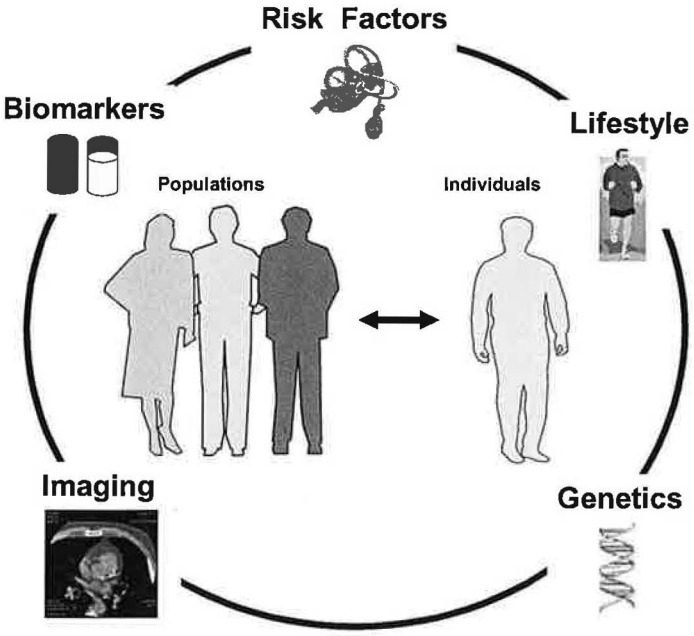
# Cardiovascular Risk Prediction: Changing Paradigms for a Persistent Problem

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## I. INTRODUCTION

Cardiovascular disease (CVD) has been the leading cause of death in the United States every year since 1900, except for during the influenza pandemic of 1918. Currently, approximately 900,000 people succumb to CVD each year, and this illness accounts for more deaths than the next four leading causes combined, including all cancers.[1] Although less widely appreciated, CVD does not discriminate between genders and is also the leading cause of death among women, with a greater number of women dying from this disease than men. In addition to the considerable toll on life, CVD imparts additional heavy burdens on society. The estimated cost of CVD in 2006 totaled to $403 billion and coronary heart disease (CHD) is the leading cause of premature, permanent disability in the U.S. labor force.[2]

CHD alone accounts for approximately 50% of the deaths from CVD and is the major etiologic factor contributing to congestive heart failure and sudden cardiac death. Despite several treatment options, currently there is no cure for this chronic illness. Equally distressing is the fact that 50% of men and 64% of women who die suddenly of CHD will have had no prior warning symptom of this disease. Ultimately, the best treatment for this illness is to prevent its occurrence and advances in the understanding of risk factors for this disease over the past half century have lead to substantial reductions in CVD mortality rates. However, as death rates stabilize, coupled with questions regarding the effectiveness of current preventive strategies and advances in technology, there is growing interest in novel strategies for heart disease prevention and risk assessment.

## II. HISTORICAL PERSPECTIVE ON CARDIOVASCULAR RISK FACTORS

In the early part of the 1900's, several changes in U.S. society contributed to significant increases in CVD death rates, which reached their peak soon after WWII (**Fig 1**).[3] Contributing factors included the transition to an urban, industrialized economy with accompanying changes in diet and reductions in physical activity.[4] The impact of these social changes was accentuated by the concomitant decline in death rates from infectious diseases. Surprisingly little was known at that time about the risk factors for CHD, the principle illness associated with the near epidemic rise in CVD death rates. It was anticipated that the identification of these factors could result in new strategies for the prevention of CVD. The use of epidemiologic methods had previously been implemented primarily in the study of infectious illness, but had not been applied to any significant degree for the study of CVD. In light of the significant public health impact of CVD, the U.S. Preventive Health Service initiated the development of an epidemiologic study of CVD in 1947.[5] The town of Framingham, Massachusetts, a predominantly white, middle-class industrial and trading center 21 miles west of Boston was selected and enrollment of the 5209 men and women began in 1948.

### Identifying coronary heart disease risk factors

Several animal studies and observational reports in the first few decades of the twentieth century suggested a link between cholesterol and dietary fat levels and atherosclerosis.[6] These experimental studies were expanded in the early 1950's and were bolstered by observations of differing CHD rates between countries, attributed to differences in diet and cholesterol levels. In

addition, several smaller epidemiologic studies and the early Framingham experience suggested an association between cholesterol, smoking, and blood pressure with CHD.[6,7] In 1959, the first public statement on CHD prevention and risk factors was presented, including mention of hypercholesterolemia, elevated blood pressure, smoking, obesity, and family history.[8] The original term "risk factor" was coined by Framingham Heart Study investigators in one of their initial manuscripts published in 1961.[9] Subsequent reports by the Framingham investigators and several other epidemiologic studies clarified and confirmed the relationship between major risk factors to incident CV events.[10,11]

The identification of major risk factors for CHD paved the way for preventive efforts to impact rates of CVD. The landmark Surgeon General report on adverse effects of smoking in 1964 hastened the significant decline in smoking rates in the 1960's and 70's.[12] In addition, campaigns to reduce dietary fat intake in the 1960's and 1970's,[13] to treat hypertension in the 1970's and 80's,[14] and to reduce blood lipid levels in the 1980's and 90's[15] have made major contributions to the dramatic reduction of CVD death rates in the past half century. However, since the 1990's, these trends have tapered, in part due to diminishing improvements in CV risk factor levels and to growing epidemics of obesity and diabetes.

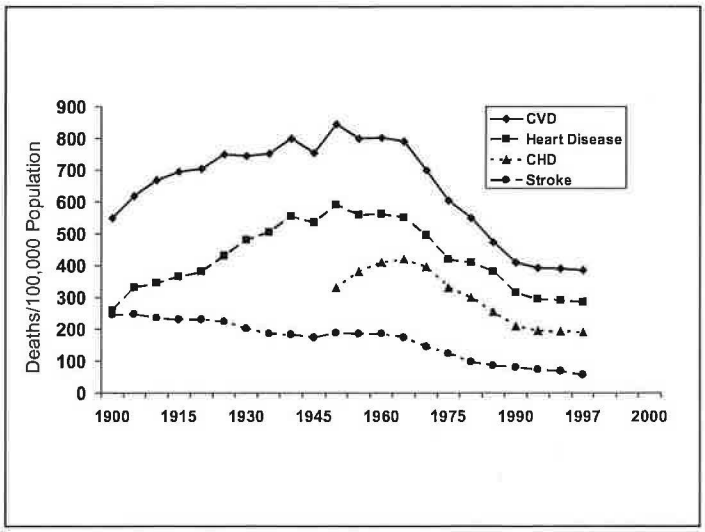



**Fig 1**. Age- adjusted mortality rates from cardiovascular diseases in the U.S. 1900-1997

## III. STRATEGIES FOR PREVENTION OF CARDIOVASCULAR DISEASE

### Population-based strategy

Two different, but complementary approaches have been utilized for the prevention of CVD (**Fig. 2**).[16] The *population-based* strategy attempts to shift the distribution of risk factors in the entire population to a lower average level (ie: shifting the mean blood pressure), often utilizing public health measures. Advantages of this strategy include the potential to make a considerable impact upon the risk for disease. Also, its successful application shifts socially accepted "normal" levels of a risk factor to a lower threshold (ie: decreased social acceptability of smoking). In support of this strategy, several studies have demonstrated that achieving optimal levels of multiple risk factors results in markedly lower rates of CVD.[17-19] Stamler et al examined over 360,000 subjects from the MRFIT and Chicago Heart Association Detection

Project in Industry studies and determined long-term outcomes in those with a low risk factor burden (serum cholesterol <200mb/dl, blood pressure ≤ 120/80 mmHg, no current smoking, no diabetes, no myocardial infarction, normal ECG).[18] Subjects with optimal levels of these risk factors had a 77-92% reduction in CHD death and an increase in life expectancy of 6-10 years compared with those without such profiles. The results of this and other similar studies suggest that shifting the distribution of risk factors in the population to optimal levels could have a major impact on CVD, and should be the cornerstone of public health efforts for CVD prevention.

