

XV International Symposium on Atherosclerosis
June 14 - 18th, 2009 • Boston, MA, USA

Satellite Symposium
June 19, 2009



RISK FACTORS FOR ATHEROSCLEROSIS

Highlights

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INTERNATIONAL
ATHEROSCLEROSIS
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RISK FACTORS FOR ATHEROSCLEROSIS

This publication is based on the lectures presented by international experts during the satellite symposium entitled "Risk Factors for Atherosclerosis" held as a satellite meeting from June 18-19, 2009 in Framingham, MA, USA, during the XV International Symposium on Atherosclerosis June 14-18, 2009, Boston, MA, USA.

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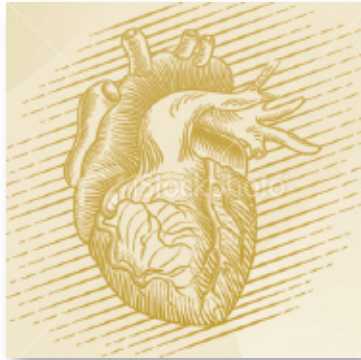
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PREFACE

Traditional risk factors for coronary heart disease (CHD) for each gender include age, hypertension, smoking, diabetes, elevated total cholesterol, and decreased high density lipoprotein (HDL) cholesterol (see Expert Panel, *JAMA* 2001, and update Grundy et al., *Circulation* 2004). However, approximately 20% of all vascular events occur in the absence of these risk factors and half of them occur in the absence of significant lipid abnormalities. Thus, existing risk stratification guidelines may not fully account for the additional risk possibly associated with novel risk factors and biomarkers. Furthermore, the uncovering of both new biological mechanisms and new technologies may impact the decision making with regards to biomarkers.

There is a need for screening modalities that can identify at-risk patients who may benefit from alternative treatment strategies for primary prevention and criteria need to be established by consensus regarding the validity and future clinical applicability of novel risk factors. This expert decision making is necessary in order to refine existing risk assessment guidelines, address disparities in risk among various patient populations from various regions and ethnic back grounds, and design and implement appropriate CHD risk reduction strategies worldwide.

CARDIOVASCULAR DISEASE RISK ASSESSMENT LESSONS FROM OBSERVATIONAL STUDIES OVER 60 YEARS

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Obesity was not considered to be a major CVD risk factor, but the NHLBI's Framingham Heart Study documented that obesity contributes the many other major risk factors (such as lipids, hypertension, and diabetes).

There have been major accomplishments in the field of epidemiology over the past 60 years leading to the proposal of the multivariable risk factor concept and its application for multivariable risk assessment. This has provided the healthcare professional with cardiovascular risk profiles which allow for the targeting of individuals who are likely to benefit from intervention.

The NHLBI's Framingham Heart Study was the first to clarify many of the major concepts in CHD risk prediction and took away many misconceptions in medicine. A few examples are: 1.) Framingham showed us that there is two-way traffic of cholesterol, which is reflected by the total cholesterol (TC)/high density lipoprotein (HDL) cholesterol ratio. 2.) Obesity was not considered to be a major CVD risk factor, but Framingham documented that obesity contributes the many other major risk factors (such as lipids, hypertension, and diabetes). 3) The American Heart Association smoking statement of 1956 quoted that "much greater knowledge is needed before any conclusions can be drawn between smoking and death due to CHD." Framingham showed that there was an increased CHD risk associated with how much you smoked per day; moreover, smoking cessation rapidly decreased CHD risk, indicating a link between smoking and thrombosis (see Kannel et al., *Ann Intern Med* 1961). Furthermore Framingham introduced multivariate modeling. By means of logistic regression a total CHD risk prediction system was already established in 1976.

Thus the NHLBI's Framingham Heart Study laid the foundation for many of today's concepts in CHD risk prediction. The final question is: do we need more risk factors? Since 40-50% of the people with CHD events are not considered to be at high risk by most risk profiles, the answer is yes. These are individuals in the intermediate risk group, and their risk needs to be better defined by biomarkers, genetic markers, and cardiovascular imaging.

