per cent of patients having the E4/E3 genotype suffered myocardial infarction before 60 yr of age while this pattern was reversed in patients with the E3/E2 genotype. Kumar P. et al. (2003) study conducted on CAD patients revealed APOE3 as the most common allele in CAD patients and in the normal subjects with the E4 allele frequency being comparable between the two groups (54), similar to the Caucasian population (55) which reported a significant decrease in the frequency of APOE4 between patients and controls, indicating a negative correlation of APOE4 with the risk of myocardial infarction. Gerdes LU. et al. (56) examined the relation between APOE genotype and a major coronary event or death in 966 Danish and Finnish survivors of myocardial infarction enrolled in the Scandinavian Simvastatin survival study. This extensive follow up study concluded that myocardial infarction survivors carrying the E4 allele had an 80 per cent accelerated risk of death compared to other patients. Further, it indicated that the APOE genotype had no predictive value on a major nonfatal coronary event.

The MONICA (Monitoring of Trends and Determinants in Cardiovascular disease) project, a multi-national study sponsored by the World Health Organization, monitored trends in cardiovascular mortality and morbidity and assessed the

relation of these trends to changes in risk factor levels and/or medical care. The project suggested that increase in the relative frequency of E4 allele increases the CAD death rate by 24.5 per 100 000 (57). Studies from Finland, Scotland and Northern Ireland have shown that populations with higher cholesterol levels and higher CAD mortality rates also have a higher frequency of E4 allele. The association between APOE2/2 genotype and type III hyperlipoproteinaemia has been evidenced since a long time (53). Overt type III hyperlipoproteinaemia occurs at a frequency of 1-5 per 5 000 whereas homozygosity for E2/E2 occurs with a frequency of 0.5-1.0 per 100 in Caucasian populations. In general, the homozygous E4/E4 genotype is used to determine the risk of coronary heart disease. Schiele F. et al. (2000) reported from the Apolipoprotein Europe Project that the total cholesterol lowering effect of E2 allele was 2-3 times higher than the cholesterol raising potential of E4 allele. The E2 allele lowers cholesterol levels by approximately 14 mg/dl and E4 raises it by approximately 8 mg/dl. This effect is evident in most populations, despite highly variable mean concentration of cholesterol. The gene products of APOE seem to function in a relatively uniform physiologic way in all populations despite differences in genetic background, diet and exercise patterns (58). Mooijaart CP. et al. (59) analyzed the relationship between plasma levels of apolipoprotein E (apo E), cardiovascular risk factors and mortality in a cohort of 561 inhabitants in a community of Leiden, and reported that elderly individuals with high plasma levels of apo E were at a higher risk of cardiovascular mortality, irrespective of their APOE genotype, lipid levels and other cardiovascular risk factors. The apo E has proinflammatory properties and thus contributes to cardiovascular disease. The concomitant inflammatory response of apo E on binding to lipid antigens adequately eliminates the lipid antigen from the circulation. Thus high plasma levels of apo E in combination with increased lipid-antigen presentation lead to chronic inflammation and these may contribute to arteriosclerosis. They also found that, as in other studies involving young populations, APOE genotypes

associate with plasma levels of apo E. It is also reported that plasma apo E levels are highly dependent on heritable factors (60). Over the past 25 years, APOE isoforms have consistently been shown to be associated with variation in plasma LDL cholesterol and apo B levels, with E4 having a greater influence that E3 and in turn, E3 having a greater influence than E2 across a 10-15 per cent range. This effect is clinically important because high levels of plasma LDL cholesterol is an indispensable risk factor for cardiovascular disease, especially CAD. The genetically-determined 5-7 per cent difference in LDL cholesterol level from the reference (wild type) E3/E3 genotype to carriers of either the E 4 (higher LDL-cholesterol levels) or E2 alleles (lower LDL cholesterol levels) becomes even more important in light of the fact that only approximately 50 per cent of individuals in most populations have the E3/E 3 genotype, with the remainder carrying at least one E4 or E2 allele (60).

Song Y. et al. (61) conducted a comprehensive meta-analysis of 48 studies on APOE genotypes and risk for coronary heart disease and found that carriers of the APOE4 allele had a higher risk for coronary heart disease than the carriers of E3/E3 genotype. On the contrary, no consistent association between the E2 allele and CAD risk was observed. However, these data were observational and confounding biases might have affected the pooled estimates. There are potential chances of argument toward the fact that the true genetic effects of APOE genotypes on CAD cannot be quantified from any pooling or meta analysis of studies with heterogeneous samples. This was answered by using multiple sensitivity analysis which produced consistent pooled estimates, although false-positive findings were possible even in stratified analyses. To sum up, this meta analysis supported the notion that the E4 allele is significantly related to an increased risk for CAD while the E2 allele has no effect (60). Humphries et al. (62) published a report hypothesizing that APOE genotype modifies the effect of smoking in CAD patients. Karvonen J. et al (63) reported the interaction between APOE genotype and smoking in relation to cardiovascular disease. Their study included hypertensive men and age-matched

normotensive controls who participated in the population based OPERA study (Olulu Project Elucidating Risk of Atherosclerosis project). In hypertensive men, there was a significant interaction between presence of the E4 allele and smoking in relation to mean carotid intima-media thickness (IMT) whereas no effect of the E4 allele on carotid IMT was seen in hypertensive non-smokers. The presence of E4 was positively associated with mean carotid IMT in hypertensive smokers, further IMT increased with age in hypertensive smokers carrying the E4 allele but to a lesser extent in non-carrier, non-smokers and normotensive subjects. The authors suggested that the interaction between APOE genotype and smoking can be due to the combined pro-oxidant effects of smoking and the decreased protection against oxidation has been attributed more to the E4 allele that the E2 and E3 allele.

Studies conducted by SinghPP. et al. (64,65) in Punjab, India, identified that the E3 allele and E3/E3 genotypes were most common in normal and angiographically-diagnosed CAD patients. Data from European populations suggested that the low frequency of the APOE4 allele in Southern Europeans was partly responsible for the low incidence and mortality of CAD in the southern population compared to the northern populations (66). Lehtimaki T. et al. (67) conducted an extensive six-year follow-up study on Finnish children and adults to analyse the relationship of APOE phenotype and lipid metabolism. Their results were similar to those of Ehnholm C. et al. (68) with a higher frequency of E4 and a lower frequency of E2 alleles among the Finnish population. The relative changes in serum total and LDL cholesterol during the study period was highest in the subjects having the APOE4/E2 phenotype. The mean concentrations of total cholesterol, LDL cholesterol and apo B were highest in the E4/4 homozygotes and the lowest concentrations were observed in E2/2 homozygotic individuals. Heide S. et al. (69). investigated the role of APOE 3/4 and APOE 4/4 genotypes in premature coronary arteriosclerosis among autopsy cases. In this study, no significant association of the APOE4 genotype and coronary heart disease was observed

both in healthy individuals and CAD patients similar to the observations of Volcik et al. (70). Kolovou GD. et al.(71) observed a lower frequency of the E4 allele in normal BMI men with CAD than in healthy controls. In this study, the normoweight CAD patients had a lower frequency of E2/E2, E3/E3 genotypes and the E2 allele compared to healthy controls. Specifically, the obese CAD patients had a higher E4 allele frequency when compared to the lean patients with CAD. Apolipoprotein E polymorphism can explain as much as 11% of the variation in lipid parameters (72). Increased plasma TC levels play an important role in the occurrence of CAD, and APOE alleles are a marker for susceptibility to increased lipid levels and atherosclerotic disease (73).

But some studies have shown that there is no correlation between lipid levels and CAD severity and that APOE polymorphism was still a CAD risk factor even after lipid factors were accounted for (74). The E4 allele is also associated with a higher restenosis rate after percutaneous transluminal coronary angioplasty (75).

## Apolipoprotein E polymorphism in Moldovan patients with acute myocardial infarction

A case-control study to research the relationship between APOE polymorphism and characteristics of diseased vessels in male CAD patients defined by angiocoronarography in Moldova was designed. Polymerase chain reaction method was used to determine the alleles in the APOE gene *(figure 6.3)*. The results showed that APOE3/3 and APOE3/4 were the most frequent genotypes (E3/3-37,3% and E3/4-29,2%). The least frequent genotype was APOE2/2- 5,6%. The frequency of the APOE4 allele was 27.0%. The APOE4 carriers had more serious stenosis, longer vessel disease, a greater number of diseased vessels, more wide-ranging vessel disease than APOE2 carriers or individuals with the E3/3 genotype.

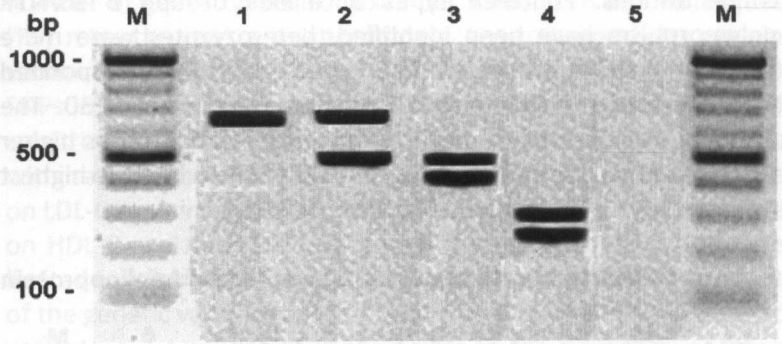



*Figure 6.3.* **Electrophoretic result of PCR products of Apolipoprotein E in Moldovan patients with coronary heart disease.** M – Marker (100 bp, Fermantas); 1 – genotype E4, 2 – genotype E3/4, 3 – genotype 3/4, 4 – genotype 2/2, 5 – negative control.

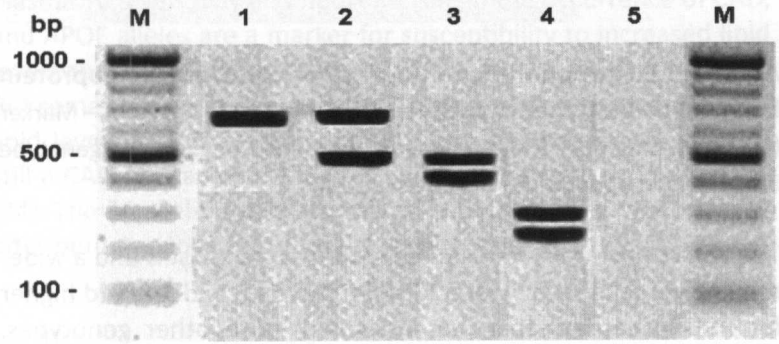
The risk of having a >75% stenosis, 3 diseased vessels and a wide-ranging vessel disease were 2.2-fold, 2.0-fold, and 1.8-fold higher for APOE4 carriers than for individuals with other genotypes. This result suggests that the APOE4 allele may serve as a genetic marker for susceptibility to CAD.

### Apolipoprotein B 3'VNTR polymorphism in Moldovan patients with coronary artery disease

Polymerase chain reaction method was used to determine the alleles in the hypervariable minisatellite region of 3' Variable Number Tandem Repeat (VNTR) close to the apolipoprotein B gene in patients with severe coronary disease *(figure 6.4)*. The amplified products were directly observed after electrophoresis in 2% agarose. The number of hypervariable elements (HVE) was evaluated and analysed in association with lipid serum

concentrations. Fourteen types of alleles of apo B 3'VNTR polymorphism have been identified, heterozygotes were more frequent than homozygotes. The larger amplicon corresponded with the allele HVE44, and the smallest one was HVE30. The frequency of the HVE 36 in the study group patients was higher than that in the control subjects ($p<0.05$). HVE36 had the highest frequency (46.7%), followed by HVE32 (40.0%).

*Figure 6.4.* **Electrophoretic result of PCR products of Apolipoprotein**




**B hypervariable repeat genotype in Moldovan patients with coronary heart disease.** M – Marker (100 bp, Fermantas); 1–3, 5, 8–10, 12–14, 17, and 18 - heterozygotes (two straps); 4, 6, 7, 11, 15, and 16 - homozygotes (one strap).

The HVE 36 alleles were the most common in both females and males. Apolipoprotein B gene polymorphisms were associated with increased levels of total cholesterol 6.7±0.2mmol/l in carriers of HVE 38. Carriers of HVE 32 had a normal level of total cholesterol ($P<0.002$), these differences have been presented in the LDL cholesterol levels.

The more recent approach has been to dramatically expand both the number of genetic polymorphisms typed within a gene and the

number of genes typed. For example, Knoblauch and colleagues (76) recently used a multicandidate gene approach to explain variance in serum LDL-C and HDL-C. By the genotyping of 93 SNPs in 13 genes known to be important in lipid metabolism and constructing 230 SNP haplotypes (a linear array of genetic variants within a chromosomal region inherited as a block), genetic variance on LDL-C explained 26% of the total variance; the genetic variance on HDL-C explained 38%. Although the results of this study are limited to a "normal" population, the ability to explain a large part of the genetic variance of LDL-C and HDL-C on the basis of genetic variants in a small set of genes points to the possibility of using such genetic information to predict CAD risk.

## Known genes- influencing lipids

### Single nucleotide polymorphism- lipid associations

In order to verify common genetic variants associated with plasma concentrations of LDL cholesterol, HDL cholesterol and triglycerides Dawn M. Waterworth et al. decided to combine genome-wide association scan data from two of their studies, including 1 874 individuals from the FUSION study of type 2 diabetes and 4 184 individuals from the SardiNIA study of aging-associated variables plus data on 2 758 individuals from the Diabetes Genetics Initiative (77). For CAD meta-analysis, they combined data from 9 studies comprising up to 9 633 cases and 38 684 controls. These studies included 2 nonoverlapping case-control studies of CAD derived from the EPIC-Norfolk cohort (78), Wellcome Trust Case Control Consortium CAD study (79), Ottawa Heart Study (80), MEDSTAR and PENN CATH studies (81,82) GEMS study (83,84) and Rotterdam study Prospective Studies Collaboration (85). There were identified SNPs at 8 loci that showed evidence for independent replication ($P<0.05$) with 1 or more lipid traits and that showed directional consistency with the discovery studies and no material

heterogeneity among studies (P>0.1). Tables 6.7-6.9 summarize the results for these SNPs. In a combined analysis of all studies 6 of these loci reached genome-wide statistical association ($P<5x10^{-8}$). These were SNPs at the MYLIP/GMPR and PPP1R3B loci for LDL-C; at the SLC39A8, TTC39B, and FADS1 loci for HDL-C; and at FADS1 for TG. The SNP rs2142672 showed strong statistical association with LDL-C levels. The C allele (74% frequency) was associated with relatively higher levels of circulating LDL-C. The SNP lies in a distinct block of high linkage disequilibrium (LD) between 2 genes- myosin regulatory light chain interacting protein (MYLIP) and guanosine monophosphate reductase (GMPR) on chromosome 6p23. The illustration suggests that this SNP is correlated with other SNPs that also show similar patterns of statistical association and that cluster around the MYLIP gene. A recent report has also implicated MYLIP (IDOL) in the regulation of circulating LDL-C levels, by its induction of low-density lipoprotein receptor degradation. The functional characteristic of identified SNPs is of high importance, in understanding of role that lipids play in the pathogenesis of CAD.

SNP rs2126259 lies upstream of the protein phosphatase 1, regulatory (inhibitor) subunit 3B gene (PPP1R3B) on chromosome 8p23 and was statistically associated with circulating LDL-C levels. The A allele (10% frequency) was associated with relatively lower levels of circulating LDL-C. The PPP1R3B protein is involved in the regulation of glycogen metabolism in both muscle and liver. This association with circulating LDL-C and very-low-density lipoprotein-cholesterol levels is a reflection of downstream effects on the bioavailability of TG (86). The C allele (14% frequency) of rs643531 at the tetratricopeptide repeat domain 39B (TTC39B) locus on chromosome 9p22 was associated with lower HDL-C levels (*table 6.8*).