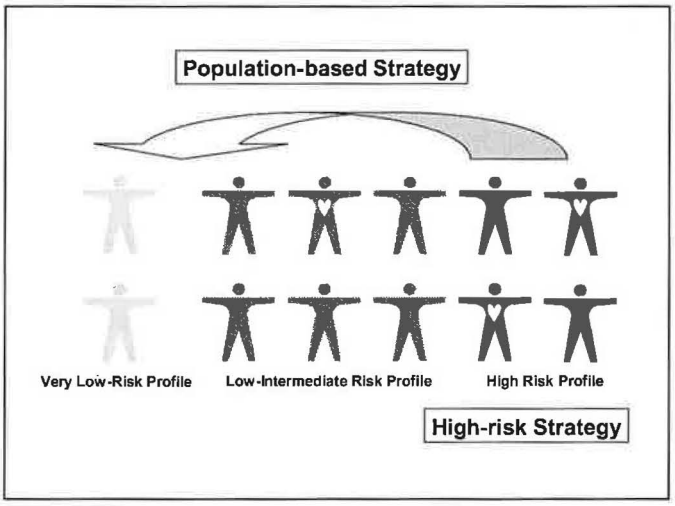



**Fig. 2** Strategies for CHD prevention. Population-based strategies shift the overall distribution of risk factors. High-risk strategies focus on those with high risk profile.

**High-risk strategy**

The population-based strategy also has many shortcomings. While the entire population is impacted by the preventive measures, only a small fraction would have actually been affected by disease, resulting in overtreatment of the majority of individuals. Such considerations are most relevant when pharmacologic therapies or other more aggressive measures are required to treat risk factors, resulting in an unfavorable risk-benefit ratio. An alternative preventive strategy for CVD is termed the *high-risk* strategy.[16] This approach involves setting a threshold of risk, and focusing treatment strategies on individuals who exceed this risk, such as treating blood pressure in a patient once they are considered hypertensive.

Traditional medical practice centers on this approach, which identifies patients with "illness" (or risk), thereby requiring treatment. There are several advantages to this strategy such as providing intervention that are appropriate to the individual, thereby creating a more favorable risk-benefit ratio. Importantly, this strategy also improves cost-effectiveness of various therapies. For example, the cost per quality adjusted life-year gained using HMG-CoA reductase inhibitors (statins) in primary prevention populations is estimated as $54,000 to $1.4 million compared with $1800 to $40,000 for secondary prevention populations that are at higher risk.[20] In addition, knowledge of underlying cardiovascular risk can enhance physician and patient motivation towards adopting preventive measures.[21] This strategy also has several shortcomings including the fact that CV risk associated with risk factors is often graded rather than dichotomous.[22] In addition, lower risk patients who cumulatively have large numbers of CV events are not treated. A major application of the high-risk strategy in CVD prevention is to determine appropriate candidates for pharmacologic lipid lowering therapy use, where the

intensity of treatment is matched to the level of risk.[23] However, a significant challenge remains in accurately assessing CV risk on which to base treatment decisions.

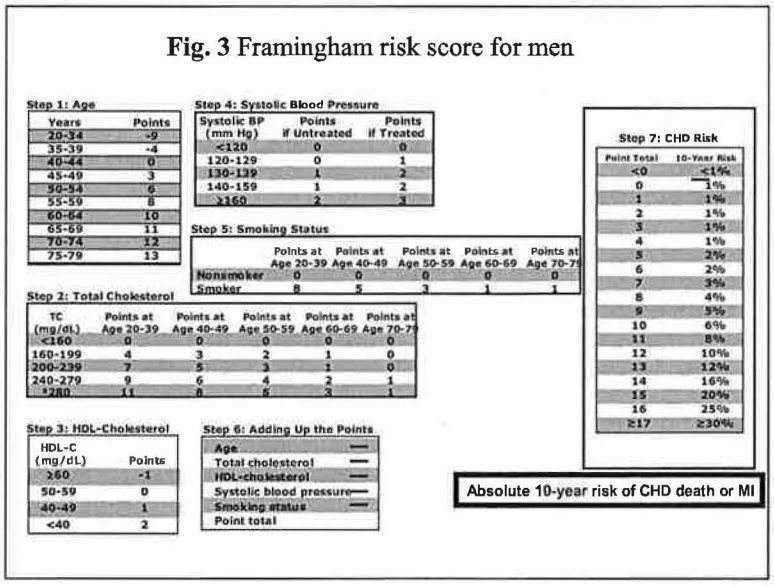
## IV. CURRENT METHODS OF ASSESSING CARDIOVASCULAR RISK

Although individual traditional risk factors may be associated with the development of CVD, they are generally poor discriminators of CV risk when used alone. One explanation for this observation is that the distribution of individual risk factors between those with and without CHD overlaps substantially.[24] In the Women's Health Study, although LDL cholesterol levels were associated with incidence CV events, 46% of these events occurred in women with LDL levels below 130mg/dl.[25] In addition, risk factor interactions are complex and magnify CHD risk, favoring more comprehensive approaches to assessing risk.

### Global risk assessment equations

The limitations of individual risk factors for evaluation of CV risk have lead to the concept of global risk assessment in the form of predictive equations.[26] These algorithms are mathematical functions derived from multivariable modeling of various weighted well-established risk factors, which provide a probability estimates of developing CHD in a given time period. The currently recommended standard for assessing CHD risk in the U.S. is the Framingham Risk Score (FRS) (**Fig. 3**).[23,27] It was derived from the Framingham Heart Study population and a modified version was endorsed by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) report.[23] The FRS consists of a point scoring system based on categories of age, total cholesterol, HDL cholesterol, systolic blood pressure and smoking status, and has separate algorithms for men and women. The point total is converted into an estimate of the 10-year (short-term) absolute risk of "hard" CHD endpoints (CHD death or myocardial infarction).

**Fig. 3** Framingham risk score for men




One routinely used technique to evaluate the utility of risk assessment strategeis is the receiver operating characteristic curve analysis (ROC). The area under the ROC curve (AUC) estimates the probability that the risk function will assign a higher value to those who will develop an

event compared to those who will not.[28] Essentially, it assesses how well the risk function can *discriminate* between affected and unaffected persons with a value of 0.5 (or 50%) being no better than chance and a value of 1.0 being perfect discrimination. An analogous term for AUC is the c-statistic, and values in the range of 0.7-0.9 are considered good while values greater than 0.9 are considered excellent for discrimination. The FRS yields an overall c-statistic of approximately 0.79-0.80 for CHD, which has been found to be reasonably consistent between men and women, and between blacks and whites.[29] However, this algorithm generally overestimates risk in subjects of Asian and possible Hispanic descent.[30,31]

The FRS has several desirable properties which lead to its broad endorsement as the primary method for CV risk assessment.[23,32] As mentioned, it has reasonable ability to discriminate risk from a population perspective and has been validated in several cohorts.[30] Its components are inexpensive to measure and it can be easily applied in an office-based setting with both paper and computer-based tools to facilitate its use.[23] Also, the components of this score, except for age, are largely modifiable risk factors which are thought to be causal in the etiology of atherosclerosis.