TRADITIONAL APPROACHES TO RISK ASSESSMENT

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There are a number of issues surrounding novel risk factors and the way risk algorithms are moved forward.

Traditional risk assessment started in 1987 when HDL was incorporated into the Framingham risk estimating equation. This led to the concept that in clinical risk models, there is a baseline state, a risk factor, and a follow-up state (see Wilson et al., *Arteriosclerosis* 1988). Genes and environment drive the baseline state and genes and environment drive the follow-up state. The baseline state may spin off risk markers which are not along the causal pathway, but do associate with the disease end point. In 1998 a risk estimation calculator in Framingham study population was presented, adding up risk factors with the ultimate goal to use these estimates for public health strategies (see Wilson et al., *Circulation* 1998, Expert Panel, *JAMA* 2001).

CHD Risk Assessment-- Framingham Points

- Gender, Age, Smoking, Systolic Blood Pressure, Total Cholesterol, HDL Cholesterol – Risk Prediction for CHD and Stroke - Point System, Also Continuous Analysis
- If fasting can calculate:
LDL cholesterol= TC-(TG/5 + HDL Cholesterol)
- Diabetes is automatically high risk
- 10 Year CHD Risk and LDL C Goals:
 - <10%: < 160 mg/dl
 - 10-20%: < 130 mg/dl
 - >20% (or CVD, PVD, or Diabetes):
 - < 100mg/dl
 - Optional < 70 mg/dl

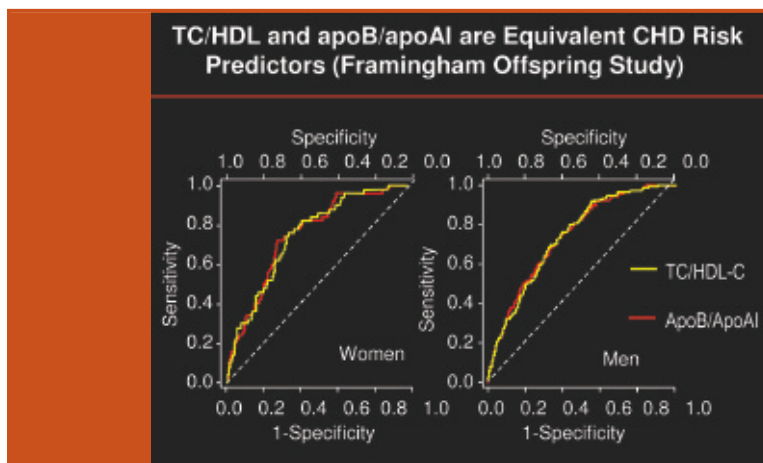
NCEP, ATP III, IV, AHA, NIH
Europeans and Canadians also recommend :
TC/HDL < 4.0 in high risk or CHD patients

Traditionally cardiovascular risk assessments came from observational studies and the placebo arms of clinical trials. All previous risk assessment models have included age and sex (unless performed in an all male or female population), blood pressure levels (and treatment, although earlier models did not include treatment data), total cholesterol (TC), and HDL-cholesterol (C) when it became available. Low density lipoprotein (LDL-C) levels, on the other hand, were shown not to have any added value over TC and HDL-C. In addition, cigarette smoking and diabetes are often included in clinical models, although for diabetes it is better to use diabetes-specific variables, which are: microalbuminuria, duration of diabetes, and hemoglobin A1C. It is interesting to note that the

risk factors in the model are dynamic; for instance, the electrocardiogram (ECG) has faded from the risk score because they are no longer suggested for initial screening unless the patient has known hypertension. Finally, the events which are used as an endpoint in risk scores are hard CHD endpoints, i.e. myocardial infarction or coronary death, but these events may be broadened to other events in the future, such as coronary artery bypass or angioplasty.