*Table 6.7*

**Associations between SNPs with LDL-C** (Waterworth et al., 2010)

| LDL-C SNP | Chr | Nearest Locus or Loci | Effect Allele | Effect Allele Frequency | P Value |
|---|---|---|---|---|---|
| rs11206510 | 1 | PCSK9 | T | 0.77 | 0.52 |
| rs660240 | 1 | CELSR2 | A | 0.21 | 0.22 |
| rs515135 | 2 | APOB | A | 0.19 | 0.47 |
| rs12916 | 5 | HMGCR | T | 0.62 | 0.80 |
| rs2954021 | 8 | TRIB1 | G | 0.50 | $1.3x10^{-4}$ |
| rs1558861 | 11 | BUD13, ZNF259, APOA5-A4-C3-A1 | T | 0.94 | $1.7x10^{-7}$ |
| rs2738459 | 19 | LDLR | C | 0.48 | 0.34 |
| rs10401969 | 19 | SF4–CILP2 | T | 0.91 | 0.26 |
| rs4420638 | 19 | APOE-C1-C4-C2 | G | 0.18 | $2.0x10^{-7}$ |

*Table 6.8*

**Associations between SNPs with HDL-C**
(Waterworth et al ., 2010)

| HDL-C SNP | Chr | Nearest Locus or Loci | Effect Allele | Effect Allele Frequency | P Value |
|---|---|---|---|---|---|
| rs10489615 | 1 | GALNT2 | G | 0.60 | $3.8x10^{-9}$ |
| rs11902417 | 2 | APOB | G | 0.78 | $3.7x10^{-7}$ |
| rs325 | 8 | LPL | T | 0.89 | $7.8x10^{-25}$ |
| rs3890182 | 9 | ABCA1 | G | 0.88 | $4.7x10^{-7}$ |
| rs964184 | 11 | ZNF259, APOA5-A4-C3-A1 | G | 0.12 | $1.6x10^{-11}$ |
| rs9943753 | 12 | MYO1H, KCTD10, UBE3B, MVK | G | 0.63 | $3.2x10^{-6}$ |
| rs261334 | 15 | LIPC | G | 0.20 | $4.9x10^{-22}$ |
| rs9989419 | 16 | CETP | G | 0.60 | $1.3x10^{-32}$ |
| rs12449157 | 16 | GFOD2–LCAT | G | 0.17 | $2.3x10^{-7}$ |
| rs2156552 | 18 | LIPG | T | 0.81 | $1.7x10^{-12}$ |

SNP rs643531 lies within intron 1 of the TTC39B gene in a modest linkage disequilibrium block that does not contain any other known or putative genes. Kathiresan S. et al. (2009) data revealed statistical association between an SNP- rs471364- at this locus and HDL-C levels (87). The 2 SNPs (rs471364 and rs643531) were correlated at an $r^2$ of 0.74 and showed directional consistent associations. The function of the TTC39B gene in humans is presently unknown.

### *SLC39A8*

SNP rs13107325 at the solute carrier family 39 (zinc transporter) member 8 (SLC39A8) locus on chromosome 4q22 showed strong statistical association with circulating levels of HDL-C (*table 6.8*). It is a nonsynonymous SNP located in exon 8 of the SLC39A8 gene, which produces a change in amino acid from alanine to threonine. The T alleles (8% frequency) were associated with relatively lower levels of circulating HDL-C and is not materially correlated with any other SNP across 100 kb of genomic sequence spanning the SLC39A8 gene in HapMap. Besecker B. et al. (2008) reported that this gene encodes a zinc transporter that has been shown to function in the cellular importation of zinc at the onset of inflammation, and its expression is induced by TNF (88). It is possible that the SLC39A8 molecule might be associated with HDL-C in an inflammatory context.

### *FADS1*

SNP rs174548 at the fatty acid desaturase 1 (FADS1) locus on chromosome 11q12 showed strong statistical association with both HDL-C and TG levels. The G allele (30% frequency) were associated with relatively higher TG and lower HDL-C levels (*table* 6.9). SNP rs174548 lies in a block of clear linkage disequilibrium that also contains the C11, FEN1 and FADS2/3 genes. Schaeffer L. et al. showed that fatty acid desaturases are involved in the metabolism of polyunsaturated fatty acids in humans and SNPs

at the FADS1/2 gene cluster have been linked to changes in the fatty acid composition of serum phospholipids in humans (89). In a combined analysis of all studies Waterworth Dawn M. et al. (77) identified an additional locus that reached genome-wide statistical association- AFF1- a novel locus for circulating TG ($P=3.1x10^{-10}$). SNP rs442177 lies in intron 10 of the AFF1 gene on chromosome 4q21 in a modest LD block with correlated SNPs showing similar levels of statistical association. The A allele (60% frequency in white European populations) was associated with relatively higher levels of circulating TG.

*Table 6.9*

**Associations between SNPs with TG at known lipid loci** (Waterworth et al.., 2010)

| TG SNP | Chr | Nearest Locus or Loci | Effect Allele | Effect Allele Frequency | P Value |
|---|---|---|---|---|---|
| rs1168013 | 1 | DOCK7, ANGPTL3 | G | 0.65 | 0.97 |
| rs6544366 | 2 | APOB | T | 0.22 | $5.3x10^{-7}$ |
| rs1260333 | 2 | GCKR | C | 0.55 | 0.08 |
| rs1178979 | 7 | BAZ1B, BCL7B, TBL2, MLXIPL | A | 0.80 | $8.0x10^{-3}$ |
| rs10105606 | 8 | LPL | C | 0.68 | $1.7x10^{-14}$ |
| rs2954029 | 8 | TRIB1 | T | 0.46 | $4.5x10^{-5}$ |
| rs4938303 | 11 | BUD13, ZNF259, APOA5-A4-C3-A1 | T | 0.75 | $9.6x10^{-8}$ |
| rs16965220 | 16 | CETP, LOC100130044, NLRC5 | C | 0.68 | 0.04 |
| rs2304130 | 19 | CILP2–ZNF101 | G | 0.09 | 0.55 |

The AFF1 gene encodes a protein involved in the regulation of cyclin-dependent kinase inhibitor CDKN1B and may therefore be involved in cell cycle regulation. Its function with respect to TG metabolism is unknown.

## Genetic loci association with blood lipids

Waterworth et al. (77) identified 6 genetic loci that showed both genome-wide statistical association with blood lipids and statistical association with CAD after adjustment for multiple testing ($P<0.0013$ after testing 36 SNPs). Specifically, they confirmed the association between variation at the CELSR2 (Samani NJ., 90) and APOB genes and variation at the APOE-C1-C4-C2 cluster, which influence mainly LDL-C levels and risk of CAD. None of the genetic variants largely or specifically associated with HDL-C showed statistical association with CAD risk after correction for multiple testing. SNPs at the ZNF259-APOA5-A4-C3-A1 cluster-which reached genome-wide statistical association-and at the TRIB1 and LPL loci, which showed strongest association with TG levels, were also statistically associated with risk of CAD after adjustment for multiple testing. The direction of association with CAD risk for all of these SNPs was consistent with their association with lipid levels. However, several of the SNPs at these loci were associated with more than 1 lipid trait. Of note, only SNPs at CELSR2 and APOB showed specific associations with LDL-C. By contrast, only SNPs at the LPL locus showed clear associations with HDL-C and TG, but they were not associated with LDL-C.

Dawn M. Waterworth et al. studies have identified 3 novel loci (PPP1R3B for LDL-C, SLC39A8 for HDL-C, and AFF1 for TG) associated with variation in circulating LDL-C, HDL-C, and TG. They also provide strong statistical evidence for 6 loci that influence levels of blood lipids and risk of CAD. In addition to those that are largely associated with LDL-C concentrations. These authors showed that genetic loci mainly associated with circulating TG

are also associated with risk of CAD. Collectively, these studies potentially provide new insights into biological regulation of lipid metabolism and the etiology of CAD (77).

Waterworth et al. (2010) provided robust statistical evidence for the association of 3 novel genetic loci with circulating LDL-C, HDL-C, and TG levels, in addition to confirming the recently reported novel associations for circulating LDL-C with SNPs at MYLIP/GMPR, HDL-C levels with SNPs at the TTC39B locus and for both circulating HDL-C and TG levels at the FADS1 locus (77). The function of the 3 novel loci, PPP1R3B, SLC39A8 and AFF1 in lipid metabolism is not known.

Cambien F.et al. (2007) provided inconsistent evidence for the APOB locus and CAD risk in line with the effect of rare, highly deleterious mutations at this gene (91). Soutar AK., Naoumova RP., (2007) discovered the genes known to be implicated in Mendelian forms of hypercholesterolemia, including LDLR and PCSK9 (92) and showed only suggestive evidence for their association with CAD risk. Dawn M. Waterworth et al. (77) provided compelling statistical data that genetic variants at loci predominantly associated with both circulating blood TG and HDL-C are also associated with risk of CAD-specifically at the ZNF259-APOA5-A4-C3-A1 cluster, TRIB1, and LPL loci. The TRIB1 locus is a recently identified lipid gene that predominantly influences TG but is also associated with LDL-C and HDL-C. One report by Willer CJ. et al. (2008) has shown suggestive evidence for an association between a SNP at this locus and CAD risk (93).

Barter PJ.et al. (2007) reported that none of the genetic loci showing reproducible and specific association with HDL-C levels (including CETP), showed strong evidence for association with CAD risk. The functional relationship of HDL-C to CAD risk is inherently complex, and plasma concentrations of HDL-C are not always a reliable marker of reverse cholesterol transport or other biological functions of HDL, including antiinflammatory effects (94).

Genes at the loci implicated in the latest GWA studies (77,87,90,93) affect the entire cycle of formation, activity and turnover of lipoproteins and triglycerides. They encode many of the apolipoproteins (ApoE, ApoB and ApoA5), but they also encode a transcription factor activating triglyceride synthesis (MLXIPL), an enzyme involved in cholesterol biosynthesis (MVK), transporters of cholesterol (ABCA1) and cholesterol ester (CETP), a lipoprotein receptor (LDLR), potential receptor-modifying glycosyltransferases (B4GALT4, B3GALT4 and GALNT2), lipases (LPL, LIPC and LIPG) and a protein involved in cholesterol degradation (MMAB), an inhibitor of lipase (ANGPTL3) and a possible endocytic receptor for LPL (SORT1).

## References:


1. Kuulasmaa, K. et al. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet. 55, 675–687,2000.
2. Clarke, R. et al. Cholesterol fractions and apolipoproteins as risk factors for heart disease mortality in older men. Arch. Intern. Med. 167, 1373–1378 (2007).
3. Grundy, S.M. et al. Implications of recent clinical trials for the National CholesterolEducation Program Adult Treatment Panel III guidelines. Circulation 110, 227–239,2004.
4. Gotto, A.M. Jr. & Brinton, E.A. Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. J. Am. Coll. Cardiol. 43, 717–724 (2004).
5. *Heath, R.B., Karpe, F., Milne, R.W., Burdge, G.C., Wootton, S.A. and Frayn, K.N. (2003) Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period.* J. Lipid Res., 44, 2065–2072.
6. *Kwiterovich, P.O., Jr (2002) Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity.* Am. J. Cardiol., 90, 30i–47i.
7. Botnaru V. Dislipidemiile: ghid de practică medicală, 2004.

8. *Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).* JAMA 285:2486–*2497,* 2001.

9. European Guidelines on CVD Prevention in clinical practice. EHJ, 2007; 28: 2375-2414., Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adult (Adult Treatment Panel III) final report. Circulation, 2002; 106: 3143-3421.

10. Ivanov V., Popovici M. Dislipidemiile. Chişinău, 2005, 90 p.

11. Reiner Z., Catapano A., De Backer G, et al.ESC/EAS Guidelines for the Management of dyslipidemias. The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS)European Heart Journal (2011) 32, 1769–1818.

12. Grundy SM, *Cleeman JI, Merz CN, et al, Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines.* Circulation. 2004;110:227-239.pmid:15249516.

13. Yadav D., Pitchumoni, C. S., M.A.C.G.Issues in Hyperlipidemic Pancreatitis. Journal of Clinical Gastroenterology: January 2003 - Volume 36 - Issue 1 - pp 54-62.

14. Weinstock PH, Bisgaier CL, Aalto-Setala K, et al: Severe hypertriglyceridemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice: Mild hypertriglyceridemia with impaired very low density lipoprotein clearance in heterozygotes. J Clin Invest 1995; 96:2555.

15. Sijbrands EJ, Westendorp RG, Defesche JC, et al. Mortality over two centuries in large pedigree with familial hypercholesterolaemia: family tree mortality study. BMJ. 2001; 322: 1019–1023.

16. Thorsson B, Sigurdsson G, Gudnason V. Systematic family screening for familial hypercholesterolemia in Iceland. Arterioscler Thromb Vasc Biol. 2003; 23: 335–338.

17. Heath KE, Humphries SE, Middleton-Price H, et al. A molecular genetic service for diagnosing individuals with familial hypercholesterolaemia (FH) in the United Kingdom. Eur J Hum Genet. 2001; 9: 244–252.

18. van Aalst-Cohen ES, Jansen ACM, Tanck MWT, Defesche JC, Trip MD, Lansberg PJ, Stalenhoef AFH, Kastelein JJP. Diagnosing familial hypercholesterolaemia: the relevance of genetic testing. Eur Heart J. 2006; 27: 2240–2246.

19. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. Am J Epidemiol. 2004; 160: 407–420.

20. Abifadel M, Varret M, Rabes J-P, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derre A, Villeger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf J-M, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003; 34: 154–156.

21. Cohen JC, Boerwinkle E, Mosley Jr TH, Hobbs HH: Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006; 354:1264.

22. Soria LF, Ludwig EH, Clarke HR, Vega GL, Grundy SM, McCarthy BJ. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. Proc Natl Acad Sci U S A. 1989; 86: 587–591.

23. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002;155:487–95.

24. George Yuan, Khalid Z. Al-Shali and Robert A. Hegele.Hypertriglyceridemia: its etiology, effects and treatment. CMAJ,2007; 176 (8).

25. Austin, M. A., K. L. Edwards, S. A. Monks,K. M. Koprowicz, J. D. Brunzell, A. G. Motulsky, M. C. Mahaney, J. E. Hixson.Genome-wide scan for quantitativetrait loci influencing LDL size and plasma triglyceride in familialhypertriglyceridemia. J. Lipid Res.2003.44:2161–2168.

26. Berge KE, Tian H, Graf GA, et al: Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000; 290:1771.

27. *Danesh J, Collins R, Peto R (2000).* "Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies". *Circulation 102 (10): 1082–5.*

28. Berglund L, Ramakrishnan R (2004). "Lipoprotein(a): an elusive cardiovascular risk factor". Arterioscler. Thromb. Vasc. Biol. 24 (12): 2219–26.

29. Rita M. Cantor; Tjerk de Bruin; Naoko Kono; Susan Napier; Atila van Nas; Hooman Allayee; Aldons J. Lusis. Quantitative Trait Loci for Apolipoprotein B, Cholesterol, and Triglycerides in Familial Combined Hyperlipidemia Pedigrees. **Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;24:1935-1941.**

30. Lewis GF, Rader DJ: New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ Res 2005; 96:1221.

31. Stephanie Trelogan. Genes Can Cause Coronary Artery Disease. Genetic Health.com, 12, 2000.

32. Sorci-Thomas MG, Thomas MJ: The effects of altered apolipoprotein A-I structure on plasma HDL concentration. Trends Cardiovasc Med 2002; 12:121.

33. Boekholdt SM, Kuivenhoven JA, Hovingh GK, et al: CETP gene variation: Relation to lipid parameters and cardiovascular risk. Curr Opin Lipidol 2004; 15:393.

34. Calabresi L, Pisciotta L, Costantin A, et al: The molecular basis of lecithin : cholesterol acyltransferase deficiency syndromes: A comprehensive study of molecular and biochemical findings in 13 unrelated Italian families. Arterioscler Thromb Vasc Biol 2005; 25:1972.