**Limitations of current cardiovascular risk assessment strategies**

Despite the many advantages of the FRS, it also has several shortcomings as a method of assessing CV risk. While discrimination ability of 80% may be suitable from a population standpoint, this level may be suboptimal for the individual, especially when faced with such a prevalent illness that is accompanied by significant morbidity and mortality. In addition, global measures of this tools utility, such as the c-statistic, do not adequately describe its accuracy in various subgroups and its functionality in clinical practice.

A recent study by Akosah et al. demonstrated these limitations of the Framingham Risk Score in a real-world setting.[33] The authors performed a retrospective study of 222 young men (≤55 years) and women (≤ 65 years) hospitalized in their institution with an acute myocardial infarction. After calculating 10-year risk based upon the FRS, only 12% of this cohort would have been considered high-risk prior to their event, with 18% categorized as intermediate risk and 70% lower risk. Furthermore, only 25% of men and 18% of women would have been eligible for statin therapy prior to their event based upon their risk category and application of the NCEP ATPIII guidelines. These findings have been supported by other studies demonstrating the inaccuracy of the Framingham Risk Score in young populations and in women.[34,35]

The perceived complexity and time involved in calculating the FRS likely contribute to its limited application by clinicians in practice. Many clinicians rely on numbers of risk factors to determine risk and the appropriateness for therapy. The hazards of this approach were demonstrated in analysis of more than 122,000 patients enrolled in clinical trials of CHD, including myocardial infarction, unstable angina, and percutaneous coronary intervention.[36] In this study, 15% of women and almost 20% of men had none of the 4 conventional CHD risk factors (hypercholesterolemia, hypertension, smoking, and diabetes) upon diagnosis of CHD at trial entry. In addition, more than 50% of women and 60% of men had only 0 or 1 of these risk factors.

Another significant limitation of current risk assessment strategies is inherent to the high-risk approach to CVD prevention in that large numbers of low risk individuals who are not actively treated ultimately have CV events. Using data from the NHANES 1999-2002 study, Ajani et al. determined the proportions of the U.S. population in the low, intermediate, and high-risk categories based on the FRS to be 76%, 11%, and 13% respectively.[37] Based upon a 3% 10-year risk of hard CHD events in the low-risk group, this would cumulatively result in almost 5 million such events over that time period in this untreated population (**Fig 4**). Efforts to better predict seemingly low risk individuals who develop CV events are needed.

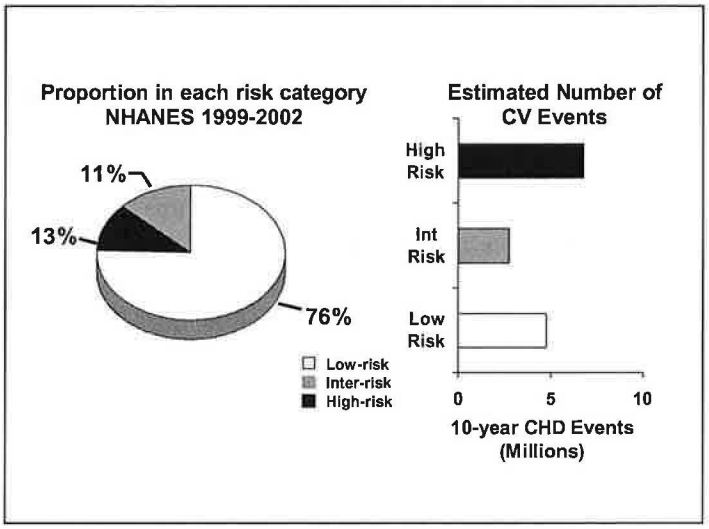



**Fig. 4** Large number of CV events in the low-risk group. The majority of the population is in the low-risk group (left). Cumulatively, this group accounts for several million CHD events over 10 years (right)

## NOVEL STRATEGIES OF CARDIOVASCULAR DISEASE RISK ASSESSMENT

As the many limitations of current CV risk assessment strategies become increasingly evident, technological and scientific advances are providing promising new tools for improving risk prediction. Numerous candidates for improving risk assessment have been proposed and can generally be grouped in three broad categories: biomarkers, atherosclerosis imaging, and genetic markers.

### I. BIOMARKERS

Biological markers, or biomarkers, can broadly be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention".[38] Thus, biomarkers include diverse measures such as blood pressure, a chest x-rays, and troponin I levels. More commonly, they refer to proteins, metabolites, or other materials that are sampled in the blood or urine and provide insights into health and disease. There are multiple potential uses for biomarkers including diagnosing disease, staging illnesses, determining prognosis, identifying responders to therapy, and determining risk for disease.[38,39] Not all biomarkers are equally well-suited for these various indications, and certain biomarkers can be useful for multiple ones.

Biomarkers for use in CV risk assessment can involve many distinct biological pathways that all impact on the propensity for atherosclerotic disease. The best characterized of these biomarkers

include traditional lipid measures such as total and LDL cholesterol. Other categories of biomarkers for CV risk assessment include those related to inflammation, oxidative stress, coagulation and thrombosis, renal function, as well as various others.[39,40] The properties of ideal biomarkers for this purpose include standardized assays, reproducibility on repeated measurements, low cost, and importantly, ability to predict future events in multiple prospective studies.[40] Ideally, these biomarkers would also maximize sensitivity and specificity and provide additional information to currently used standards such as the Framingham Risk Score. Of the many candidate biomarkers currently under evaluation for CV risk prediction, C-reactive protein (CRP) appears the most promising for meeting these criteria.[41]

**C-reactive protein and atherosclerotic disease**

Increasing evidence supports a close link between inflammation and atherosclerotic diseases.[42] Inflammation plays a critical role in all stages of the atherosclerotic process including initiation, progression, and complications of this illness. CRP is a systemic acute phase reactant protein involved in innate immunity that can serve as a barometer for pathologic inflammatory processes related to CVD. It is a pentameric 23 kDa member of the pentraxin family of plasma proteins that was originally named for its ability to precipitate the C-polysaccharide of *Streptococcus pneumonia*.[43] CRP elevation is not specific for atherosclerotic disease and can occur with a broad range if inflammatory stimuli such as infection, autoimmune diseases, malignancy, and trauma. Its initial utility in CVD was hampered because the major range of blood levels in the population were below the threshold of detection of older assays, a deficiency that was remedied by introducing newer, high-sensitivity assays that could detect values in the "normal" range of <3 mg/L.[44]

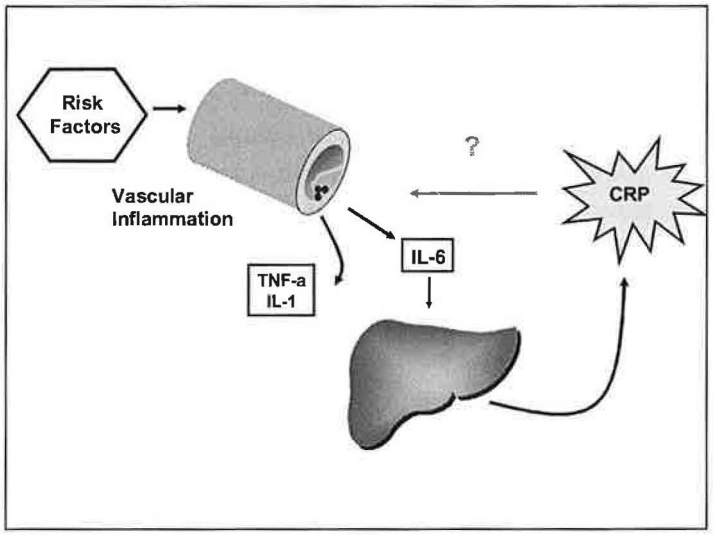



**Fig. 5** Relationship between CRP and CV events. Activated leukocytes in atherosclerotic plaque release IL-6 which stimulate CRP release from the liver.