There are a number of issues surrounding novel risk factors and the way risk algorithms are moved forward. Today biological samples are stored at -80 degrees and are well monitored, but this has not always been the case. In the past samples have been “mistreated” and therefore it is important to know how the specimens were collected and stored. In addition, bio-variability and lab-variability is a big issue; for example, fibrinogen has shown a great biological variability and this is one of the reasons it has not been included in the initial risk estimates. Also, there is the discussion whether to include or stratify for variables such as social class, race, and ethnicity in risk prediction models.

Another key question which is being raised for the traditional variables is: towards what audience are the models targeted? In general the traditional risk factor models are simple clinical models designed for risk factor screening to be used for calculating the individual risk of a person. However, current research is challenging these traditional risk factor models by adding new variables to existing simple models and testing them for predictive value. One of the ways to compare the efficacy of models is to use Receiver Operating Characteristic (ROC) curves.



These curves represent the sensitivity-plotted versus the 1-specificity: if you flip a coin, the area under the curve will be 50%, represented by the diagonal line in the plot, and better risk prediction will result in the line bending towards the upper left corner of the plot. In this particular analysis, the data from Framingham indicates that the ratio of total cholesterol/HDL cholesterol is as good as the apoB/apoA-I ratio in terms of predicting future CHD events (see Ingelsson et al., *JAMA* 2007). For successful application of an ROC one needs clinical judgment and a certain level of conservatism, especially if the results are going to be used by others, because what might work in this specific model may not work in a different population.

Another ongoing discussion in guideline development is how to express risk, as a life-time risk or a 10-year risk? The main concept is that as people get older, their life-time risk gets closer to their 10-year risk; however, for younger people risk estimation is a bigger issue for prevention and motivation. Ten-year CHD risk assessment from the NHLBI's Framingham Heart Study using gender, age, systolic blood pressure, treatment for hypertension, smoking, total cholesterol, and HDL cholesterol can be accessed through www.framinghamheartstudy.org. In this analysis, diabetes already puts a person in the very high risk category for CHD of > 20% over 10 years. This is the standard model as recommended by the third Adult Treatment Panel of the National Cholesterol Education Program (see Expert Panel, *JAMA* 2001).

In summary, there are certain aspects of risk assessment which require further attention. In the evaluation of a risk model, one should ask the following questions: 1) What is the predictive accuracy of a risk factor model (i.e. how good is the discrimination, or the ability of the model to really function for risk assessment); 2) Do the models work in other groups (not just the groups in which they were developed); 3) What are the cost of risk prediction using a new biomarker; and 4) What is the safety aspect, i.e. is screening for the biomarker safe for the patient?

DEVELOPMENT OF THE REYNOLDS RISK SCORE IN MEN AND WOMEN

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The current risk prediction models leave room for improvement.

The Framingham Risk Score has been widely used in the cardiovascular field, but there is always the question whether new biomarkers can contribute to risk prediction. Studies have been published which indicate that all CHD cases have at least one risk factor, but most control subjects also have at least one major risk factors (only 19% have no risk factors in the control group), indicating that this risk factor model is not very specific. Such data suggested to us that the current risk prediction models leave room for improvement.

The goal of the Reynolds Risk Score was to come up with a better fitting model by comparing new emerging risk factors to the traditional risk factor variables using data from the Women's Health Study. The first round of analyses generated the so-called "best fitting model" which included: hemoglobin A1C (only in the diabetics as there was no effect in the non-diabetics), apolipoprotein (apoA1) and apoB (these variables were stronger predictors than the HDL-C and LDL-C), and new variables like C-reactive protein (CRP), family history of premature myocardial infarction (MI), and the interaction of lipoprotein(a) or Lp(a) and apoB (elevated Lp(a) was important when apoB values were greater than 100 mg/dL). Next, the "best fitting model" was simplified to make it more accessible, creating the clinically relevant model also known as the Reynolds Risk Score (see P Ridker et al., *JAMA* 2007). This model included all variables used for the Framingham model, plus family history of CHD prior to age 60 and CRP.