35. Susanne M. Clee, Aeilko H. Zwinderman, James C. Engert, Karin Y. Zwarts; Henri O. F. Molhuizen, Kirsten Roomp, J. Wouter Jukema, Michel van Wijland, Marjel van DamThomas J. Hudson, Angela Brooks-Wilson, Jacques Genest, Jr, John J. P. Kastelein, Michael R. Hayden Common Genetic Variation in ABCA1 Is Associated With Altered Lipoprotein Levels and a Modified Risk for Coronary Artery Disease *Circulation.* 2001;103:1198.

36. Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science. 2004; 305: 869–872.

37. Tregouet D-A, Ricard S, Nicaud V, Arnould I, Soubigou S, Rosier M, Duverger N, Poirier O, Macé S, Kee F, Morrison C, Denefle P, Tiret L, Evans A, Deleuze J-F, Cambien F. In-depth haplotype analysis of ABCA1 gene polymorphisms in relation to plasma ApoA1 levels and myocardial infarction. Arterioscler Thromb Vasc Biol. 2004; 24: 775–781.

38. Lee CY, Lesimple A, Denis M, et al: Increased sphingomyelin content impairs HDL biogenesis and maturation in human Niemann-Pick disease type B. J Lipid Res 2006; 47:622.

39. Anderson GL, Limacher M, Assaf AR: Effects of conjugated equine estrogen in post-menopausal women with hysterectomy: The Women's Health Initiative randomized controlled trial. JAMA 2004; 291:1701.

40. Meigs JB, Wilson PW, Fox CS, et al: Body mass index, metabolic syndrome and risk of type 2 diabetes or cardiovascular disease. J Clin Endocrinol Metab 2006; 91:2906.

41. Kahn R, Buse J, Ferrannini E, et al: The metabolic syndrome: Time for a critical appraisal: Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2005; 28:2289.

42. Knoblauch H, Bauerfeind A, Toliat MR, Becker C, Luganskaja T, Gunther UP, Rohde K, Schuster H, Junghans C, Luft FC, Nurnberg P, Reich JG. Haplotypes and SNPs in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol. Hum Mol Genet. 2004; 13: 993–1004.

43. Wang X, Paigen B. Genetics of variation in HDL cholesterol in humans and mice. Circ Res. 2005; 96: 27–42, Hobbs HH, Graf GA, Yu L, Wilund KR, Cohen JC. Genetic defenses against hypercholesterolemia. Cold Spring Harb Symp Quant Biol. 2002; 67: 499–505.

44. Ward H., Mitrou P. N., Bowman R., Luben R., Wareham N. J., Khaw K.-T., and Bingham S. **APOE Genotype, Lipids, and Coronary Heart Disease Risk: A Prospective Population Study.**Arch Intern Med, August 10, 2009; 169(15): 1424 - 1429.

45. Lehtinen S, Lehtimäki T, Sisto T, Salenius JP, Nikkilä M, Jokela H, koivula T, et al. Apolipoprotein E, serum lipids, myocardial infarction and severity of angiographically verified coronary artery disease in men and women. Atherosclerosis 1995; 114 : 83-91.

46. Brscic E, Bergerone S, Gagnor A, Colajanni E, Matullo G, Scaglione L, et al. Acute myocardial infarction in young adults: prognostic role of angiotensin converting enzyme, angiotensin II type 1 receptor, apolipoprotein E, endothelial constitutive nitric oxide synthase, and glycoprotein IIIa genetic polymorphisms at medium term follow-up. Am Heart J 2000; 139 : 979-84.

47. van Bockxmeer FM, Mamotte CDS. Apolipoprotein epsilon 4 homozygosity in young men with coronary heart disease. Lancet 1990; 340 : 879-80.

48. Stengard JH, Zerba KE, Pekkanen J, Ehnholm C, Nissinen A, Sing CF. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 1995; 91 : 265-9.

49. Mc Carron MO, Delong D, Alberts MJ. Apo E genotype as a risk factor for ischemic cerebro disease: A meta analysis. Neurology 1993; 53 : 1308-11, 1999.

50. Kumar P, Luthra K, Dwivedi M, Behl VK, Pandey RM, Misra A. Apolipoprotein E gene polymorphisms in patients with premature myocardial infarction: a case-controlled study in Asian Indians in north India. Ann Clin Biochem 2003; 40: 382-7.

51. Ashavaid TF, Todur SP, Nair KG. Apolipoprotein E4 polymorphism as risk factor for coronary heart disease among Indian subjects. IJCB 2002; 17: 83-93.

52. Venkatramana P, Reddy PC. Effects of apolipoprotein E polymorphism on the lipid and lipoprotein levels related to risk for cardiovascular disease. ICMR Bull 1998; 28 : 66-7.

53. Lenzen HJ, Assmann G, Buchwalsky R. Association of Apo E polymorphism, low density lipoprotein cholesterol and coronary artery diseases. Arteriosclerosis 1983; 3: 310-5.

54. Luthra K, Bharghav B, Chabbra S, Das N, Misra A, Agarwal DP, et al. Apolipoprotein E polymorphism in Northern Indian patients with coronary heart disease: phenotype distribution and relation to Serum lipids and lipoproteins. Mol Cell Biochem 2002; 232: 97-102.

55. Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. The LDL- receptor-related protein LRP is an apolipoprotein E binding protein. Nature 1989; 341: 162-4.

56. Gerdes LU, Gerdes C, Kervinen K, Savolainen M, Klausen IC, Hansen PS, et al. The apolipoprotein ε allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction. A sub study of the Scandinavian simvastatin survival study. Circulation 2000; 101 : 1366-71.

57. Stengard JH, Weiss KM, Sing CF. An ecological study of association between coronary heart disease mortality rate in men and the relative frequencies of common allelic variations in the gene coding for apolipoprotein E. Hum Genet 1998; 103 : 234-41.

58. Schiele F, De Bacquer D, Vincent-Viry M, Beisiegel U, Ehnholm C, Evans A, et al. Apolipoprotein E serum concentration and polymorphism in six European countries: the Apo Europe project. Arteriosclerosis 2000; 152 : 475-88.

59. Mooijaart SP, Berbe´e JFP, van Heemst D, Havekes LM, de Craen AJM, Slagboom PE, et al. Apo E plasma levels and risk of cardiovascular mortality in old age. PLoS Med 2006; ( 6): e 176: 0874-83.

60. Gutman CR, Strittmatter WJ, Weisgraber KH, Matthew WD. Apolipoprotein E binds to and potentiates the biological activity of ciliary neurotrophic factor. J Neurosci 1997; 17 : 6114-21.

61. Song Y, Stampfer MJ, Liu S. Meta analysis: Apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 2004; 141 : 137-47.

62. Humphries SE, Talmud PJ, Hawe E, Bolla M, Day INM, Miller GJ. Apolipoprotein E4 and coronary heart disease in middle aged men who smoke: a prospective study. Lancet 2001; 358 : 115-9.

63. Karvonen J, Kauma H, Kervinen K, Ukkola O, Rantala M, Päivänsalo M. Apolipoprotein E polymorphism affects carotid artery atherosclerosis in smoking hypertensive men. J Hyperten 2002; 20 : 2371-8.

64. Singh PP, Singh M, Bhatnagar DP, Kaur TP, Gaur SK. Apolipoprotein E polymorphism and its relation to plasma lipids in coronary heart disease. Indian J Med Sci 2008; 62 : 105-12.

65. Singh PP, Singh M, Mastana SS. Apo E distribution in world populations with new data from India and the U.K. Ann Hum Biol 2006; 33 : 279-308.

66. Moghadasian MH, McManus BM, Godin DV, Rodrigues B, Frohlich JJ. Pro-atherogenic and anti-atherogenic effects of probucol and phytosterols in apo E deficient mice: possible mechanisms of action. Circulation 1999; 99 : 1733-9.

67. Lehtimaki T, Moilanen T, Viikari J, Akerblom HK, Ehnholm C, Ronnemaa T, et al. Apolipoprotein E phenotypes in finnish youths: a cross sectional and six year follow-up study. J Lipid Res 1990; 31 : 487-95.

68. Ehnholm C, Lukka M, Kuusi T, Nikkila E, Utermann G. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. J Lipid Res 1986; 27 : 227-35.

69. Heide S, Manfred K, Glaser C, Schulz S. Apolipoprotein E (apo E) polymorphism: A risk factor for fatal coronary sclerosis. Forensic Sci Int 2009; 192 : 62-6.

70. Volcik KA, Barkley RA, Hutchinson RG, Mosley TH, Heiss G, Sharrett AR, et al. Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC study participants. Am J Epidemiol 2006; 164 : 342-8.

71. Kolovou GD, Anagnostopoulou KK, Kostakou P, Giannakopoulou V, Mihas C, Hatzigeorgiou GI, et al. Apolipoprotein E Gene polymorphism and obesity status in middle – aged men with coronary heart disease. In vivo 2009; 23 : 33-40.

72. Lahoz C, Schaefer EJ, Cupples LA, et al. Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study. Atherosclerosis. 2001;154:529–537.

73. Friedlander Y, Leitersdorf E, Vecsler R. The contribution of candidate genes to the response of plasma lipids and lipoproteins to dietary challenge. Atherosclerosis. 2000;152(1):239–248.

74. Scuteri A, Bos AJG, Znderman AB. Is the apo E4 allele an independent predictor of coronary events? Am J Med.2001;110:28–32.

75. Tada H. The E4 allele of apolipoprotein E is associated with increased restenosis after coronary angioplasty. Tokai J Exp Clin Med. 2001;26(3):81–92.

76. Knoblauch H, Bauerfeind A, Toliat MR, Becker C, Luganskaja T, Gunther UP, Rohde K, Schuster H, Junghans C, Luft FC, Nurnberg P, Reich JG. Haplotypes and SNPs in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol. Hum Mol Genet. 2004; 13: 993–1004.

77. Waterworth Dawn M. Waterworth; Sally L. Ricketts; Kijoung Song Genetic Variants Influencing Circulating Lipid Levels and Risk of Coronary Artery Disease. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2010;30:2264.

78. Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, Wareham N. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer. 1999; 80 (suppl 1): 95–10.

79. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447: 661–678.

80. Stewart AF, Dandona S, Chen L, Assogba O, Belanger M, Ewart G, LaRose R, Doelle H, Williams K, Wells GA, McPherson R, Roberts R. Kinesin family member 6 variant Trp719Arg does not associate with angiographically defined coronary artery disease in the Ottawa Heart Genomics Study. J Am Coll Cardiol. 2009; 53: 1471–1472.

81. Kathiresan S, Voight BF, Purcell S, Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009; 41: 334–341.

82. Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, Schunkert H, Erdmann J, Reilly MP, Rader DJ, Morgan T, Spertus JA, Stoll M, Girelli D, McKeown PP, Patterson CC, Siscovick DS, O'Donnell CJ, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Melander O, Altshuler D, Ardissino D, Merlini PA, Berzuini C, Bernardinelli L, Peyvandi F, Tubaro M, Celli P, Ferrario M, Fetiveau R, Marziliano N, Casari G, Galli M, Ribichini F, Rossi M, Bernardi F, Zonzin P, Piazza A, Mannucci PM, Schwartz SM, Siscovick DS, Yee J, Friedlander Y, Elosua R, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Kathiresan S, Meigs JB, Williams G, Nathan DM, MacRae CA, O'Donnell CJ, Salomaa V, Havulinna AS, Peltonen L, Melander O, Berglund G, Voight BF, Kathiresan S, Hirschhorn JN, Asselta R, Duga S, Spreafico M, Musunuru K, Daly MJ, Purcell S, Voight BF, Purcell S, Nemesh J, Korn JM, McCarroll SA, Schwartz SM, Yee J, Kathiresan S, Lucas G, Subirana I, Elosua R, Surti A, Guiducci C, Gianniny L, Mirel D, Parkin M, Burtt N, Gabriel SB, Samani NJ, Thompson JR, Braund PS, Wright BJ, Balmforth AJ, Ball SG, Hall AS, Schunkert H, Erdmann J, Linsel-Nitschke P, Lieb W, Ziegler A, Konig I, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Schunkert H, Samani NJ, Erdmann J, Ouwehand W, Hengstenberg C, Deloukas P, Scholz M, Cambien F, Reilly MP, Li M, Chen Z, Wilensky R, Matthai W, Qasim A, Hakonarson HH, Devaney J, Burnett MS, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein SE, Rader DJ, Scheffold T, Berger K, Stoll M, Huge A, Girelli D, Martinelli N, Olivieri O, Corrocher R, Morgan T, Spertus JA, McKeown P, Patterson CC, Schunkert H, Erdmann E, Linsel-Nitschke P, Lieb W, Ziegler A, Konig IR, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Holm H, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Engert JC, Do R, Xie C,

Anand S, Kathiresan S, Ardissino D, Mannucci PM, Siscovick D, O'Donnell CJ, Samani NJ, Melander O, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Altshuler D. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009; 41: 334–341.

83. Stirnadel H, Lin X, Ling H, Song K, Barter P, Kesaniemi YA, Mahley R, McPherson R, Waeber G, Bersot T, Cohen J, Grundy S, Mitchell B, Mooser V, Waterworth D. Genetic and phenotypic architecture of metabolic syndrome-associated components in dyslipidemic and normolipidemic subjects: the GEMS Study. Atherosclerosis. 2008; 197: 868–876.

84. Wyszynski DF, Waterworth DM, Barter PJ, Cohen J, Kesaniemi YA, Mahley RW, McPherson R, Waeber G, Bersot TP, Sharma SS, Nolan V, Middleton LT, Sundseth SS, Farrer LA, Mooser V, Grundy SM. Relation between atherogenic dyslipidemia and the Adult Treatment Program-III definition of metabolic syndrome (Genetic Epidemiology of Metabolic Syndrome Project). Am J Cardiol. 2005; 95: 194-198.

85. Rotterdam study Prospective Studies Collaboration Blood cholesterol and vascular mortality by age, sex and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet, 370, 1829-1839 (2007).

86. Chasman DI, Pare G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Hamsten A, Kathiresan S, Malarstig A, Ordovas JM, Ripatti S, Parker AN, Miletich JP, Ridker PM. Forty-three loci associated with plasma lipoprotein size, concentration and cholesterol content in genome-wide analysis. PLoS Genet. 2009; 5: e1000730.

87. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, Voight BF, Bonnycastle LL, Jackson AU, Crawford G, Surti A, Guiducci C, Burtt NP, Parish S, Clarke R, Zelenika D, Kubalanza KA, Morken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kuusisto J, Bergman RN, Sundvall J, Laakso M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, de Bakker PI, O'Donnell CJ, Chambers JC, Kooner JS, Hercberg S, Meneton P, Lakatta EG, Scuteri A, Schlessinger D, Tuomilehto J, Collins FS, Groop L, Altshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, Mohlke KL, Cupples LA. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet. 2009; 41: 56–65.

88. Besecker B, Bao S, Bohacova B, Papp A, Sadee W, Knoell DL. The human zinc transporter SLC39A8 (Zip8) is critical in zinc-mediated cytoprotection in lung epithelia. Am J Physiol Lung Cell Mol Physiol. 2008; 294: L1127–L1136.

89. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, Demmelmair H, Illig T, Koletzko B, Heinrich J. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet. 2006; 15: 1745–1756.

90. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Blankenberg S, Balmforth AJ, Baessler A, Ball SG, Strom TM, Braenne I, Gieger C, Deloukas P, Tobin MD, Ziegler A, Thompson JR, Schunkert H. Genomewide association analysis of coronary artery disease. N Engl J Med. 2007; 357: 443–453.

91. Cambien F, Tiret L. Genetics of cardiovascular diseases: from single mutations to the whole genome. Circulation. 2007; 116: 1714–1724.

92. Soutar AK, Naoumova RP. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nat Clin Pract Cardiovasc Med. 2007; 4: 214–225.

93. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet. 2008; 40: 161–169.