Various biologic mechanisms explain how CRP may reflect CV risk (**Fig 5**). Activated leukocytes in the evolving atheroma release a host of cytokines, including interleukin-6 (IL-6), TNF-α, and IL-1.[42] These cytokines then act on the liver to stimulate release of acute phase response proteins including CRP, serum amyloid A, and fibrinogen. IL-6 is the principle cytokine involved in CRP release from the liver, [43] and up to one-third of circulating IL-6 may derive from adipose tissue,[45] possibly accounting for the close correlation between obesity and CRP.[26] Small quantities of CRP may also be produced by vascular smooth muscle cells.[46]

Thus, vascular inflammation stimulated by various risk factors and other insults results in CRP production in the liver that is sampled in the blood. As such, CRP may be a better predictor of rupture-prone, inflammatory plaque with activated macrophages rather than the burden of atherosclerotic disease.[47]

An area of contention is whether or not CRP itself is directly involved in the atherosclerotic process.[48] Several lines of evidence suggest that CRP may be a *risk factor* for CVD rather than just a *risk marker*. *In vitro* cell culture experiments and animal studies have provided insights as to how CRP may promote atherothrombosis including inducing the expression of cellular adhesion molecules, decreasing levels of endothelial nitric oxide synthase, increasing levels of endothelin 1 and tissue factor, and promoting the uptake of oxidized LDL.[49] However, the findings of many of these studies have been recently questioned and possibly attributed to the contaminants lipopolysaccharide and azide that are commonly found in commercially prepared CRP preparations.[50,51]

Transgenic mice studies offer additional insights into these issues by providing models of endogenous CRP production. An initial experiment by Paul et al. evaluated CRP hemizyogous transgenic mice on an apolipoprotein E null background and discovered that aortic atherosclerotic lesions were larger in the CRP transgenic mice compared with those without the transgene.[52] Despite this intriguing finding, two subsequent investigations of CRP transgenic mice have found no association with atherosclerosis.[53,54] Human genetic studies of CRP have also provided conflicting reports. Miller et al. resequenced the *CRP* gene in 192 subjects in the Women's Health Study and identified 6 sequence polymorphisms that were consistently related to CRP levels in additional cohorts.[55] However, none of these variants were associated with cardiovascular events in a direction consistent with their effect on CRP in over 1300 cases and controls from the Physician's Health Study. Recently, investigators from the Cardiovascular Health Study recently evaluated the relationships of 5 tag single-nucleotide polymorphisms (SNP) in the *CRP* gene to CRP levels, subclinical atherosclerosis, and CV events. The investigators found three polymorphisms to be associated with CV events in whites, and one in blacks, but none of these polymorphisms were associated with subclinical atherosclerosis measures.[56]

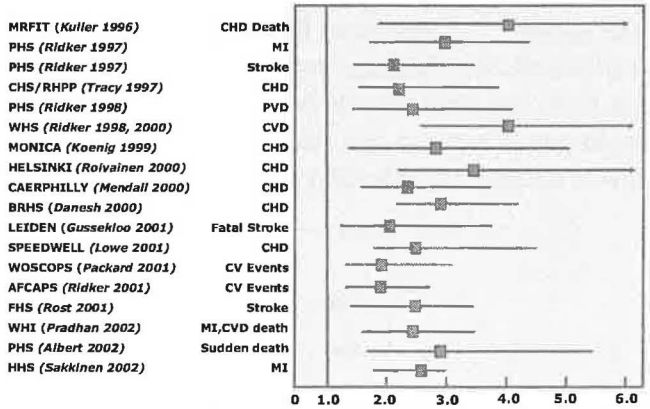
Regardless of any etiologic role in atherogenesis, CRP may still be an important risk marker for CVD. Several biologic and chemical properties of CRP contribute to its appeal as a clinically useful biomarker. CRP levels are quite stable in plasma, with a half-life of approximately 19 hours and they have no diurnal variation.[43] In addition, the correlation of CRP levels over time in individuals is approximately 0.6, which is similar to that for LDL levels.[57] Assays for CRP have been internationally standardized and validated, and the cost of clinical measurement is similar to that for lipid measurements (~ $50).

**Clinical studies of C-reactive protein and cardiovascular events**

Currently, there are more than 24 prospective epidemiologic studies that have evaluated the relationship between CRP levels and the risk for adverse CV events (**Fig. 6**). These studies have demonstrated that higher CRP levels are associated with an increased *relative* risk of a diverse range of CV events including myocardial infarction, stroke, peripheral vascular disease, and sudden cardiac death.[58-68] These relationships are also consistent in cohorts of men and women,

and in various populations in Europe and the United States.[62,64,66] Importantly, these findings appear consistent above and beyond traditional risk factor assessments.

**Fig. 6** Prospective studies of CRP and CV events




Analyses from the Women's Health Study demonstrated that while both CRP and LDL cholesterol levels were predictive of incident CV events, the increment in risk with higher quartiles of CRP was greater than that of LDL.[25] In addition, CRP was able to further stratify risk amongst those with high or low LDL levels. Further analyses revealed that CRP conveyed additional information about CV risk within each category of FRS.[25,69] The findings that CRP predicts risk independent of traditional risk factor components of the FRS have been observed in at least 10 large studies.[70] In addition, several studies have demonstrated an association between CRP and CV events beyond components of metabolic syndrome.[71,72]

Due to the robust evidence relating CRP to the risk of CV events, clinical practice recommendations from the Centers for Disease Control and American Heart Association (CDC/AHA) issued in 2003 supported the use of CRP testing in selected patients to assist in the assessment of CV risk.[41] These recommendations endorsed the optional use of CRP for additional risk assessment in patients considered intermediate risk for CV events. They also outlined thresholds for CRP that could be used clinically to define categories of risk: <1mg/L, lower risk; 1-3, intermediate risk; >3 higher risk), which were based upon approximate tertiles of CRP in populations of >40,000 people who had been evaluated to that point.

**Limitations of C-reactive protein in cardiovascular risk assessment**

Despite the great enthusiasm for CRP testing preceding the issuing of these clinical practice recommendations, several subsequent findings have highlighted many important limitations regarding the clinical utility of CRP.[73] It should be noted that the majority of earlier studies regarding CRP and CV events consisted of select research and clinical trial populations which may have had important differences compared with the general population. One particular concern relating to this observation was whether or not CRP thresholds outlined in the CDC/AHA statement were reflective of the actual distribution of CRP levels in the population. Few studies had previously been performed directly comparing the distribution of CRP between blacks and whites, and women and men. Investigators from the Dallas Heart Study (DHS) recently explored this issue by evaluating if there were race and gender difference in CRP levels

in their large, population-based sample.[26] This study revealed that blacks had significantly higher CRP levels than whites (median 3.0 v. 2.3 mg/L; p<0.001) and women had significantly higher CRP levels than men (median 3.3 v. 1.8 mg/liter; p<0.001). The proportion of subjects with CRP levels >3 mg/L (high risk) was 31%, 40%, 51%, and 58% in white men, black men, white women, and black women, respectively (p<0.05 each group vs. white men) (**Fig 7**). The findings of gender [74,75] and racial[76,77] differences in CRP have been supported by other studies and highlight limitations of translating findings regarding biomarkers from research studies to the general population. Indeed, the DHS results demonstrate that reliance on CRP to predict CV risk may result in overtreatment of women compared with men, as greater than 50% of the former have high CRP levels despite significantly lower CV event rates.