94. Barter PJ, Puranik R, Rye KA. New insights into the role of HDL as an anti-inflammatory agent in the prevention of cardiovascular disease. Curr Cardiol Rep. 2007; 9: 493–498.

*Every human being is the author of his own health or disease.*

*Buddha quotes, Hindu Prince Gautama Siddharta,*
*the founder of Buddhism, 563-483 B.C.*

# Chapter 7.
# Systemic hypertension

## Familial nature of hypertension

The familial nature of hypertension has long been recognized. During the 1950s, the St. Mary's Study was the first to convincingly document that the occurrence of hypertension is more common among family members with increased blood pressure (BP) than among the general population (1). This study recruited first-degree relatives of probands with high BP (diastolic BP 100 mmHg) and normal BP (diastolic BP 85 mmHg). Familial aggregation of BP in families of high BP probands was similar to normal BP probands. Subsequent studies have verified and refined these observations: Overall, first-degree relatives of a person of any age with hypertension are at a 2.3-fold greater risk of developing hypertension before age 49 than the general population. This risk increases to almost fourfold when an individual has two or more family members diagnosed with hypertension before age 55 (2). Twin and adoption studies have demonstrated that familial

correlation in blood pressure is influenced by both shared genes and shared environment (3). The heritability (i.e., the proportion of a trait's variation in a population that has a familial basis) of BP estimated from twin studies is around 60% (4), because twin studies integrate both genetic and environmental influences, heritability estimates of about 30% from pedigree studies are more reasonable estimates of population variability due to genetic factors (5). The importance of hypertension as a risk factor for stroke, chronic kidney disease, and cardiovascular disease; the considerable prevalence of hypertension in many populations; and the well-documented heritable nature of hypertension have made it an important phenotype for genetic and genomic study. Although considerable progress has been made in characterizing monogenic forms of hypertension, the complex nature of essential hypertension has made it more resistant to genetic dissection.

## Mendelian disorders resulting in hypertension

The contribution of Mendelian forms of hypertension to blood pressure variation in populations as a whole is small; however, the study of these single-gene disorders with clear Mendelian patterns of inheritance has revealed much about the primary mechanisms of blood pressure and volume control. The best illustration of the potential role of rare gene variants in the pathogenesis of essential hypertension is found in a study of 3 125 participants from the Framingham study performed by Lifton and colleagues (6) in which they carefully sequenced 3 genes known to be involved in renal electrolyte transport and BP regulation. Previous studies by Lifton and colleagues had uncovered mutations in these genes that caused rare recessive diseases characterized by very large reductions in BP. Remarkably, one of every 64 subjects in this Framingham cohort was found to carry a mutation of potential functional significance in one of these 3 genes: SLC12A3 (NCCT), SLC12A1 (NKCC2) and KCNJ1 (ROMK)-causing rare recessive

diseases featuring large reductions in blood pressure. Very few of the carriers shared the same variant and the tendency was for each person to carry his or her own particular mutation so that each specific mutation was rare. On average, the systolic BP at age 60 in those carrying one of these rare mutations was 9 mm Hg lower than in the noncarriers, and having one of these rare mutations reduced the risk for hypertension at the age of 60 years by ≈60%.

Fewer than a dozen forms of monogenic hypertension have been described; all tend to have large effects on blood pressure, and most act via a physiologic pathway in the kidney by altering renal salt reabsorption (7). Mutations that increase salt reabsorption necessarily increase water reabsorption and vascular volume, and thereby increase blood pressure. The cascade of events leading from a single mutation to hypertension is often complex, but for many such mutations the pathophysiologic chain is well characterized. For example, glucocorticoid remediable aldosteronism (GRA) is an autosomal dominant disorder resulting from an unequal crossing over between two closely related genes on chromosome 8 that are involved in adrenal steroid biosynthesis. The coding region of the cytochrome P450, subfamily XIB, polypeptide 2 gene (CYP11B2, commonly known as the aldosterone synthase gene) forms a meiotic mismatch with the promoter region of the cytochrome P450, subfamily XIB, polypeptide 1 gene (CYP11B1, commonly known as the 11-β-hydroxylase gene), resulting in a chimeric protein with composite properties of the two parent enzymes. Aldosterone synthase is the rate-limiting enzyme in aldosterone synthesis; aldosterone, in turn, is the steroid hormone that regulates the mineralocorticoid receptor that regulates epithelial sodium channel activity and, ultimately, salt reabsorption and blood pressure. Increased aldosterone normally results in increased blood pressure. The 11-β-hydroxylase enzyme is involved in the synthesis of cortisol, a corticosteroid hormone whose expression is regulated by the adrenocorticotropic hormone

(ATCH). The chimeric enzyme resulting from the mismatched genes takes on the synthetic role of aldosterone synthase but, like 11-β-hydroxylase, it is regulated by ATCH. As a result, in the process of maintaining normal cortisol levels, ATCH also boosts aldosterone synthase activity with the expected commensurate increase in aldosterone, plasma volume, and blood pressure. The salt and water retention suppresses the secretion of renin, but, because aldosterone is effectively under the control of ATCH and not the normal renin–angiotensin–aldosterone pathway, the secretion of aldosterone remains unchecked. GRA has a clinical expression ranging from mild blood pressure elevation to severe, early-onset hypertension, often diagnosed in childhood. Treatment with a glucocorticoid such as dexamethasone often reduces blood pressure in young patients. Adults are more variable in their response and are typically treated with additional antihypertensive medications such as thiazide diuretics. Other Mendelian disorders are similarly documented in the excellent review of Mendelian forms of hypertension with special emphasis on clinical diagnosis offered by Luft FC.,2003 (8).

## Complex genetic forms of hypertension

Although the characterization of monogenic forms of hypertension is a bright spot in the field of clinical genetics, the predominance of essential hypertension and the development of increasingly powerful genetic and genomic methods have focused efforts on explicating essential hypertension and its polygenic nature. Both linkage and candidate gene association studies have been used to identify genes with modest effects on the BP phenotype that may contribute to essential hypertension. Table 7.1 summarizes candidate genes in the causal pathways of blood pressure regulation that may influence susceptibility to cardiovascular disease (9). Linkage studies have pointed to regions on all human chromosomes that appear to contribute to hypertension and BP-

related traits. Although the LOD (logarithm to the base 10 of the odds) score signals in most studies offer only statistically suggestive evidence of linkage, a number of meta-analyses have attempted to integrate findings across multiple studies, populations, and ethnic groups (10).

*Table 7.1*

**Candidate genes for hypertension** (Ding K. Kullo I.. Circulation. 2009)

| Gene | Signature of Selection | Mode of Selection | Molecular Function |
|---|---|---|---|
| Blood pressure | | | |
| ACE2 | Increased nucleotide diversity | Balancing | Regulates cardiovascular and renal function; an insertion/ deletion polymorphism is associated with hypertension |
| AGT | Increased derived allele frequency | Positive | Regulates salt metabolism; a regulatory polymorphism (A-6G) is associated with hypertension |
| CYP3A5 | High FST; reduced nucleotide diversity | Positive | Involved in cortisol and/or aldosterone metabolism; the CYP3A5*3 polymorphism is associated with hypertension |
| GNB3 | Long haplotypes | Positive | Mediates G protein–coupled signaling; the C825T polymorphism is associated with hypertension |
| GRK4 | High FST (blacks vs Asians); long haplotypes | Positive | Phosphorylates the activated forms of G protein–coupled receptors; a nonsynonymous variant (R65L) is associated with hypertension |
| SCG2 | Reduced nucleotide diversity; increased derived allele frequency | Positive | Neuropeptide with potent angiogenic activity; a common regulatory variation has been implicated in blood pressure control and susceptibility to hypertension |

HDL indicates high-density lipoprotein. ACE2 is the angiotensin I-converting enzyme 2 gene; AGT, angiotensinogen gene; CYP3A5, cytochrome P450, family 3, subfamily A, polypeptide 5 gene; GNB3, guanine nucleotide binding protein (G protein), β polypeptide 3 gene; GRK4, G protein–coupled receptor kinase 4 gene; SCG2, secretogranin II gene.

Candidate gene association studies have sought to connect specific genes with high BP or hypertension. To date, at least 40 genes have been investigated for association with BP-related traits (11). As has been the case with other cardiovascular phenotypes, however, almost every published positive hypertension- gene variant association result has been followed by a published negative result (12). The reasons for these inconsistencies may include genetic heterogeneity, confounding by environmental factors or phenotypic heterogeneity.

Genes frequently investigated in association studies include those for α1b-adrenergic receptor, β2-adrenergic receptor, angiotensin I-converting enzyme (ACE) and endothelin 1 (13,14). As with linkage studies, meta-analyses have been conducted in an effort to integrate findings from independent association studies. In a meta-analysis of 127 studies, Sethi and colleagues concluded that the angiotensin I (AGT) M235T genotype was associated with an increased risk of hypertension in white and Asian population. In their meta-analysis of the four networks of the National Heart, Lung, and Blood Institute (NHLBI) Family Blood Pressure Program, Province et al. (15) concluded that the AGT 6 G–A polymorphism had minimal to no effect on interindividual variation of BP levels.

## Gene–gene and gene–environment interactions

Blood pressure is influenced by multiple genes interacting with each other and with the environment. Gene-gene interaction, or

"epistasis," occurs when the actions of two or more genes influence a phenotype. Much of the difficulty in the genetic dissection of essential hypertension stems from the epistatic nature of the phenotype. However, recent research has begun to explain the complex interplay of genes influencing BP. Kardia and colleagues (14,16) recently found significant evidence of interaction between the ACE in/del and the AGT 6 G–A polymorphisms and systolic BP in men and women.

Gene-environment interaction occurs when the same genotype produces a different phenotype under different environmental exposures, such as age, salt consumption, or a pharmacological treatment such as an ACE inhibitor or diuretic. Weder AB. (2007) has summarized hypotheses that have been put forth to explain hypertension in the framework of evolution (17). Differential susceptibility to hypertension is likely in part due to our history of adaptation to climatic changes (18). For example, the sodium hypothesis (19) posits that sodium-conserving mechanisms conferred a survival advantage among our ancestors in the hot and humid climate of Africa but may lead to hypertension in temperate climates. Thus, sodium-conserving genotypes in US blacks may predispose to excessive sodium retention and sodium-sensitive hypertension in their current milieu. The sodium-conserving alleles show strong latitudinal gradients in allele frequency (clines) and are more prevalent in Africans than in populations from northern Europe, in whom signatures of positive selection (eg, high levels of LD and low haplotype diversity) are noted for the derived allele. The genetic patterns observed in candidate genes affecting sodium handling could have implications for the diagnosis and treatment of hypertension in geographically and ethnically defined populations around the world (20).

The geographic distribution of the A(-6)G variant in the promoter region of the human angiotensinogen (AGT) gene suggests that the G(-6) variant has been selectively advantageous outside Africa. The G(-6) variant, the derived allele, is present at higher frequency in Asians and Europeans than in Africans, and evidence exists of a selective sweep in the vicinity of the polymorphism because haplotypes carrying the derived G(-6) allele showed elevated levels of LD in non-African populations. Genetic drift is not a likely explanation because frequencies of other AGT alleles are not similarly affected (21). Other examples of correlation between latitude and "heat-adapted" alleles include variants in CYP3A5 (22), GNB3, ADRB2, and SCNN1A (*table 7.1).* Among these alleles, the variant 825T in GNB3 may account for a significant portion of worldwide variation in blood pressure (23). Blood pressure response to a low-sodium diet has been shown to vary by polymorphisms of renin–angiotensin–aldosterone system genes, in particular the AGT 6 G–A polymorphism. Results from the Dietary Approaches to Stop Hypertension (DASH) study showed the AGT 6 AA genotype is associated with a significant decrease in blood pressure (6.93 mmHg systolic and 3.68 mmHg diastolic) for individuals on the DASH diet (24). Similarly, in the Treatment of Hypertension Prevention Trial, the incidence of hypertension was significantly lower after sodium reduction for persons with the AA genotype [relative risk 0.57 (95% confidence intervals 0.34, 0.98) versus usual care] but not for persons with the GG genotype [relative risk 1.2 (95% confidence intervals 0.79, 1.81), test for trend $P<0.02$] (25). Based on such results, individuals with the AGT 6 AA genotype could be placed on a low-sodium diet prior to exhibiting elevated BP, thereby avoiding hypertension and consequent organ damage.

## Genetic loci asssociated with hypertension

The proponents of genome-wide association (GWA) studies assert that such research has already provided initial insights into the genetic basis of hypertension and that larger genome-wide studies are warranted in the future (26). To see that we have learned very little about the genetic basis of hypertension from the large genome-wide association studies conducted to date, it is helpful to consider what the results of GWA studies have actually shown so far and what was known about the genetic architecture of hypertension before the advent of GWA studies.

Recently, the results of 2 large GWA studies (26) relevant to hypertension have been reported: 8 loci (chromosome regions) were found to be associated with effects on blood pressure (BP) at a genome-wide level of statistical significance. They identified association between systolic or diastolic blood pressure and common variants in eight regions near the CYP17A1 ($P= 7 X10^{-24}$), CYP1A2 ($P = 1 X 10^{-23}$), FGF5 ($P = 1 X 10^{-21}$), SH2B3 ($P= 3 X 10^{-18}$), MTHFR ($P= 2 X 10^{-13}$), c10orf107 ($P= 1 X 10^{-9}$), ZNF652 ($P= 5 X 10^{-9}$) and PLCD3 ($P= 1 X 10^{-8}$) genes. All variants associated with continuous blood pressure were associated with dichotomous hypertension. The implicated loci had only tiny effects on BP (<1 mm Hg or so) and typically accounted for <0.2% of the overall BP variation in the study populations. Each of these loci was associated with miniscule effects on BP, with each accounting for <0.1% of the total variation in BP.

For SBP, the strongest evidence for association was at 10q24 (rs11191548, MAF = 0.09, 1.16 mm Hg higher per major allele, $P = 7X10^{-24}$, *table 7.2*). This SNP is part of a large cluster of associated SNPs spanning a ~430-kb region at 10q24. The locus includes six genes, most notably CYP17A1, which encodes the cytochrome P450 enzyme CYP17A1 (also known as P450c17) that mediates steroid 17-a-hydroxylase and 17,20-lyase activity.

The first enzymatic action is a key step in the biosynthesis of mineralocorticoids and glucocorticoids that affect sodium handling in the kidney and the second is involved in sex-steroid biosynthesis. Missense mutations in CYP17A1 cause one form of adrenal hyperplasia characterized by hypertension, hypokalemia and reduced plasma renin activity (27).

The second locus associated with SBP was at 1p36 (rs17367504, MAF 0.14, 0.85 mm Hg lower SBP per minor allele, P= 2 $X10^{13}$, *table 7.2*). This SNP is located in an intron of the MTHFR (methylenetetrahydrofolate reductase) gene in a region with many plausible candidate genes, including MTHFR, CLCN6, NPPA, NPPB and AGTRAP. The strongest signal in the locus is 6.4 kb away from and uncorrelated with rs1801133 (C677T, A222V, $r^2$ CEU = 0.06), a coding variant that has been related to higher plasma homocysteine concentration, pre-eclampsia, and variably hypertension (28). In Global BP gen study the locus rs1801133 was associated with 0.08 mm Hg higher SBP per T allele (P= 0.56), 0.24 mm Hg higher DBP (P= 0.01) and an odds ratio for hypertension of 1.00 (95% CI = 0.94–1.05, P= 0.90).

The natriuretic peptides encoded by NPPA and NPPB genes, also located within the 1p36-associated interval, have vasodilatory and natriuretic properties and the NPPA knockout mouse has salt-sensitive hypertension (29). A recent study found that the minor allele of rs5068 (43 kb from rs17367504, $r^2$ CEU= 0.26), in the 3' untranslated region of NPPA, was associated with higher plasma atrial and B-type natriuretic peptide, as well as lower SBP, DBP and odds of hypertension (30). The less well-characterized gene CLCN6, also at the 1p36 locus, encodes a neuronally expressed chloride channel that has not previously been implicated in blood pressure physiology, although rare mutations in other renally expressed chloride channels are associated with extremes of blood pressure

(31). Lastly, AGTRAP (encoding angiotensin II receptor-associated protein) negatively regulates angiotensin II signaling by interacting with the angiotensin II type 1 receptor, a critical component of the renin-angiotensin-aldosterone system (32).

The third locus associated with SBP was at 17q21 (rs12946454, MAF 0.28, 0.57 mm Hg higher SBP per minor allele, $P= 1 X 10^{-8}$, *table 7.2)*. This SNP is located in an intron in PLCD3 (phospholipase C-delta isoform), and is part of a cluster of associated SNPs. PLCD3 is a member of the phospholipase C family of enzymes, important in vascular smooth muscle signaling and activated by the vasoactive peptides angiotensin II and endothelin (33).

The DBP SNP with the strongest association evidence on joint analysis was rs1378942 (MAF=0.36, 0.43 mm Hg higher per minor allele, $P=1X10^{-23}$, *table 7.2*), which is in an intron of CSK at 15q24 and is one of a cluster of associated SNPs spanning ~72 kb. Genes in the region include CYP1A2 (cytochrome P450 enzyme), CSK (c-src tyrosine kinase), LMAN1L (lectin mannose-binding1 like) and ARID3B (encoding AT-rich interacting domain protein). Other nearby genes include CYP1A1 (~60 kb) and CYP11A1 (~418 kb). Cytochrome P450 enzymes are responsible for drug and xenobiotic chemical metabolism in the liver and cellular metabolism of arachidonic acid derivatives (34), some of which influence renal function, peripheral vascular tone and blood pressure. CYP1A2 is widely expressed, representing 15% of CYP450 enzymes produced in the liver and mediating the metabolism of multiple medications. A correlated SNP, rs762551 (MAF= 0.31, $r^2= 0.63$, HapMap CEU) in an intron of CYP1A2 has been found to influence caffeine metabolism (35). The ARID3B gene is embryonic lethal when knocked out in mouse, with branchial arch and vascular developmental abnormalities (36), but is potentially interesting because of the presence of ARID5B at the 10q21 locus.

*Table 7.2.*

**Relationship of SNPs at 8 genome-wide significant loci to both blood pressure traits**

| SNP ID | Chr | Coded allele | Non-coded allele | Coded allele frequency | N (effective) | Trait | P |
|---|---|---|---|---|---|---|---|
| rs17367504 | 1 | G | A | 0.14 | 34,158 | SBP | $1\ 10^{-5}$ |
| | | | | | | **DBP** | $\mathbf{3\ 10^{-5}}$ |
| rs11191548 | 10 | T | C | 0.91 | 33,123 | SBP | $3\ 10^{-7}$ |
| | | | | | | **DBP** | $\mathbf{2\ 10^{-4}}$ |
| rs12946454 | 17 | T | A | 0.28 | 32,120 | SBP | $4\ 10^{-6}$ |
| | | | | | | **DBP** | $\mathbf{6\ 10^{-4}}$ |
| rs16998073 | 4 | T | A | 0.21 | 26,106 | DBP | $7\ 10^{-9}$ |
| | | | | | | **SBP** | $\mathbf{1\ 10^{-5}}$ |
| rs1530440 | 10 | T | C | 0.19 | 32,718 | DBP | $3\ 10^{-6}$ |
| | | | | | | **SBP** | $\mathbf{7\ 10^{-3}}$ |
| rs653178 | 12 | T | C | 0.53 | 30,853 | DBP | $1\ 10^{-7}$ |
| | | | | | | **SBP** | $\mathbf{3\ 10^{-4}}$ |
| rs1378942 | 15 | C | A | 0.36 | 34,126 | DBP | $6\ 10^{-8}$ |
| | | | | | | **SBP** | $\mathbf{2\ 10^{-6}}$ |
| rs16948048 | 17 | G | A | 0.39 | 34,052 | **DBP** | $\mathbf{2\ 10^{-6}}$ |
| | | | | | | **SBP** | $\mathbf{2\ 10^{-3}}$ |

For each of eight SNPs, the upper row shows association statistics for the blood pressure trait used for the analysis in which they were selected (SBP or DBP). The lower row (in boldface) shows the equivalent association statistics for the alternate blood pressure trait. Results are shown for the 34,433 individuals in the stage 1 Global BPgen GWAS samples.

The second DBP SNP is rs16998073 (MAF= 0.21, 0.50 mm Hg higher per minor allele, P = 1 $X10^{-21}$, *table 7.2*), which lies 3.4 kb upstream of FGF5 (fibroblast growth factor 5) on 4q21. The FGF5 protein is a member of the fibroblast growth factor (FGF)

family that stimulates cell growth and proliferation in multiple cell types, including cardiac myocytes, and has been associated with angiogenesis in the heart (37).

The third DBP SNP, rs653178 (MAF = 0.47, 0.46 mm Hg lower DBP per major allele, P = 3 $X10^{-18}$, *table 7.2*) at 12q24 is in an intron of the ATXN2 gene. This SNP was perfectly correlated with a missense SNP in exon 3 of SH2B3 (rs3184504, R262W). The minor allele of rs3184504, associated with higher DBP, has recently been associated with increased odds of type 1 diabetes (38), myocardial infarction, hypertension and higher eosinophil and other blood cell counts (39). There were no other SNPs previously reported to be associated with type 1 diabetes, celiac disease or myocardial infarction and blood pressure. SH2B3 is expressed in hematopoietic precursor cells and in endothelial cells (40). Murine knockout of the SH2B3 gene (also known as lymphocyte-specific adaptor protein, LNK) was associated with increased hematopoietic progenitors of several lineages (41), suggesting that the minor allele of the missense SNP in humans results in a loss of SH2B3 function. In response to inflammatory stimuli, LNK seems to be a negative regulator of inflammatory signaling pathways in the endothelial cell, a cell type central to both blood pressure regulation and the process of atherosclerosis.

The fourth DBP SNP, rs1530440 (MAF= 0.19, 0.39 mm Hg lower per minor allele, P = 1$X10^{-9}$, *table 7.2*) at 10q21 was intronic and one of a cluster of SNPs in C10orf107, an open reading frame of unknown function. Nearby genes include ARID5B (A- rich interactive domain 5B (MRF1 like)), TMEM26 (transmembrane protein 26), RTKN2 (RhoA GTPase effector, rhotekin-2) and RHOBTB1 (RhoBTB GTPase). The Rho family of GTPases converts guanine triphosphate to inactive guanine diphosphate. The actions relating to other GTP-modulating enzymes may modulate salt-sensitive hypertension (42). The ARID5B gene is a member of the AT-rich interaction domain family of transcription factors and

is highly expressed in cardiovascular tissue and involved in smooth muscle cell differentiation (43).

The fifth DBP SNP, rs16948048 (MAF 0.39, 0.34 mm Hg higher DBP per minor allele, P = 5 $X10^{-9}$, *table 7.2*) at 17q21 was upstream of ZNF652 (zinc finger protein 652) and PHB (prohibitin). Neither gene has previously been implicated in hypertension or other cardiovascular phenotypes.

## References:


1. Hamilton M, Pickering GW, Roberts JA, Sowry GS. The aetiology of essential hypertension. The role of inheritance. Clinical Science (London) 1954; 13: 273–304.
2. Williams RR, Hunt SC, Hasstedt SJ et al. Are there interactions and relations between genetic and environmental factors predisposing to high blood pressure? Hypertension 1991; 18 (3 Suppl): I29–37.
3. Feinleib M, Garrison RJ, Fabsitz R et al. The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. American Journal of Epidemiology 1977; 106: 284–285.
4. Hunt SC, Hasstedt SJ, Kuida H et al. Genetic heritability and common environmental components of resting and stressed blood pressures, lipids, and body mass index in Utah pedigrees and twins. American Journal of Epidemiology 1989; 129: 625–638.
5. Longini IM, Jr., Higgins MW, Hinton PC et al. Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. American Journal of Epidemiology 1984; 120: 131–144.
6. Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. Nat Genet. 2008; 40: 592–599.
7. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell. 2001; 104: 545–556.

8. Luft FC. Mendelian forms of human hypertension and mechanisms of disease. Clinical Medicine and Research 2003; 1: 291–300.

9. Ding Keyue, Kullo Iftikhar J. Evolutionary Genetics of Coronary Heart Disease. *Circulation.* 2009;119:459-467.

10. Wu X, Kan D, Province M et al. An updated meta-analysis of genome scans for hypertension and blood pressure in the NHLBI Family Blood Pressure Program (FBPP). American Journal of Hypertension 2006; 19: 122–127.

11. Luft FC. Present status of genetic mechanisms in hypertension. Medical Clinics of North America 2004; 88: 1–18, vii.

12. Oparil S, Weber MA. Hypertension: a Companion to Brenner and Rector's The Kidney. W.B. Saunders, Philadelphia, 2000.

13. Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. Arteriosclerosis Thrombosis and Vascular Biology 2003; 23: 1269–1275. 37.

**14. Curocichin Gh. Complexul dereglărilor metabolice la pacienţii hipertensivi: caracteristica clinico-genetică. Teza de doctor habilitat în medicină, 2009.**

15. Province MA, Boerwinkle E, Chakravarti A et al. Lack of association of the angiotensinogen-6 polymorphism with blood pressure levels in the comprehensive NHLBI Family Blood Pressure Program. National Heart, Lung and Blood Institute. Journal of Hypertension 2000; 18: 867–876.

16. Kardia SL, Bielak LF, Lange LA et al. Epistatic effects between two genes in the renin- angiotensin system and systolic blood pressure and coronary artery calcification. Medical Science Monitor 2006; 12: CR150–158.

17. Weder AB. Evolution and hypertension. Hypertension. 2007; 49: 260–265.

18. Hancock AM, Witonsky DB, Gordon AS, Eshel G, Pritchard JK, Coop G, Di Rienzo A. Adaptations to climate in candidate genes for common metabolic disorders. PLoS Genet. 2008; 4: e32.

19. Gleiberman L. Blood pressure and dietary salt in human populations. Ecol Food Nutr. 1973; 1: 143–156.

20. Nakajima T, Wooding S, Sakagami T, Emi M, Tokunaga K, Tamiya G, Ishigami T, Umemura S, Munkhbat B, Jin F, Guan-Jun J, Hayasaka I, Ishida T, Saitou N,

Pavelka K, Lalouel JM, Jorde LB, Inoue I. Natural selection and population history in the human angiotensinogen gene (AGT): 736 complete AGT sequences in chromosomes from around the world. Am J Hum Genet. 2004; 74: 898–916.

21. Danziger RS. Hypertension in an anthropological and evolutionary paradigm. Hypertension. 2001; 38: 19–22.

22. Thompson EE, Kuttab-Boulos H, Witonsky D, Yang L, Roe BA, Di Rienzo A. CYP3A variation and the evolution of salt-sensitivity variants. Am J Hum Genet. 2004; 75: 1059–1069.

23. Young JH, Chang YP, Kim JD, Chretien JP, Klag MJ, Levine MA, Ruff CB, Wang NY, Chakravarti A. Differential susceptibility to hypertension is due to selection during the out-of-Africa expansion. PLoS Genet. 2005; 1: e82.

24. Svetkey LP, Moore TJ, Simons-Morton DG et al. Angiotensinogen genotype and blood pressure response in the Dietary Approaches to Stop Hypertension (DASH) study. Journal of Hypertension 2001; 19: 1949–1956.

25. Hunt SC, Cook NR, Oberman A et al. Angiotensinogen genotype, sodium reduction, weight loss, and prevention of hypertension: trials of hypertension prevention, phase II. Hypertension 1998; 32: 393–401.

26. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Wellcome Trust Case Control C, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Doring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvanen AC, Tognoni G, Lakatta

EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dorr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Volker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Volzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009; 41: 666–6)).,( Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Kottgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009; 41: 677–687.

27. Martin, R.M. et al. P450c17 deficiency in Brazilian patients: biochemical diagnosis through progesterone levels confirmed by CYP17 genotyping. J. Clin. Endocrinol. Metab. 88, 5739–5746 (2003).

28. Qian, X., Lu, Z., Tan, M., Liu, H. & Lu, D. A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. Eur. J. Hum. Genet. 15, 1239–1245 (2007).

29. John, S.W. et al. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. Science 267, 679–681 (1995).

30. Newton-Cheh, C. et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat. Genet. 41, 348–353 (2009).

31. Simon, D.B. et al. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K+ channel, ROMK. Nat. Genet. 14, 152–156 (1996).

32. Daviet, L. et al. Cloning and characterization of ATRAP, a novel protein that interacts with the angiotensin II type 1 receptor. J. Biol. Chem. 274, 17058–17062 (1999).

33. Suh, P.G. et al. Multiple roles of phosphoinositide-specific phospholipase C isozymes. BMB Rep. 41, 415–434 (2008).

34. Nebert, D.W. & Dalton, T.P. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat. Rev. Cancer 6, 947–960 (2006).

35. Sachse, C., Brockmoller, J., Bauer, S. & Roots, I. Functional significance of a C-A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br. J. Clin. Pharmacol. 27, 445–449 (1999).

36. Takebe, A. et al. Microarray analysis of PDGFR alpha+ populations in ES cell differentiation culture identifies genes involved in differentiation of mesoderm and mesenchyme including ARID3b that is essential for development of embryonic mesenchymal cells. Dev. Biol. 293, 25–37 (2006).

37. Vatner, S.F. FGF induces hypertrophy and angiogenesis in hibernating myocardium. Circ. Res. 96, 705–707 (2005).

38. Todd, J.A. et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat. Genet. 39, 857–864 (2007).

39. Gudbjartsson, D.F. et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat. Genet. 41, 342–347 (2009).

40. Fitau, J., Boulday, G., Coulon, F., Quillard, T. & Charreau, B. The adaptor molecule Lnk negatively regulates tumor necrosis factor-alpha-dependent VCAM-1 expression in endothelial cells through inhibition of the ERK1 and -2 pathways. J. Biol. Chem. 281, 20148–20159 (2006).

41. Velazquez, L. et al. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. J. Exp. Med. 195, 1599–1611 (2002).

42. Du, Y.H., Guan, Y.Y., Alp, N.J., Channon, K.M. & Chen, A.F. Endothelium-specific GTP cyclohydrolase I overexpression attenuates blood pressure progression in salt-sensitive low-renin hypertension. Circulation 117, 1045–1054 (2008).

43. Watanabe, M. et al. Regulation of smooth muscle cell differentiation by AT-rich interaction domain transcription factors Mrf2alpha and Mrf2beta. Circ. Res. 91, 382–389 (2002).

*The doctor of the future will give no medicines, but will interest his patients in the care of the human frame, in diet and in the cause and prevention of disease."*

Thomas A. Edison

# Chapter 8. Pharmacogenetics

## Introduction

Pharmacogenetics is the study of genetic basis of variability in response to drugs, including drug action and risk for side effects. Given the large number of drugs currently available for the treatment and prevention of coronary artery disease (CAD) even small sources of variation in drug efficacy and safety have important implications for clinical and public health (1).

The aim of pharmacogenetics and pharmacogenomics is to define the genetic basis of variable drug response, with the posibility to utilize genetic information in drug therapy decision-making (2). Pharmacogenetics is often used to describe the influence of single genes on drug response, while pharmacogenomics often refers to approaches that study large numbers of genes for associating genetic polymorphisms with drug response variability. There are several potential areas in which genetic information might be used to optimize drug therapy. First, response rates across a variety of therapeutic drug classes average about 50% (3). Thus, if it would be possible to identify those who will not benefit from therapy based on their genetic make-up, alternative therapeutic approaches could be pursued. Similarly, drugs are associated

with certain risks, some of which are serious, and identification of those at risk would also be beneficial. Finally, understanding the relationship between drug efficacy and toxicity and genotype might help expedite drug discovery and drug development. In the case of drug discovery, knowledge of the genetic determinants of disease can help identify potential drug targets.