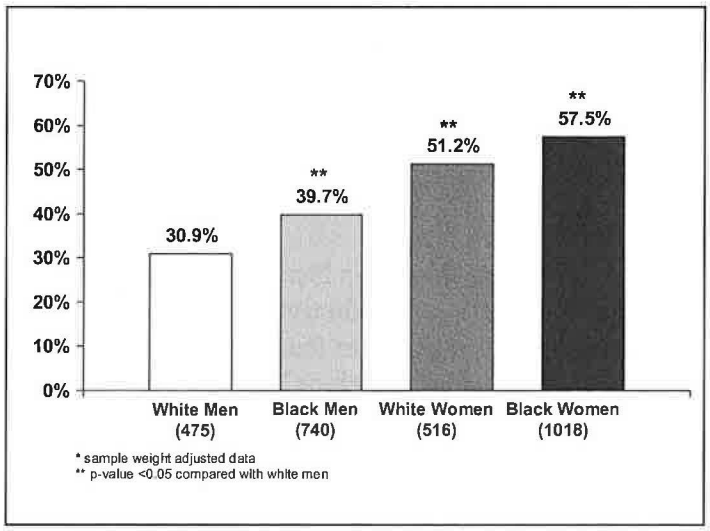



**Fig. 7** Proportion of subjects with high relative-risk CRP levels (>3mg/L) in each demographic group in the Dallas Heart Study.

Subsequent studies have also challenged the magnitude of the association between CRP and CV events.[59] A meta-analysis of 11[78] studies performed prior to 2000 revealed adjusted odds ratios of 2.0 for CV events with elevated CRP levels, while a recent updated meta-analysis of 22 trials revealed a more modest odds ratio of 1.5.[59] These authors also evaluated the predictive capacity of CRP in the Reykjavik Study with 2459 incident CHD cases, the largest such study to date.[59] They demonstrated that magnitude of risk with CRP (OR 1.45) was less than that for total cholesterol (OR 2.35) and smoking (1.87). CRP also added little *incremental* benefit to standard risk factors as it minimally affected AUC measurements. These cautionary findings were echoed by other reports.[79-81] The Framingham Heart Study investigators observed a modest association between CRP levels >3 and the risk of CV events (OR 1.6), which was no longer significant after adjustment for traditional risk factors. In addition, traditional risk factors provided an AUC of 0.78 for the discrimination of CV events, which was unchanged with the addition of CRP.[81] Similarly in the Atherosclerosis Risk in Communities (ARIC) study, the AUC for traditional risk factors for the prediction of CV events was 0.767 which was minimally increased to 0.770 when CRP was taken into account.[79]

These instructive findings regarding CRP raise important issues about how to appropriately evaluate emerging biomarkers for clinical utility.[73] While sizeable odds ratios are often interpreted as a surrogate for validity, higher levels of evidence such as *independence* from traditional risk factors and *incremental* utility over standard risk assessment strategies must be

evaluated.[82] From a statistical standpoint, reliance solely on AUC measures to validate biomarkers may ignore other valuable contributions to risk assessment such as increased sensitivity, specificity, and calibration, especially since any single biomarker is unlikely to make a major change in AUC.[83] Furthermore, from a clinical standpoint, additional questions must be posed about applicability to subgroups, useful clinical thresholds, appropriate implementation, and cost-effectiveness. These challenges are not unique to biomarkers or CRP, but are relevant to all novel risk assessment tools.

## II. ATHEROSCLEROSIS IMAGING

Imaging of the vasculature to determine CV risk has many inherent attractive characteristics. Atherosclerosis is the underlying etiology of adverse CHD events in the preponderance of cases, and the majority of all people dying from CHD have significant quantities of atherosclerosis.[84] Atherogenesis is a complex process involving interactions between risk factors, the endothelium and inflammatory mediators,[85] and atherosclerosis imaging integrates all of these data into a tangible and measurable output. Unlike traditional risk factors and biomarkers which are conceptually more distant from the outcome of interest, atherosclerosis itself is a more concrete and proximal manifestation of CHD risk. Indeed, knowledge of the presence of atherosclerosis has been shown to improve patient motivation towards preventive measures.[21]

**Table 1** Atherosclerosis imaging techniques

| Vulnerable Plaque Imaging | Plaque Burden Imaging |
|---|---|
| CT Angiography | Coronary Artery Calcium Scanning |
| Coronary/ Carotid MRI | Carotid Intimal Medial Thickness |
| Molecular Imaging | CT Angiography |
| Intravascular Ultrasound | Coronary/ Carotid MRI |
| Optical Coherence Tomography | |
| Thermography | |

Many different techniques are being evaluated for atherosclerosis imaging (**Table 1**). Conceptually, these techniques can broadly be categorized into two groups: vulnerable plaque imaging, and plaque burden imaging. While it was previously believed that CHD events usually derived from obstructive coronary lesions, it is now appreciated that the majority (approximately 70%) of myocardial infarctions result from nonobstructive plaques.[86,87] Thus, unlike exercise treadmill testing or nuclear perfusion imaging, atherosclerosis imaging for primary prevention of CHD does not rely upon detecting hemodynamically significant plaques. Histologically, vulnerable plaques that rupture and cause acute coronary syndromes commonly consist of a large lipid-filled necrotic core and a thin fibrous cap.[88] Non-invasive techniques for vulnerable plaque imaging include multidetector computed tomography (MDCT) coronary angiography, coronary and carotid magnetic resonance imaging (MRI), and molecular imaging, and attempt to determine the histology or biological characteristics of atherosclerotic lesions to infer which are

rupture-prone. Currently, these non-invasive techniques are still undergoing development for clinical use.[89,90] Invasive techniques including intravascular ultrasound, angioscopy, optical coherence tomography, and thermography are also in developmental phases, but will not be applicable for wide spread screening in primary prevention.[91]

The basis of plaque burden imaging is to determine the overall quantity of atherosclerosis in a particular vascular territory. Higher quantities of atherosclerosis burden are consistently associated with increased CV event rates,[92,93] likely because a greater overall burden of atherosclerosis results in a greater absolute number of vulnerable plaques. Three techniques currently employed in clinical practice are carotid intimal medial thickness imaging, coronary artery calcium (CAC) imaging, and CT angiography.[94,95] CAC imaging is the most widely used and evaluated technique, with a recently published AHA Scientific Statement supporting its use in select cases for CV risk assessment.[94]

**Coronary artery calcium scanning- methodology**

It has long been appreciated that calcification in the arteries is intimately associated with atherosclerosis. Calcification itself is an innate response to injury and repair in the body, but is deemed to be an organized, regulated process in atherosclerotic vascular lesions.[96] The presence of calcification in the arteries is synonymous with atherosclerosis, but not all atherosclerotic plaques have calcifications. Several studies have demonstrated a close correlation between the quantity of coronary calcium and burden of atherosclerotic plaque.[97,98] In a seminal study by Rumberger et al., 38 coronary arteries from 13 autopsied hearts underwent calcium measurements by electron-beam CT (EBCT) scanning and atherosclerotic plaque measurement by direct histological assessment.[97] Each artery was sectioned into 3mm segments and the sum of calcium areas and sum of histological plaque areas were highly correlated (r=0.90) (**Fig. 8**). Importantly, a few arteries had evidence of atherosclerotic plaque, but no corresponding calcium. While CAC tracks closely with plaque burden, it has poor specificity to detect obstructive CAD.[99]

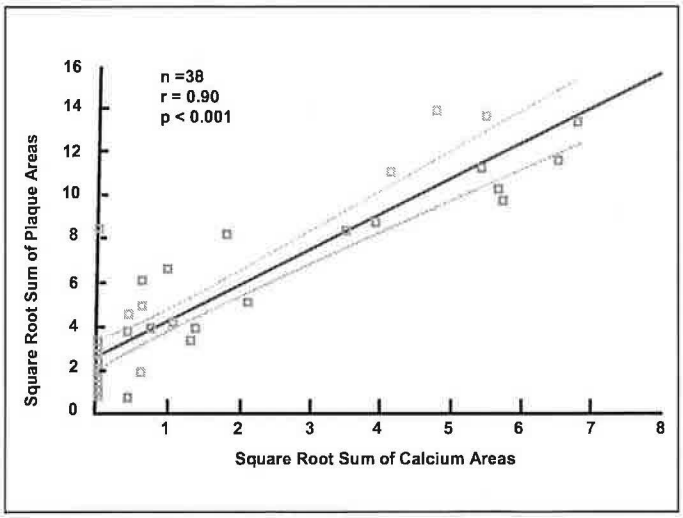



**Fig. 8** Close correlation between EBCT measured coronary artery calcium and atherosclerotic plaque in 38 coronary arteries.