## Research approaches in pharmacogenetics and pharmacogenomics

Pharmacogenetics also has the potential to influence drug development and clinical trial design to achieve greater efficiency, thereby allowing a larger number of more highly effective and cheaper drugs to be available for treatment and prevention of CAD. There are different approaches that can be taken in identifying the genetic variants associated with variable drug response. Historically, the primary approach was to focus on a candidate gene, meaning the gene for a protein that might be reasonably expected to be associated with response variability. Initially, this approach focused on only one or two genes, and a few single nucleotide polymorphisms (SNPs) within those genes. With advances in genotyping technologies, the candidate gene approaches have expanded to include tens to hundreds of genes. Additionally, based on findings from the International HapMap project the studies no longer focus on a few SNPs, but often utilize an approach that selects tag SNPs to explain variability across the entire gene (4). In general the candidate gene approach has been fruitful in pharmacogenetics, owing in part to the fact that the pharmacology and pharmacokinetic properties of the drugs are typically well defined and thus genes that influence response may be easier analyzed. More recently, genome-wide association approaches have become more common. In this approach, 300 000 to 1 million SNPs across the genome are genotyped and then tested for association with the relevant drug response. The goal

here is to identify additional genes that might contribute to drug response variability, particularly those that would not have been intuitive *a priori* in the candidate gene approach.

## Coronary artery disease pharmacogenetics

Antiplatelet, anticoagulant, thrombolytic and antilipidemic drugs are essential to preventing and treating CAD. Whereas the potential for gene-guided warfarin therapy is fairly far advanced, pharmacogenetic studies are just now emerging for aspirin and clopidogrel, and a handful have investigated genetic predictors of response to heparin and glycoprotein IIB/IIIA inhibitors. Pharmacogenetics could also allow for personalized dosing and monitoring of antiplatelet and anticoagulant drugs, and identify potential nonresponders in whom alternative agents or combination therapy might be beneficial. Approximately 5–60% of patients taking aspirin and about 4–30% of patients taking clopidogrel will have cardiovascular events or fail to exhibit adequate inhibition of platelet function *ex vivo*, a phenomenon referred to as antiplatelet "resistance" (5). The glycoprotein IIIA PlA polymorphism (Leu33Pro) is the most commonly studied candidate for aspirin or clopidogrel resistance, although the data are conflicting (6,7). Genetic variations in the target of aspirin (PTGS1) have been associated with aspirin response (8), although polymorphisms (P2Y12) in the target of clopidogrel (9) and other platelet surface glycoproteins (10) have generally not proven to be informative markers of antiplatelet response. Interestingly, since clopidogrel is metabolically activated *in vivo*, cytochrome P450 2C9 (CYP2C9) isoenzyme polymorphisms that confer reduced activity have been linked to diminished clopidogrel response, resulting in higher cardiovascular event rates after coronary stenting (11). Warfarin will likely be the first cardiovascular drug with genotype-guided dosing to reach the clinic. Numerous studies have documented that the vitamin K epoxide reductase (VKORC1) and CYP2C9

variants are important determinants of warfarin dose variability, bleeding risk, and time to stable warfarin therapy. Indeed, in mid-2007, the US Food and Drug Administration (FDA) changed the warfarin label to indicate that physicians should consider lower initial doses in certain genetically defined populations. A number of other strong biological candidates have been studied, but no others have been consistently associated with warfarin dose (12). Early studies focused on the drug metabolizing enzyme CYP2C9, but the subsequent characterization of the target protein's gene, vitamin K epoxide reductase. Along with consideration of other clinical and demographic factors it possible to explain 50–60% of warfarin dosing variability (13). One of the most well-studied areas in pharmacogenetics is altered bioavailability of the active compounds of the enzymes that influence drug effects and genetic variants in the cytochrome P450 system (14). Genetic variants in this system of metabolizing enzymes have the capacity to affect the metabolism of a number of commonly used CAD medications. For example, CYP2D6 facilitates oxidative metabolism of CAD drugs, including antiarhythmics and ß-blockers (15). Depending on the population studied, 1% to 10% of subjects have a variant coding for multiple copies of the gene, producing a rapid or extensive metabolizing phenotype.

Other variants produce unstable, diminished, or absent enzyme activity, yielding a poor drug-metabolizing phenotype. Similarly, the enzymatic metabolism of the S enantiomer of warfarin and losartan is reduced in persons with certain allelic variants of the cytochrome P450, subfamily IIC, polypeptide 9 gene (CYP2C9) that occur in 2% to 13% of subjects studied (16).

Despite the evidence of enhanced or impaired inactivation or clearance of these drugs, there are surprisingly few data concerning the impact of these variants on the clinical safety or efficacy of the drugs in question. The major exception concerns the use of warfarin. Persons with 1 or 2 copies of the ile359leu allele in the CYP2C9

gene require lower warfarin doses to achieve anticoagulation and are at a higher risk for hemorrhagic complications (17). Table 8.1 lists examples of gene variants that influence the safety or efficacy of cardiovascular medications.

*Table 8.1*

**Genes implicated in variable outcomes of drug therapy** (Roden DM, 2003)

| Medicine | Gene | Reported association |
|---|---|---|
| Digoxin | ABCB1 | Variable drug levels resulting from variable bioavailability and clearance |
| Warfarin | CYP2C9 | Greater anticoagulation with hypofunctional alleles |
| Procainamide | NAT2 | Poor acetylators at greater risk for drug-induce |
| QT-prolonging drugs | KCNH2, KCNE2, KCNQ1, KCNE1 | Increased torsade de pointes risk |
| ß-Blockers<br><br>Metoprolol | ADRB1, ADRB2<br><br>CYP2D6 | Altered extent of heart rate slowing or blood pressure lowering<br>Poor metabolizers display greater ß-blockade |
| Lipid-lowering therapy<br><br>Fluvastatin<br>Pravastatin | LIPC<br><br>ABCA1<br>CETP | Variable lipid lowering<br>Fluvastatin resistance<br>Variable regression of atherosclerosis |
| Antiplatelet drugs | ITGB3 | Variable antiplatelet effects ex vivo |
| Antihypertensive drugs<br>ACE inhibitors<br>Losartan, irbesartan | AGTR1<br><br>ACE<br>CYP2C9 | No relation to antihypertensive effects<br>Decreased response in subjects with the DD genotype<br>Greater blood pressure drop with hypofunctional alleles |
| Diuretics | ADD1 | Variable stroke incidence, variable blood pressure response |

There are also examples of gene variants that affect the primary target for drugs or key steps in drug-effector pathways. For example, Chasman et al. (18) identified 2 SNPs in the hydroxymethylglutaryl coenzyme A reductase gene that are associated with significantly smaller reductions in cholesterol in subjects treated with pravastatin.

The interaction of genetic variation with the treatment response of ACE-inhibitors has previously been investigated in a few small studies, which were not randomized or lacked a placebo-control group and, therefore, the reported relations were largely inconclusive. The PERindopril GENEtic association study (PERGENE), a substudy of the EUROPA-trial (2010) randomized 12 218 stable CAD patients to perindopril (8 mg/day) or placebo demonstrated,that the treatment benefit of ACE-inhibitor therapy by perindopril may be modified by variation in two genes in the renin-angiotensin-aldosterone system and kallikrein-bradykinin (BK) systems: the angiotensin-II type I (AT1) receptor gene, and the BK1 receptor gene. In the bradykinin type I -receptor gene; rs12050217 was a strong modifier of the treatment benefit of perindopril. Patients with the AA genotype (62%) had a 7.3% risk of cardiovascular death or myocardial infarction when using perindopril whereas patients with this genotype in the placebo group had a 10.8% risk, which is a 36% event reduction with perindopril [HR 0.64 (95% CI:0.55-0.78)]. For the AG (33.2%) and the GG genotypes (4.7%) the HR for reduction of treatment benefit were 1.02 (0.79-1.29 and 1.10 (0.56-2.19), respectively. The P-values for interaction were 0.004 (empirical) and 0.012 (permuted). In the AT1 receptor gene, rs275651 and rs5182 significantly modified the treatment benefit of perindopril, with empirical P-values of 0.008 and 0.011, and permutated P-values of 0.049 and 0.054 (borderline), respectively. The haplotype analysis confirmed the association between the identified SNPs and

treatment effect modification observed in single SNP analysis. In both genes, the haplotype analysis identified similar alleles to be associated with a decreased treatment benefit of perindopril as the single SNP analysis. These findings show that three out of four patients had an enhanced benefit of ACE-inhibitor therapy (33% reduction of cardiovascular death or MI, up to 54% in patients with pharmacogenetic score= 0). In contrast, one out of four patients experienced no treatment benefit and a possible adverse outcome with perindopril. The latter patients, with the highest score, had the lowest risk in the placebo group (19). Brugts J., et al. (2010) concluded that, by developing a pharmacogenetic score related to treatment response, patients most likely to benefit from such treatment can be selected and distinguished from those without benefit, or even with an adverse response to preventive therapy.

Pharmacogenetics supplies the experimental basis to understand the variability in response to drugs as a function of an individual's genetic makeup. Genetic polymorphisms may influence drug response through a number of mechanisms including pharmacokinetic interactions, pharmacodynamic gene-drug interactions that involve gene products expressed as receptors, and genes that are in the causal pathway of disease and are able to modify the effects of drugs.

## Dyslipidemia pharmacogenetics

Lowering serum lipid levels has been demonstrated to slow progression or even induce regression in atherosclerosis. However, as with any other drug treatment, the magnitude of plasma lipid responses to drug therapies varies considerably among individuals. Several genetic polymorphisms that may play a role in the different responses to lipid-lowering therapy have been identified recently

and thus may predict individual successes in hypolipemic drug treatment.

Variation in response to statins on disease progression also has been associated with genetic polymorphisms. Dyslipidemia is an important worldwide modifiable risk factor for first MI, and is amenable to drug therapy with such agents as HMG-CoA reductase inhibitors (statins), peroxisome proliferator-activated receptor (PPAR-α) agonists (fibrates), niacin, bile acid resins, and cholesterol absorption inhibitors, such as ezetimibe (20). Despite widespread use of drugs to correct lipoprotein abnormalities, there is tremendous variability in drug response in terms of both lipoprotein and inflammatory protein changes and cardiovascular risk reduction (21). Because of the healthcare resources expended on dyslipidemia management and uncertainty in drug responses, pharmacogenetics-enhanced treatment algorithms may prove to be important in streamlining primary and secondary disease prevention. While pharmacogenetics studies have been performed for nonstatin agents, the majority of evaluations have been conducted in cohorts of statin-treated individuals (22).

A polymorphism for the cholesteryl ester transfer protein gene has been associated with the effectiveness of pravastatin on disease progression. About 16% of the Regression Growth Evaluation Statin Study (REGRESS) trial population (men with angiographically documented atherosclerosis) had the B2/B2 genotype, and these subjects did not show any benefit from the use of statins on the progression of atherosclerosis, whereas individuals without this genotype did demonstrate benefit (23). In the pravastatin-randomized patients in the REGRESS trial, the risk of clinical events was associated with a common polymorphism in the promotor of the matrix metalloproteinase stromelysin-1 gene (24). These effects were independent of the effects of pravastatin on lipid levels.

Candidate genes in statin pharmacogenetic studies include those involved in drug metabolism (e.g., CYP enzyme genes), drug transport, cholesterol biosynthesis, and lipoprotein metabolism (25). Furthermore, because of growing interest in the low-density lipoprotein (LDL)-independent effect of statins, candidate genes have grown to include related enzyme targets such as the gene which encodes endothelial nitric oxide synthase. While relatively small published studies are increasing in number, two large studies remain the hallmarks of statin pharmacogenetics to date. The first is a 10-candidate-gene association study of the prospectively executed Pravastatin Inflammation/CRP Evaluation (PRINCE) study (26). In this retrospective analysis, investigators explored whether any of 148 SNPs in the ABCG5, ABCG8, ApoB, ApoE, CETP, CYP3A4, CYP3A5, FDFT1, HMGCR, and LDLR genes were associated with variable lipoprotein responses to pravastatin 40 mg/day among the 1536 individuals treated for 24 weeks. It was found that two highly linked SNPs in the HMGCR gene (whose encoded protein is the target of statin therapy) were associated with variable lipid responses; carriers had roughly 20% smaller reductions in total cholesterol and LDL than individuals with two common copies of the gene. This association remained robust even after rigorously controlling for multiple comparisons. In an even larger study, Thompson et al. (27) investigated whether SNPs in 16 candidate genes were associated with lipoprotein responses to a variety of statins studied in the Atorvastatin Comparative Cholesterol Efficacy and Safety Study (ACCESS) program. In addition to many of the genes studied in PRINCE, the investigators also explored ABCB1, ACE, ApoA1, CYP7A1, LIPC, and LPL. Analysis of this 2735-patient database revealed that the well-studied triallelic polymorphism in APOE (E2/E 3/E4) was associated with variable LDL responses to statins. Several studies have revealed significant interactions between an individual's APOE genotype and plasma lipoprotein lipid changes with statin therapy. In these studies, subjects with

the E2 allele, and sometimes subjects with the E3 allele, were more likely to respond to statin therapy with a favorable reduction in total cholesterol and LDL cholesterol than were subjects with at least 1 E4 allele (28). In addition to these and other fairly large analyses, novel modeling approaches to exploring genetic determinants of statin efficacy (and myotoxicity) are being employed (29). For example, Ruano et al. (30) conducted an analysis of 10 vascular function genes and found that creatine kinase activity in patients taking statins was associated with variation in endothelial nitric oxide synthase and the angiotensin II type 1 receptor genes. These evolving methods highlight increasing activity in the area of statin pharmacogenetic research.

## Clinical implications for pharmacogenetics in dyslipidemia

As with pharmacogenetics in general, there are limitations to the currently published statin pharmacogenetic studies. For example, there is a paucity of whole genome studies in this area, the impact of variable lipoprotein responses on clinical outcomes is uncertain, pharmacogenetic studies of statin pleiotropic effects are limited, and replication of significant genetic associations is inconsistent. However, integrative research programs that include clinicians, basic and translational scientists, statistical geneticists, and bioinformaticians are increasingly being developed. Consequently, it is conceivable that, for such widely used drugs as the statins, future policy interventions (e.g., at the health system or managed care levels) would include genotype-enhanced therapeutic decisions. Particularly relevant to clinicians, there are ongoing efforts to identify the genetic risks for statin-mediated myalgia and myopathy (31). Clinical investigations will likely serve as paradigms for the conduct of pharmacogenetic evaluations of severe, rare adverse drug events. Furthermore, it is clear that LDL lowering is not the sole predictor of statin cardioprotection.

Ongoing pharmacogenetic evaluations of statin antiinflammatory effects may identify an at-risk genotype group that requires clinical intervention with a statin regardless of baseline LDL or lipoprotein treatment response.

## Paradigm for translation of pharmacogenetics knowledge into clinical practice

Genetic variability that influences drug response was first identified through genetic association studies (through either a candidate gene or genome-wide approach) and the importance of the single nucleotide polymorphism/gene was documented in several studies (31-32). At some point it is optimal if there is an understanding of the functional basis for the genetic polymorphism and its clinical association. Next, there must be an ability to sufficiently explain clinical response variability (likely with both genetic and nongenetic factors), and this often requires knowledge of the contribution of multiple genes to the variable response. Finally, in many cases it is necessary to document in a controlled clinical trial that a genotype-guided approach is superior in order for there to be broad uptake in practice.

Clopidogrel is a prodrug that requires bioactivation to an active metabolite, a process for which cytochrome P450 2C19 (CYP2C19) plays a major role. The CYP2C19 gene contains several reduced function polymorphisms, the most common of which is the allele, which leads to a loss of enzymatic activity. Three papers, published simultaneously, reported that carriers of reduced function variant CYP2C19 alleles had increased risk for adverse cardiovascular outcomes (32). Data for these papers arose from both controlled clinical trials and population cohorts, providing insight into the external validity of the findings. These consistent and compelling data suggest CYP2C19 genotyping could quickly become a

standard of care for patients being initiated on clopidogrel therapy. Additionally, certain drugs that inhibit CYP2C19 (e.g. proton pump inhibitors) can, in essence, convert patients to the same phenotype as the genetic polymorphisms. Thus, careful attention to drugs interacting with clopidogrel's metabolism via CYP2C19 are probably also warranted.

The CYP2C19 gene is associated with the conversion of clopidogrel in its active metabolite. Variations in these gene include the loss-of-function CYP2C19 (CYP2C192 and CYP2C193), which slow this conversion and the gain-of-function CYP2C19 allele (CYP2C1917) which facilitates the metabolism of clopidogrel to its active form. In the Clopidigrel in Unstable Angina to Prevent Reccurent Events (CURE) trial, were examined 5 059 patients and clopidogrel provided a similar magnitude of protection against the primary endpoint compared with placebo in carriers of loss–of-function allele and in those who did not carry a loss-of-function allele (33).

In the Atrial Fibrillation Clopidigrel Trial with Irbesartan for Prevention of Vascular Events (ACTICE A) trial, no differences in clopidogrel efficacy were observed between carriers and noncarriers of CYP2C19 loss-of-function allele or between carriers and noncarriers of the gain-of-function CYP2C19 allele (34). In the important investigation PLATO genetic substudy, Wallentin Lars et al. (35) randomized 18 624 patients with acute coronary syndrome and were able to confirm earlier results from smaller trials with respect to the importance of disorders in the CYP2C19 gene. From these data, it might be concluded that personalized therapy targeting patients who carry these genetic variants might help to improve clinical outcome after stent implantation. However, for the clinical role of genetic profiling, multiple unknown factors still remain. While in the majority of trials CYP2C19 genetic polymorphisms have been shown to reduce clopidogrel metabolism

and its clinical effectiveness while other genetic variants remained less important, there are no prospective studies demonstrating a clinical benefit to personalizing antiplatelet therapy based on genotyping.

Commercially available genetic tests that can determine CYP2C19 genotype variants are not routinely reimbursed and point of care assays for patients with ACS are lacking at the moment. Moreover, it is important to point out that CYP2C19 polymorphisms account for only approximately 12% of variability in clopidogrel platelet response, the positive predictive value of CYP2C19 loss-of-function polymorphisms for cardiovascular events in patients with ACS undergoing PCI is low, approximately 12% to 20% and other clinical factors and risk constellations might be of greater clinical importance.

Among 5 752 patients of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) study, Iakoubova O. et al. assessed the effect of pravastatin, compared with placebo, on coronary events according to 719Arg carrier status using proportional hazards models (36). They reported that pravastatin therapy significantly reduced events in 719Arg carriers [hazards ratio (HR): 0.66, 95% CI: 0.52–0.86] but not in noncarriers (HR: 0.94, 95% CI: 0.69–1.28, P=0.09) for interaction between treatment and carrier status. Among those without prior disease, no significant benefit was observed in either carriers or noncarriers.

There was promise for genome-wide association studies, but no such studies had been completed in cardiovascular pharmacogenetics. Published studies now include evaluation of the LDL response to statins, the blood pressure lowering with a thiazide, and statin-induced myopathy. It is the latter for which the findings are most striking. The investigators discovered a

common polymorphism in an organic anion transporter gene (SLCO1B1) associated with statin-induced myopathy, with the data suggesting a 4.5-fold increased risk for myopathy per copy of variant allele (37). This gene is one for which there was mounting evidence from smaller pharmacokinetic studies of a potential role in the disposition of certain statins. Thus, an understanding of the functional underpinnings of the association, along with replication of the association with myopathy in a separate clinical trial, add confidence that the finding is real. Further population-based cohorts will likely need tested to provide greater insight into the clinical utility of testing for this polymorphism prior to statin therapy. But the literature in hand suggesting the use of this information in the clinic may be around the corner. In summary, in the short period between submission to proof stage, there were two major cardiovascular pharmacogenetic findings reported. This high-lights the speed with which additional advances in this field might come in the future.

## Hypertension pharmacogenetics

The complexity of the blood pressure (BP) phenotype itself can lead to discrepancies in findings, and all pharmacogenetic studies should dissect the chain of intermediate phenotypes leading from gene polymorphism to final hypertensive phenotype (38). Given the complexity and number of pathways (and their interactions) regulating BP, the number of genes involved in these pathways (39), and the fact that the pharmacodynamics of some antihypertensives are incompletely understood, implementing this suggestion presents its own substantial challenges. The fact that the genetic basis of essential hypertension can be attributed to many common variants with individually small phenotypic effects certainly also demands that future studies be adequately powered. In terms of clinical relevance, given the incontrovertible

evidence of the familial nature of hypertension, screening family members of probands with hypertension should be a top priority for all clinicians. There are five antihypertensive drug classes recommended as first-line therapy, although data suggest that, despite the plethora of agents available to treat the disease, blood pressure control is far from optimal. Specifically, estimates suggest that 42% of treated hypertensives do not have their BP controlled (i.e., 140/90 mmHg), with another 16% who know they have hypertension but are untreated. These sobering statistics may be influenced in part by the fact that any given antihypertensive will produce only a reasonable antihypertensive response in about 50% of the population when used as monotherapy (40), but there are few mechanisms for identifying which patients will respond to which medications. In the case of treated but uncontrolled hypertensives, it may be possible to improve BP control if those genetically predisposed to respond to a given therapy could be identified prior to therapy. Additionally, data suggest that failure to persist with antihypertensive therapy is high among newly diagnosed hypertensives, and a potential cause of poor persistence is that the prescribed therapy either caused adverse effects or failed to reduce their blood pressure. Additionally, hypertension is treated to reduce the incidence of the long-term adverse cardiovascular outcomes (myocardial infarction, stroke, heart failure, renal failure and while it is possible to measure the BP response to a drug in an individual patient, it is not possible to measure prevention of these adverse outcomes in an individual patient. Thus, there are numerous potential benefits associated with identification of the genetic variants associated with blood pressure and event reduction with antihypertensive drugs. Among the first-line antihypertensive agents, the beta-blockers have the most compelling pharmacogenetic data to date. Numerous (although not all) studies that have investigated the influence of common SNPs in the β1-adrenergic receptor gene (ADRB1) have

shown that the Arg389 homozygous genotype is associated with significantly better BP lowering than those who carry a Gly389 allele (41). The polymorphisms in ADRB1 may have future clinical utility in identifying those hypertensives who are the best candidates for beta-blocker therapy.

Despite many studies on the genetic associations with angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (ARB) response, there have been few consistent findings across pharmacogenetic studies evaluating the drug targets, ACE, and the angiotensin II receptor (42). Two of these large studies found no association of the ACE insertion/deletion (I/D) polymorphism with treatment response for different antihypertensive drugs. There have been some recent papers suggesting that the angiotensinogen gene (AGT) may represent an interesting marker for BP response and outcomes in hypertension, although further work is needed because the directions of the associations are not always consistent. Similarly, in the PERGENE study, the proxy of the ACE I/D polymorphism, rs4343, was not related with the treatment benefit of perindopril. A limitation of the previous studies was that they focused on one single polymorphism, which does not account for the complexity of the RAAS and KB systems. To allow truly meaningful conclusions, it is necessary to comprehensive coverage of all RAAS and KB systems genes, with multiple tagging SNPs within multiple candidate genes in a common pathway.
For an initial replication of PERGENE study findings, the reseachers had the opportunity to use data of 1051 European patients of PROGRESS studying the ACE-inhibitor perindopril. The PROGRESS study was designed to optimize BP treatment in 1051 European stroke patients. The treatment benefit in PROGRESS was known to be contingent on the combination with indapamide (2.5 mg). The combined P-values improved for all three SNP's by adding PROGRESS to the EUROPA data set, which lent support to the PERGENE study findings. Despite the similar trend in interaction

effect, the individual interaction terms of the three SNP's (in the BK I-receptor gene: rs12050217 ans in the AT1 receptor gene: rs275651 and rs5182 ) in PROGRESS did not reach statistical significance. This lack of significance is most likely related to limited power because of the relatively small number of patients which could be analysed in PROGRESS (n=1051, 526 perindopril, 525 placebo). Furthermore, the genetic risk score showed concordance in the treatment interaction effect in PROGRESS and PERGENE studies. The relative change in treatment benefit associated with the genetic variants was identical in both trials (19).

Regarding the diuretics, there have been several studies (but not all) suggesting an association with the α-adducin gene (ADD1) and BP response (43), along with one suggesting different outcomes based on genotype among diuretic-treated hypertensives (44). Recently, carriers of some ADD1 alleles taking diuretic therapy were found to be at lower risk of MI or stroke than individuals using other types of antihypertensive medications (45). However, the latter findings were not replicated in genetic substudies from the very large ALLHAT (46) and INVEST (47) studies.

Other studies have reported interactions with respect to blood pressure response between diuretics and variants in the nitric oxide synthase 3 gene (48), ß-blockers and the guanine nucleotide-binding protein (GNB3) ß3 gene (49) and angiotensin II type 1 receptor antagonists and angiotensin-converting enzyme.

## Clinical implications for pharmacogenetics in hypertension

Pharmacogenetics has a high potential clinical value in hypertension, through helping to identify the drug that either would lead to the best BP lowering or would be associated with the greatest event reduction. At present ADRB1 polymorphisms

(notably Arg389Gly) represent the only example with potential for near-term translation to practice. For this gene, there have been numerous genetic association studies suggesting the Arg389 homozygous genotype has the greatest blood pressure lowering, with more recent data suggesting that beta-blockers provide superior reduction in adverse cardiovascular events for those with this genotype (50). If the latter findings can be replicated in independent cohorts, this would suggest that those with the target genotype/haplotype should receive beta-blockers to reduce adverse cardiovascular outcomes. For ADD1 and the diuretic response, several studies suggest an association with blood pressure response, but this does not appear to translate into differences in outcomes. The other potentially interesting gene at present is AGT, which may influence response to ACE inhibitors (and presumably ARBs), but further work is required before such information could be translated to practice.

Many trials would appear to have great potential for translation to clinical practice in the next few years including beta-blockers in hypertension, clopidogrel and statins (for statin induced myopathy). In each of these cases, the accrued evidence is near the top of the pyramid. At the top of the knowledge pyramid are clinical trials to document the superiority of a genotype-guided approach. Others are likely to follow, but will require additional research to move the knowledge base around them to higher steps of the pyramid. There is also great potential for pharmacogenetics to aid in drug development. While there are numerous challenges ahead, it appears that the use of genetic information to improve drug or dose selection and outcomes with cardiovascular drugs is realistic in the next decade.

## References:


1. Arnett Donna K., Alison E. Baird, Ruth A. Barkley, Craig T. Basson, Eric Boerwinkle, Santhi K. Ganesh, David M. Herrington, Yuling Hong, Cashell Jaquish, Deborah A. McDermott, Christopher J. O'Donnell, Relevance of Genetics and Genomics for Prevention and Treatment of Cardiovascular Disease. *Circulation.* 2007;115:2878-2901.
2. Piquette-Miller M, Grant DM. The art and science of personalized medicine. Clinical Pharmacology and Therapeutics 2007; 81: 311–315.
3. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. Trends in Molecular Medicine 2001; 7: 201–204.
4. de Bakker PI, Burtt NP, Graham RR et al. Transferability of tag SNPs in genetic association studies in multiple populations. Nature Genetics 2006; 38: 1298–1303.
5. Geisler TMD, Gawaz MMD. Variable response to clopidogrel in patients with coronary artery disease. Seminars in Thrombosis and Hemostasis 2007; 33: 196–202.
6. Lev EI, Patel RT, Guthikonda S et al. Genetic polymorphisms of the platelet receptors P2Y(12), P2Y(1) and GP IIIa and response to aspirin and clopidogrel. Thrombosis Research 2007; 119: 355–360.
7. Macchi L, Christiaens L, Brabant S et al. Resistance in vitro to low-dose aspirin is associated with platelet PlA1 (GP IIIa) polymorphism but not with C807T(GP Ia/ IIa) and C-5T Kozak (GP Ibalpha) polymorphisms. Journal of the American College of Cardiology 2003; 42: 1115–1119.
8. Maree AO, Curtin RJ, Chubb A et al. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. Journal of Thrombosis and Haemostasis 2005; 3: 2340–2345.
9. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E et al. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme to variability in individual responsiveness to clopidogrel. Arteriosclerosis Thrombosis and Vascular Biology 2006; 26: 1895–1900.
10. Jefferson BK, Foster JH, McCarthy JJ et al. Aspirin resistance and a single gene. American Journal of Cardiology 2005; 95: 805–808.
11. Suh JW, Koo BK, Zhang SY et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking

clopidogrel. CMAJ: Canadian Medical Association Journal 2006; 174: 1715–1722.

12. Suh JW, Koo BK, Zhang SY et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. CMAJ: Canadian Medical Association Journal 2006; 174: 1715–1722.
13. Schwarz UI, Stein CM. Genetic determinants of dose and clinical outcomes in patients receiving oral anticoagulants. Clinical Pharmacology and Therapeutics 2006; 80: 7–12.
14. Meyer UA, Zanger UM. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu Rev Pharmacol Toxicol. 1997; 37: 269–296.
15. Yoon YR, Cha IJ, Shon JH, Kim KA, Cha YN, Jang IJ, Park CW, Shin SG, Flockhart DA, Shin JG. Relationship of paroxetine disposition to metoprolol metabolic ratio and CYP2D6*10 genotype of Korean subjects. Clin Pharmacol Ther. 2000; 67: 567–576.
16. Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol. 1998; 45: 525–538.
17. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet. 1999; 353: 717–719.
18. Chasman DI, Posada D, Subrahmanyan L, Cook NR, Stanton VP Jr, Ridker PM. Pharmacogenetic study of statin therapy and cholesterol reduction. JAMA. 2004; 291: 2821–2827.
19. Brugts J., Isaacs A., Boersma E., et al.Genetic determinants of treatment benefit of the angiotensin-converting enzyme-inhibitor perindopril in patients with stable coronary artery disease. Eur Heart J (2010) 31 (15): 1854-1864.
20. Yusuf S, Hawken S, Ounpuu S et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364: 937–952.
21. Libby P. The forgotten majority: unfinished business in cardiovascular risk reduction. Journal of the American College of Cardiology 2005; 46: 1225–1228.

22. Johnson JA, Zineh I. Pharmacogenetics and personalized medicine. In: Dzau VJ, Liew CC, ed. Cardiovascular Genetics and Genomics for the Cardiologist. Blackwell Publishing; Oxford, England 2007: 250–276.

23. Kuivenhoven JA., Jukema JW., Zwinderman AH., et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. N Engl J Med. 1998; 338: 86–93.

24. de Maat MP, Jukema JW, Ye S, et al. Effect of the stromelysin-1 promoter on efficacy of pravastatin in coronary atherosclerosis and restenosis. Am J Cardiol. 1999; 83: 852–856.

25. Zineh I. HMG-CoA reductase inhibitor pharmacogenomics: overview and implications for practice. Clinical Cardiology 2005; 1: 191–206.

26. Chasman DI., Posada D., Subrahmanyan L. et al. Pharmacogenetic study of statin therapy and cholesterol reduction. JAMA: the Journal of the American Medical Association 2004; 291: 2821–2827.

27. Thompson JF., Man M., Johnson KJ. et al. An association study of 43 SNPs in 16 candidate genes with atorvastatin response. Pharmacogenomics Journal 2005; 5: 352–358.

28. Zineh I. Pharmacogenetics of response to statins. Current Atherosclerosis Reports 2007; 9.

29. McCarty C, Wilke R, Giampietro P et al. The Marshfield Clinic Personalized Medicine Research Project (PMRP): design, methods and initial recruitment results for a population-based DNA Biobank. Personal Medicine 2005; 2: 49–79.