CAC scanning can be performed using two different methods, EBCT and MDCT.[94,100] EBCT or ultrafast-CT was the initial method that was used in the clinical development of CAC scanning. This technology utilizes scanning electron beams rather than rotating x-ray tubes, which provides for very rapid image acquisition times and fewer motion artifacts.[100] MDCT or helical CT

scanning is rapidly evolving as the preferred clinical method for CAC scanning, partially due to its greater versatility for performing other imaging tests. This technique involves x-rays tubes mounted on a rotating gantry and can acquire multiple (4 to 64) slices simultaneously, but with slower imaging times that EBCT scans. These two techniques correlate reasonably well, although there may be some discrepancies at lower levels of calcium.[100] Radiation dosing is an important consideration for using such tests in primary prevention, asymptomatic populations. The effective radiation dose for CAC scanning using EBCT scanners is approximately 0.7-1.3mSv, while it is slightly higher with MDCT methods, approximately 1.0-1.8 mSv.[101,102] By comparison, the effective radiation dose is approximately 0.05mSv for a PA and lateral chest x-ray, 8-11 mSv for an abdomen and pelvis CT, and 3.6 for the average annual background radiation in the US.[102] In addition the average charges for such tests range from $100-$400.

**Coronary artery calcium and cardiovascular events**

Several retrospective[103-105] and more recently, prospective studies[93,106-109] have evaluated the predictive ability of CAC scanning for CV events. The South Bay Heart Watch Study, started in 1990, is a large study of predominantly male subjects with the longest prospective follow up to date.[93] 1461 subjects >45 years of age (mean 66 years) with at least one coronary risk factor and no know CHD underwent CAC scanning at baseline, with 84 of them experiencing CHD death or MI in 8.5 years of follow-up. The hazard ratio for CHD events in those with CAC scores >300 compared with a score of 0 was 3.9 (95% CI 2.1-7.3). Moreover, higher CAC scores were able to stratify CHD risk within categories of risk determined by FRS, particularly the intermediate risk group (10-20% 10-year risk) ($p<0.001$). Another larger prospective study of 4613 subjects, the St. Francis Heart Study, evaluated an apparently healthy cohort that was not required to have risk factors as inclusion criteria.[106] In 4.3 years of follow-up, a CAC score ≥100 compared with <100 conferred a relative risk of CHD death or MI of 9.2 (95% CI 4.9-17.3), and the relationship between CAC and CV events persisted after adjusting for traditional risk factors and CRP ($p=0.01$). Similarly, CAC scores were able to further stratify risk amongst each category of FRS determined risk. The Aerobics Center Longitudinal Study (ACLS) is a prospective study of CAC scanning in 10,746 adults and included a wide age range (22-96) and separate analyses of men and women.[107] After an average of 3.5 years of follow-up, the hazard ratio for hard CHD events in the highest tertile of CAC compared with a score of 0 was 20.0 (95% CI 5.8-69.6) in men and 9.3 (1.2-18.9) in women (**Fig 9**). These associations persisted after adjustment for or stratification by risk factors. Similar associations between CAC and CHD events have been observed in prospective studies in Europe[109] and in cohorts of younger subjects.[107,108]

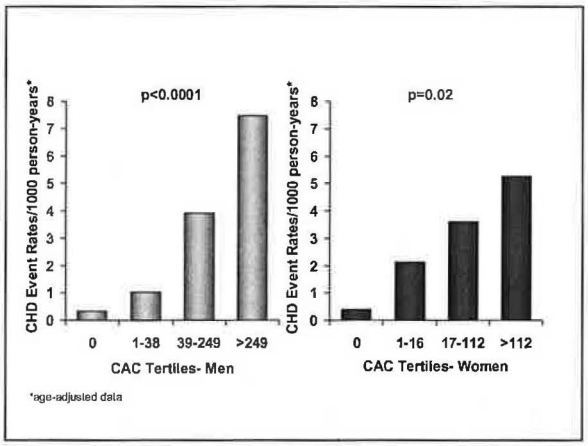



**Fig. 9** Aerobics Center Longitudinal Study. Increased rate of CHD events with increasing CAC tertiles in over 10,000 men and women, 3.5 year follow-up

An important consideration is the utility of CAC scanning relative to traditional risk assessment strategies such as the Framingham Risk Score. As discussed, most prospective studies have reported consistent associations after adjustment for components of the FRS. In addition, CAC scores appear to add predictive information within categories of the FRS.[93,106] Unlike CRP, CAC scores have also provided incremental information regarding CHD risk beyond the FRS, with an increase in AUC in the South Bay Heart Watch study from 0.63 to 0.68 ($p<0.001$)[93] and in the Rotterdam Study from 0.749 to 0.774 ($p=0.02$).[109] In the St. Francis Study, the predictive value of CAC scores for CHD was greater than that of the FRS (AUC 0.79 v. 0.68, $p<0.001$).[106]

**Challenges for the clinical use of coronary artery calcium scanning**

Despite the potential for improving CV risk assessment, the incorporation of CAC scanning into clinical practice presents many challenges. Since studies have reported differing CAC thresholds to denote higher CHD risk,[93,106-109] the interpretation of an individual patient's CAC results remains problematic. One strategy for the clinical interpretation of these results is to utilize absolute score thresholds to determine risk, regardless of age. Proposed thresholds of 0-100, >100-400, and >400 to represent low, intermediate and high-risk appear to correspond to recommended 10-year risk categories of <10%, 10-20%, and >20% (**Fig. 10**).[109,110] However, age-sex specific percentile distributions must also be considered. For example, a 45 year-old male with a calcium score of 35 may be considered to be at low absolute risk based upon these absolute thresholds. However, this score represents the 75th percentile for this patient's age and sex,[111] and he may be at a higher relative risk and on a worse long-term trajectory than others in his age group. The intensity of preventive measures, including the use of pharmacologic agents, would have to be considered in relation to his underlying risk profile, but reassurance would not be an appropriate response to this score.