30. Ruano G., Thompson PD., Windemuth A. et al. Physiogenomic analysis links serum creatine kinase activities during statin therapy to vascular smooth muscle homeostasis. Pharmacogenomics 2005; 6: 865–872.

31. Jacquelyn K. Beals Gene Variant May Predict Response to Fenofibric Acid Therapy American Society of Human Genetics (ASHG) 60th Annual Meeting: Abstract 163. Presented November 4, 2010.

32. Simon T., Verstuyft C., Mary-Krause M., et al. Genetic Determinants of Response to Clopidogrel and Cardiovascular Events. N Engl J Med 2008.

33. The Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with

acute coronary syndromes without ST-segment elevation (CURE). *N Engl J Med*. 2001;345:494-502.

34. Guillaume Pare, Shamir R. Mehta, Salim Yusuf, Sonia S. Anand, Stuart J. Connolly, Jack Hirsh, Katy Simonsen, Deepak L. Bhatt, Keith A.A. Fox, John W. Eikelboom CURE ACTIVE: Efficacy and Safety of Clopidogrel compared with Placebo according to CYP2C19 Genotype in over 6000 patients with Non-ST-elevation Acute Coronary Syndromes (CURE trial) and atrial fibrillation (ACTIVE trial), ESC Congress 2010.
35. Wallentin L, James S, Storey RF, Armstrong M, Barratt BJ, Horrow J, Husted S, Katus H, Steg PG, Shah SH, Becker RC; PLATO investigators.Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. Lancet. 2010 Oct 16;376(9749):1320-8.
36. Iakoubovab O., Robertson M,; Tong C., et al. KIF6 Trp719Arg polymorphism and the effect of statin therapy in elderly patients: results from the PROSPER study. European Journal of Cardiovascular Prevention & Rehabilitation: August 2010 - Volume 17 - Issue 4 - pp 455-461.
37. Link E, Parish S, Armitage J, et al. SLCO1B1 variants and statin-induced myopathy–a genomewide study. N Engl J Med 2008; 359: 789–799.
38. Filigheddu F, Troffa C, Glorioso N. Pharmacogenomics of essential hypertension: are we going the right way? Cardiovascular and Hematological Agents in Medicinal Chemistry 2006; 4: 7–15.
39. Chobanian AV, Bakris GL, Black HR et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report. JAMA: the Journal of the American Medical Association 2003; 289: 2560–2571.
40. Materson BJ, Reda DJ, Cushman WC et al. Single-drug therapy for hypertension in men. A comparison of six antihypertensive agents with placebo. The Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents New England Journal of Medicine 1993; 328: 914–921.
41. Johnson JA, Zineh I, Puckett BJ et al. Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clinical Pharmacology and Therapeutics 2003; 74: 44–52.

42. Johnson JA, Turner ST. Hypertension pharmacogenomics: current status and future directions. Current Opinion in Molecular Therapeutics 2005; 7: 218–225.

43. Sciarrone MT, Stella P, Barlassina C et al. ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. Hypertension 2003; 41: 398–403.

44. Psaty BM, Smith NL, Heckbert SR et al. Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. JAMA: the Journal of the American Medical Association 2002; 287: 1680–1689.

45. Davis BR, Arnett DK, Boerwinkle E, Ford CE, Leiendecker-Foster C, Miller MB, Black H, Eckfeldt JH. Antihypertensive therapy, the alpha-adducin polymorphism, and cardiovascular disease in high-risk hypertensive persons: the Genetics of Hypertension-Associated Treatment Study. Pharmacogenomics J. 2007; 7: 112–122.

46. Davis BR, Arnett DK, Boerwinkle E et al. Antihypertensive therapy, the alpha-adducin polymorphism, and cardiovascular disease in high-risk hypertensive persons: the Genetics of Hypertension-Associated Treatment Study. Pharmacogenomics Journal 2007; 7: 112–122.

47. Gerhard T, Gong Y, Beitelshees AL et al. Cardiovascular outcomes, diuretic therapy and the alpha-adducin polymorphism: results for the International Verapamil SR-Trandolapril Study Genetic Substudy (INVEST-GENES). Circulation 2005; 112: II-608.

48. Turner ST, Chapman AB, Schwartz GL, Boerwinkle E. Effects of endothelial nitric oxide synthase, alpha-adducin, and other candidate gene polymorphisms on blood pressure response to hydrochlorothiazide. Am J Hypertens. 2003; 16: 834–839.

49. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. C825T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. Hypertension. 2001; 37 (pt 2): 739–743.

50. Pacanowski MA, Gong Y, Cooper–Dehoff RM et al. Clin Pharmacol Ther 2008; 84: 715–721.

## Summary

Coronary artery disease (CAD) represents the most important cause of sudden cardiac death. More than 80% of sudden cardiac deaths are caused by atherosclerotic CAD, and in the Republic of Moldova, more than 23 647 people die from a cardiac cause each year and this represents 56.1% of all deaths. The data suggest that the CAD epidemic is not currently being properly controlled in the low and middle-income countries, although this trend could be reversed by timely intervention. CAD presents many clinical challenges, which may vary by region or culture due to differences in lifestyle, treatment options and opportunities for prevention. Most of the causes of CAD are related to lifestyle and many of these risk factors are modifiable. According to multinational (52 countries representing every inhabited continent) INTERHEART study, the 9 major CAD risk factors are raised: smoking, dyslipidemia, apoB/apoA ratio, hypertension, diabetes, abdominal obesity, psychological factors, physical inactivity, inadequate vegetable and alcohol consumption. These common modifiable risk factors account for 90% of myocardial infarctions. These findings offer some hope, because they demonstrate that CAD and CAD-related mortality can be prevented.

There is strong evidence that CAD, particularly diseases of early onset, have a genetic basis. However, much less is known about the genetic underpinnings of the common, complex forms of CAD and much work remains to be done in this area. The main objective of human genetic research is to discover new mechanisms of disease. When the search for genes that predispose to CAD started >20 years ago, it was anticipated that genetic polymorphisms might be analogous to the already known CAD risk factors and could be incorporated in a risk model such as the Framingham score to assess the risk of an individual and adopt preventive or therapeutic measures accordingly. Despite years of

intensive research, not a single genetic risk factor is used for risk assessment. The new strategy of genome-wide association studies coupled with the availability of very large cohorts of patients is starting to reveal novel genetic factors that contribute to disease risk. Whether these variants will be clinically more useful than those that were derived from the study of candidate genes still needs to be demonstrated. As time passes, the interest for genetic research on common CAD moves progressively from the direct expectation of risk stratification to the more fundamental understanding of disease origins, their indirect diagnostic and therapeutic implications. The discovery of new drug targets as a consequence of genetic research may considerably modify the therapeutic approach of cardiovascular disorders in middle and long terms. The concept of pharmacogenetics to individualize medicine is emerging rapidly and clinically highly relevant as it has the potential to revolutionize clinical practice. This concept of tailored-therapy by pharmacogenetics may have large impact on future clinical practice. Through pharmacogenetic profiling, physicians may be able to predict the response to preventive treatment and distinguish 'positive responders' and 'negative responders' before the start of drug therapy. Taken together, such pharmacogenetic analyses open up a perspective to individualize preventive therapy which may avoid unnecessary treatment, and considerably reduce health care costs.

In addition it cannot be excluded that genetic testing may be useful to help the clinician in the diagnostic and prognostic assessment of patients. It is probably in the area of pharmacogenetics that the benefit of genotyping for the patient will be the most important. In many instances, however, phenotypic biomarkers that naturally integrate multiple genetic and nongenetic influences are likely to be preferred to genetic biomarkers because the integration of genotypic information and its translation into medical decision will be very challenging.

# Glossary of terms used in genetics

**Allele**: one of the different forms of a gene occupying the same locus (position) on a chromosome.

**Alleles**: variant forms of the DNA sequence at a specified locus. For example, alleles at a SNP are characterized by the nucleotide that is changing. Combination of 2 alleles at a locus forms a genotype.

**Candidate genes**: genes suspected of involvement in the disease process.

**Complex disease**: a disease whose pattern of familial aggregation differs from that expected from the Mendelian inheritance of a single genetic defect.

**Epigenetics**: the study of heritable changes in gene activity that occur without a change in the sequence of the genetic material.

**Gene–environment interaction**: the effect of environmental factors on an association between a genotype and a phenotype. This can also be expressed in the reverse way (eg, in the case of pharmacogenetics where the focus is on how genetic factors affect the effects of a drug).

**Genome-wide association (GWA) study**: a study that investigates the statistical associations between a phenotype and a very large number of genetic markers supposed to inform on the global variability of the genome. Because GWA studies do not rely on *a priori* knowledge, they may lead to the discovery of new causes of disease.

**Genotype**: the genetic constitution of an organism, which it has inherited from its parents. More specifically, the genotype refers to the particular combination of alleles at specified loci present in an organism. At a specified locus (eg, a single base pair on homologous chromosomes), homozygous individuals have identical alleles and heterozygous individuals have different alleles.

**Haplotype**: a combination of alleles that are located at closely linked loci and tend to be inherited together.

**Linkage analysis**: a statistical method that aims to locate a gene causally related to a disease by identifying genetic markers of known chromosomal location that are coinherited with the trait of interest. Linkage analyses are conducted in families with several affected members and may use sets of markers that encompass the whole genome or specific regions.

**Linkage disequilibrium (LD):** polymorphisms in the human genome are often not independent of one another. When a mutation arises, it is associated with particular variants present on the same chromosome. Recombination subsequently acts to erode this association, but for physically close polymorphisms (eg, within a gene), the correlation, known as LD, persists over time. For this and other reasons related to population history and selection, strong statistical associations between nearby polymorphisms are often observed at the population level.

**Locus:** a specific region within a DNA sequence.

**Logarithm of odds of linkage (LOD):** a statistical estimate (relative probability) of whether two loci are linked. A higher LOD score indicates greater evidence of linkage. A LOD score of 3 or higher generally indicates that two loci are close to each other on the chromosome.

**Mendelian disease**: a disease with a pattern of familial aggregation that reflects the inheritance of a single genetic defect.

**Mini- or microsatellite**: a variable locus where alleles are characterized by different numbers of repetitions of the same motif in tandem. Mini- and microsatellites differ by the length of the repeated motif (sequence of 2 to 5 nucleotides for microsatellites, >10 nucleotides for minisatellites).

**Minor allele:** the less common of two or more alleles at a locus.

**Mutation**: in the field of genetic epidemiology, a mutation is a genetic variation with a very low allele frequency (eg, mutations responsible for Mendelian diseases).

**Phenotype**: the phenotype is the actual appearance of an organism or, more specifically in the research context, of a trait of interest. It may be a binary trait (eg, the presence/absence of a disease) or a quantitative trait (eg, the level of blood pressure).

**Polymorphism:** any variation in the sequence of DNA among individuals. In the field of genetic epidemiology, a polymorphism is a common genetic variation (allele frequency >1%).

**Single nucleotide polymorphism (SNP):** the most common type of polymorphism, in which the alleles differ at the level of a single nucleotide.

**Structural variants**: structural rearrangements present in the genome, such as copy-number variants (CNV), segmental duplications, large insertions and deletions, inversions, translocations, or large tandem repeats.

# Abbreviations

ACE- angiotensin I-converting enzyme

ATCH- adrenocorticotropic hormone

ADD1- adducin 1 gene

ATP transporters -binding cassette transporters

ABCA1- via the LXR/RXR nuclear receptor

ABCG5, ABCG8- mutant allele

ABCG5 and ABCG8-adenosine triphosphate binding cassette G5 and G8 genes

adCAD1- the first genetic locus for autosomal dominant form of coronary artery disease,

ALOX5AP- the gene encoding the 5'-lipoxygenase activating protein

ApoB-apolipoprotein B

ApoE--apolipoprotein E

ASK1-apoptosis signal-regulating kinase 1

ARH -activation receptor homology sequence 1

ASK1-apoptosis-signal regulating kinase 1

BH4- tetrahydrobiopterin

BMI- Body Mass Index

BP- blood pressure

CAD- coronary artery disease

CAMs- cellular adhesion molecules

CDKN2A-ARF-CDKN2B- locus

Chr- chromosome

CNPs- copy number polymorphisms

CVD- cardiovascular disease

CD36- receptors that bind modified lipoprotein

CD14- high-affinity receptor for endotoxin

CMV- cytomegalovirus

CETP- cholesteryl ester transfer protein

CSF2-colony-stimulating factor 2 gene

CXCR2- chemokine (C-X-C motif) receptor 2

CRF- conventional risk factors

CRP- C-reactive protein

hsCRP- high-sensitivity C-reactive protein

EEL - external elastic lamina

ESR1-estrogen receptor 1 gene

CCL11-eotaxin

FCHL- familial combined hyperlipidemia

FLAP- 5-lipoxygenase-activating protein

HDL- high-density lipoprotein

HMGB1- high mobility group 1 protein

HVE- hypervariable elements

IEL- internal elastic lamina

ICAM-1- intercellular adhesion molecule-1

IL-1 = interleukin-1

IFN- γ- interferon gamma, secrete cytokines

IMT- intima media thickness

IP-10, I-TAC, and MIG- lymphocyte-selective chemokines

KLF-2- Krupple-like factor-2, transcription factor

GM-CSF- granulocyte-macrophage colony stimulating factor

GJA4- gap junction protein, α-4, also known as connexin 37

GMPR- guanosine monophosphate reductase

GWAS- genome-wide association study (GWA study)

GMPR- guanosine monophosphate reductase

GRA- glucocorticoid remediable aldosteronism

JNK- suppressing Jun kinase

LPL- lipoprotein lipase

LTA- lymphotoxin-alpha gene

LDL - low-density lipoprotein

LDLR -low-density lipoprotein receptor

LOD log - base 10 of the likelihood ratio under the hypotheses of linkage and nonlinkage

LQTS - long QT syndrome

LCAT- lecithin-cholesterol acyltransferase

Lp(a)- lipoprotein (a)

M-CSF- macrophage-colony stimulating factor

MF - macrophage

MI- myocardial infarction

MCP- monocyte chemoattractant protein

M-CSF- macrophage colony-stimulating factor

M-CSF - macrophage colony stimulating factor;

MCP-1 - monocyte chemoattractant protein-1

MTP -microsomal triglyceride transfer protein

MMP3-matrix metalloproteinase-3 also known as stromelysin-1

MTHFR- methylenetetrahydrofolate reductase

NPC1- Niemann-Pick type C disease

NFkB- nuclear factor kappa B

NADH/NADPH- nicotinamide adenine dinucleotide phosphate

OX40- ligand gene

OxLDL -oxidized LDL

PAI-1- plasminogen activator inhibitor-1

PlGF- placental growth factor

PDGF- platelet-derived growth factor

PCSK9 gene - proprotein convertase subtilisin-kexin type 9

PCI- percutaneous coronary interventions

PON1-paraoxonase 1

PPAR-α - peroxisome proliferator-activated receptor agonists (fibrates)

QTL- Quantitative trait locus

ROS1- gene

SMCs -smooth muscle cells

ASK1-signal-regulating kinase 1 an activator of JNK

SNP- single-nucleotide polymorphism

SREBP- cleavage activating protein (SCAP)

SCARB1- scavenger receptor class B member 1 gene

SMPD-1- sphingomyelin phosphodiesterase-1 gene

TGF–β, a constituent of platelet granules

TIMPs- tissue inhibitors of metalloproteinases

THBS- thrombospondin gene

Treg- Regulatory T cells

Th - T helper

TF- tissue factor procoagulant

Txnip- thioredoxin-interacting protein

TGF-b- transforming growth factor

TNF-α - tumor necrosis factor-alpha

t-PA- tissue plasminogen activator

VNTR- variable-number tandem repeat

VCAM-1- vascular cell adhesion molecule-1

VLA-4- integrin, very late antigen-4

VEGF- vascular endothelial growth factor

VLDL- very low-density lipoprotein

## CONTENTS