**Fig. 10** CAC categories and 10-year CHD incidence

| | Coronary Calcium Score | | | |
|---|---|---|---|---|
| | 0 | 1-99 | 100-400 | >400 |
| ACLS | 0.35% | | | |
| PACC† | 0.5% | | | |
| South Bay | 5.8% | 9.3% | 12.6%* | 19.7%** |
| St. Francis† | 1.3% | 2.3% | 12.8% | 32.6% |
| Rotterdam | | 3.0% | 8.7% | 21.6% |

† indicates all CV events
*CAC 100-300
** CAC>300
††CAC 0-100

Another area of concern is determining the appropriate population in which to perform CAC scanning for risk assessment. The cost of scanning which is significantly more than current risk factor assessment, combined with the risks of radiation exposure create a complex decision algorithm for population wide screening. These decisions are further complicated by considerations of incidental findings and the potential for overutilization of subsequent diagnostic tests such as cardiac catheterization. Current recommendations suggest the use of

CAC scanning for CV risk assessment only in those patients deemed intermediate risk (10-20% 10 year risk) by the FRS.[94] The appeal of this approach is intuitive based on Bayesian theory, in that the post-test probability of disease is most favorably altered in those with intermediate pretest probability.[112] Furthermore, indiscriminant scanning of the large number of apparently low-risk patients to pick up the small number with elevated calcium scores would not be cost effective or warrant the radiation exposure. Scanning high risk subjects is also unnecessary given that they should already be treated maximally with current preventive therapies. However, there are significant limitations to focusing CAC scanning solely on the intermediate risk group.

In the Dallas Heart Study, we have examined the implications of current imaging recommendations for CV risk assessment in a large, population-based study. Among the 2699 participants ages 30-65, only 15% of men and 1% of women were categorized as intermediate risk based upon the FRS, and thus eligible for CAC scanning (**Fig. 11**). Altering the age threshold to 45-65 year old subjects resulted in 27.9% of men being in the intermediate risk category compared with only 1.9% of women. Only 3.6% of men and 0.5% of women ages 30-65 were intermediate risk and had severely elevated CAC scores (> 400 or >75th percentile), thus changing risk category to high risk. In addition, 4% of men and 3.2% of women were in the low risk group with significant amounts of calcium, and would not have been detected by current imaging algorithms. Thus, using current recommendations, few women are eligible for CAC scanning in the general population and the distribution of risk categories changes minimally in this group. Furthermore, more subjects with clinically significant amounts of calcium were missed in the apparently low risk group than those who would have been detected and promoted to a higher risk status. Clearly, applying CAC imaging to the entire apparently low risk group would not be prudent, but further studies are needed to determine in which apparently low risk patients the use of CAC scanning may be warranted.

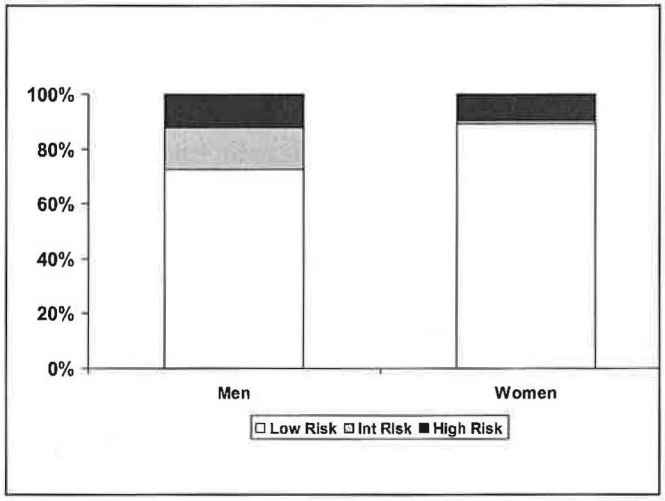



**Fig. 11** Distribution of risk categories in 2699 subjects ages 30-65 in Dallas County. Few women are considered intermediate-risk.

## III. GENETIC MARKERS

Twin and family studies have provided the convincing evidence for the role of heritable factors in CVD. A seminal study by Marenberg et al. examining 3298 monozygotic and 5964 dizygotic twins and demonstrated that the risk of death from CHD was increased 8 fold in men and 15 fold in women when an identical sibling died from premature CHD (**Fig 12**).[113] This risk was also

increased, but somewhat attenuated for dizygotic twins, with relative risks of 3.8 and 2.6 respectively. Family history studies have also consistently demonstrated an increased risk of CHD when ones parents or siblings are affected by this disease.[114-117] The risk is greater with increasing number of affected family members and with younger ages at the time of their disease onset.[118] In the Dallas Heart Study, we have demonstrated that family history of CHD also increases the risk of subclinical atherosclerosis, with a two-fold increase in the odds of CAC in those with a first degree relative who suffered a myocardial infarction.[119] A significant heritable component also exists for many of the risk factors for CHD including dyslipidemia, hypertension, and diabetes.[120]

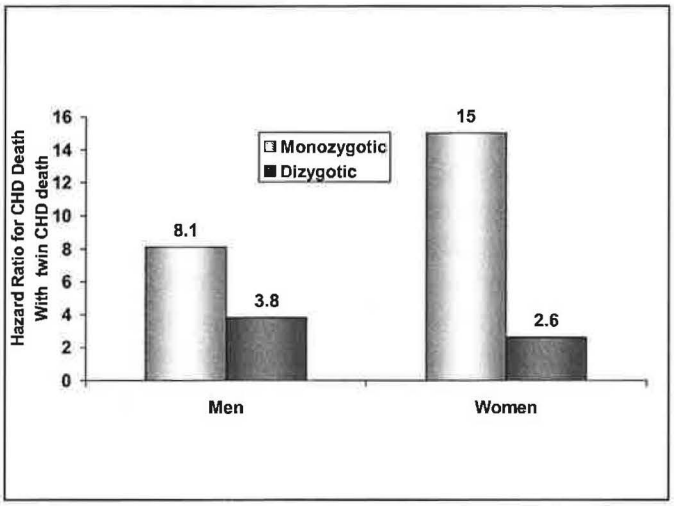



**Fig. 12** Risk for CHD in surviving twin with sibling premature CHD death. The hazard ratio is significantly greater in monozygotic than dizygotic twins.

Identifying the genetic variants that contribute to CVD can impact this illness in many important ways.[121] Discovery of new mutations can elucidate the pathophysiology of diseases and provide new targets for drug therapy, such as work done by Drs. Hobbs and Cohen in identifying mutations in *PCSK9* that affect LDL cholesterol levels.[31] Genetic information can also assist in the diagnosis of disease as well as clarify the prognosis. An emerging application is pharmacogenomics, which evaluates interactions between an individual's genetic markers and his/her responses and reactions to therapy.[122]

There is also great promise for the use of genetic markers in CV risk assessment. New technologies have greatly facilitated and lowered the cost of measuring these markers, particularly for single nucleotide polymorphisms (SNP's), which are genetic variants resulting from alterations in a single base pair. An inherent benefit of using genetic markers is that they can be detected at an early age, potentially magnifying the effects of preventive therapies by earlier initiation.[123] Also, unlike biomarkers, the measurement of this marker does not fluctuate over time and is less affected by assay. Nevertheless, the use of genetic markers for CV risk assessment faces sizeable challenges, including the difficulty in identifying sequence variants related to the susceptibility of CHD and rational approaches to the application of this information.

**Genetics of atherosclerotic disease**

The most dramatic examples of genetic contributions to atherosclerotic disease involve relatively rare single gene disorders with marked effects.[124] The classic example of such a disorder is

familial hypercholesterolemia (FH), which was characterized in the seminal work by Drs. Brown and Goldstein.[125] Using cultured fibroblasts from patients afflicted with this disease, they discovered the LDL receptor and demonstrated that mutations in the gene encoding this receptor resulted in the illness. Patients that are homozygous for these mutations have exceedingly high LDL cholesterol levels (600-1200 mg/dl), and death from myocardial infarction usually ensues prior to age 30.[126] Heterozygotes occur with a frequency of approximately 1 in 500 in the population, and have a less severe form of hypercholesterolemia with LDL levels averaging 350 mg/dl and atherosclerotic disease manifesting in the 4th and 5th decades. Despite the strong association with CVD, population screening for LDL receptor mutations to assess CV risk is not likely useful as over 400 different mutations in the LDL receptor gene can result in this disease, and these genetic abnormalities are usually clearly manifested in the phenotype of markedly elevated LDL cholesterol. Other single gene disorders that have significant effects on atherosclerotic disease have been identified, mostly involving heritable lipid abnormalities.[127,128]

Although these single gene, Mendelian forms of atherosclerotic disease provide major insights to the disease, they collectively account for a small portion of all patients with CHD. For the majority of cases, atherosclerosis is a complex, polygenic disorder involving the interactions of multiple genes in several different pathways.[129,130] Indeed, atherogenesis involves the interplay of lipoproteins, endothelial cell processes, leukocytes, and inflammatory mediators in the milieu of additional risk factors and environmental stimuli.[42] Genes involved in each of these pathways collectively contribute towards the susceptibility for this disease, but contribution of each gene is usually quite small. It is estimated that at least hundreds of genes contribute to the heritability of atherosclerosis, and these genes often translate into risk factors that interact with each other and environmental factors to promote atherosclerosis.[130]

**Identifying sequence variations**

The complexity of atherosclerosis coupled with the complexity of the human genome create great challenges for the identification of genetic mutations that confer susceptibility to CHD.[131] SNP's occur with a frequency of approximately 1 in every 1000 nucleotides and most have no affect on gene function. There are several approaches for identifying genetic mutations of complex disease, but three general categories include candidate gene association studies, linkage analysis, and genome-wide association studies.[132-134]

Candidate-gene association studies have been widely used in the study of atherosclerosis.[135] This approach involves evaluating mutations in a predetermined set of genes that have been selected by their presumed role in atherosclerosis. The mutations in these genes are identified and assessed for their associations with atherosclerosis using case-control studies. This method is useful for the study of complex diseases since it has the ability to detect gene variants with modest effects on disease susceptibility. However, candidate-gene association studies have been plagued by a large number of false-positive reports,[136] and are not suited for the discovery of novel genes or rare mutations involved in atherosclerosis.

Linkage analysis involves analyzing family data and has the advantage of using an unbiased approach in order to discover new genes that are associated with disease.[132] This method uses numerous markers spaced throughout the genome to identify excess sharing of regions of the genome that are related to illness. Major limitations of this strategy are the limited power to

detect genes with modest effects, and challenges with pinpointing pathologic mutations within regions of interest.[131] Like linkage analysis, genome-wide association studies can also be a powerful tool for identifying novel genes associated with CHD.[131,133] This emerging technique can be performed in unrelated individuals and involves the analysis of hundreds of thousands of marker SNP's in a case-control fashion. Advantages of this approach involve its unbiased approach and the ability to identify variants with modest effects on CHD. However, efforts are ongoing to clarify the methodological and analytic techniques necessary to analyze the sizeable amounts of data generated in these studies, and to minimize false positive results associated with performing thousands of statistical tests.[131]

There are several recent examples of successful application of these techniques to discover novel genetic variants associated with CHD. Ozaki et al. performed a genome-wide association study using 92,788 randomly selected SNP's in a case-control study of patient with a history of myocardial infarction and unaffected subjects from general population.[137] They identified a polymorphism in the lymphotoxin-α gene (*LTA*) that was associated with myocardial infarction, with consistent findings after replication in two additional cohorts. *LTA* is involved in the inflammatory response, and the investigators performed *in vitro* assays confirming that the identified mutation resulted in changes in gene expression and function of the protein product, with increased induction of cellular adhesion molecules.

Investigators from the deCODE group performed linkage analysis in 296 Icelandic families, consisting of 713 individuals with myocardial infarction.[138] The identified a region of interest in the long arm of chromosome 13 (13q12-13), and narrowed the region down to one candidate gene, *ALOX5A* which encodes arachidonate 5-lipoxygenase-activating protein (FLAP). They determined that a particular haplotype, or set of closely linked genetic variants inherited together, of *ALOX5A* conferred a 1.8-fold increase risk for myocardial infarction, as well as 1.7-fold increase in stroke. These findings were validated in a British cohort, and another haplotype involving the same biochemical pathway was associated with CHD risk in multiple African American cohorts from the U.S.[139] The investigators also reported higher leukotriene B4 production (a product of the 5-lipoxygenase pathway) from stimulated neutrophils of carriers with the risk haplotype compared with controls.[138]

**Table 2** Genes potentially associated with atherosclerosis

| Lipids | Inflammation | Endothelial Function | Thrombosis | General |
|---|---|---|---|---|
| LPL | PON1 | NOS3 | GP3A | ACE |
| APOE | ALOX5A | ELAM | MTHFR | AGTR1 |
| APOC3 | LTA | CX3CR1 | PAI1 | AGT |
| CETP | MMP3 | SELE | THBS1 | |
| APOA1 | IL6 | | THBS2 | |
| LIPC | MCP1 | | | |

**Application of genetic markers to cardiovascular risk assessment**

Despite the challenges in identifying genes associated with the susceptibility for CHD, several potential candidates have been described (**Table 2**). Ongoing advances in the techniques used to identify these mutations will likely translate into a significant increase in newly reported gene variants. While advances are made in the discovery of variants, applications of genetic markers to CV risk assessment has lagged behind.

Most of the issues pertaining to incorporation of biomarkers in CV risk assessment strategies also apply to the use of genetic markers. The salient considerations include reproducibility of CVD associations in multiple cohorts, independence and incremental utility to traditional risk factors, and cost-effectiveness.[140] However, due to the importance of ancestry in hereditary diseases, more careful evaluation of applicability of genetic markers to various ethnic groups is necessary. In addition, many mutations are quite rare and would be less informative and cost effective for population-level screening. Advancing technologies and reduced costs of genotyping raise the interest in multiplex assays for large numbers of mutations.[140] Yet, methods of creating an aggregate measure of risk from these multiple genotypes have not been carefully evaluated.

One interesting paradigm for the integration of multiple genetic markers is the creation of a genetic risk score. Investigators from the ARIC study recently described the incorporation of five gene variants that were independently associated with CHD into a score, with each variant given equal weight.[141] They reported that the hazard ratio for incident CHD in 13 years of follow-up with a high score was 1.60 after adjustment for traditional risk factors. This relationship to CHD was of a comparable magnitude to that of traditional risk factors.

## IV. CONCLUSIONS

Tremendous progress has been made in past half century in reducing death rates from CVD. The identification and modification of major CV risk factors have greatly contributed to this effort. However, stabilization of death rates, technological advances, and an appreciation for the limitations of current CV risk assessment strategies contribute to growing interest in novel strategies for CV risk prediction. Current strategies have been developed and evaluated for populations, while emerging strategies have greater applications for the individual and are in line with the vision of the future of medicine advocated by Dr. Elizabeth Nabel, Director of the NIH, which will be "predictive, preventive, preemptive, and personalized." Achieving these goals will require collaborative efforts and ongoing developments in discovery as well as application of these new tools.

**Return to the Dallas Heart Study**

The Dallas Heart Study is a population-based, probability sample of approximately 3500 Dallas County residents which began in 2000. A critical design element was exquisite phenotyping of the cohort including demographics, anthropometric measures, blood samples for plasma and DNA, and multiple CV and atherosclerosis imaging techniques. Several important discoveries related to CVD have already been made, but next phase of the study holds even greater promise. We will soon bring back the cohort after an approximate 7 year interval and repeat these biologic measures. In integrating all of these data, we aim to make important contributions to the discovery of new tools and development of new methods to more accurately predict those who are at risk for CVD.

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