Framingham Risk Score	Best Fitting Model A	Simplified Model B Reynolds Risk Score
Age	Age	Age
Blood Pressure	Blood Pressure	Blood Pressure
Diabetes	HbA1c% if Diabetic	HbA1c% if Diabetic
Smoking	Smoking	Smoking
Total cholesterol	Apo-B	Total cholesterol
HDL cholesterol	Apo-A	HDL cholesterol
	hsCRP	hsCRP
	Parental history MI	Parental history MI
	Lp(a) if apo-B>100	

A similar model has also been developed for men (see Ridker et al., *Circulation* 2008). The Reynolds Risk Score was evaluated on the basis of the aspects of discrimination and calibration. First of all, discrimination is the ability to correctly separate two outcome classes, cases versus non-cases, the most popular measure of which is the C statistic or the area under the ROC curve. Calibration, on the other hand, shows how close the predicted probabilities compare to the actual outcomes, the most popular measure of which is the reclassification calibration test based on the Hosmer-Lemeshow statistic. When comparing the Framingham Risk Score versus the Reynolds Risk Score in the Women's Health Study, the Framingham variables showed a lack of fit, i.e. the observed and the expected were significantly different, while the Reynolds Risk score showed a better fit. Using the Physicians Health Study data a Reynolds Risk Score was developed for men, again leading to an improved fit over the traditional variables. The Reynolds Risk Score for both men and women can be accessed at www.reynoldsriskscore.org. This risk score includes gender, age, systolic blood pressure, diabetes, smoking, total cholesterol, HDL cholesterol, C-reactive protein, and family history of premature CHD.

In conclusion, model evaluation should depend on its purpose. The C statistic may not be the best measure to evaluate the model and other predictive values need to be taken in account. Calibration is more important for risk prediction and reclassification and can directly compare models in the important categories. Ultimately one should use cost effectiveness to determine the clinical utility and the value of screening.

STATISTICAL ISSUES IN RISK REDUCTION

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Most variables are tremendously statistically significant, but do not add to the overall performance of the risk factor model.

Cardiovascular risk estimation is a multi-factorial process which develops over time, assessing the value of a risk factor at one point in time and following that variable for the development of cardiovascular disease at a later point in time. Multiple risk factors can be combined to form a so-called risk score. Framingham has presented these risk scores in the early 60's in the paper by Dr. W. Kannel (see Kannel, et al, *Ann Int Med* 1961), and since then a number of risk functions have been developed. The general acceptance of the risk score, however, came in the 1980's with the coronary heart disease risk score such as that presented by Dr. Wilson (see Wilson et al, *Circulation* 1998).

Despite the growing popularity there are a number of important aspects to bear in mind when dealing with risk scores. First of all, one should consider the population at risk. Most risk scores are developed for people free of CHD and risk profiles are different for someone who has had a heart attack than for someone from the general population. Next, one should keep the endpoint in mind. As an example, the Framingham risk score was calculated for general CHD. Using this score for hard CHD will overestimate the risk. Also keep in mind the follow-up time. A 10-year risk prediction requires a follow-up time longer than 10 years for accurate prediction. Age is also an important factor since risk factors have a different profile at higher age. The Framingham risk score for instance is developed for individuals with an average age between 35-74 years. The risk score is not going to work very well when used in the elderly since they have a different risk profile (see Expert Panel et al, *JAMA* 2001).

For mathematical risk predictions the Cox proportional hazard model is the most commonly used model. Risk models are tested on the bases of: 1) significance (are the risk factors in the model really important?); 2) discrimination (can you separate those who develop disease from those who do not); and 3) calibration (is the probability right, i.e. comparing the predicted probability versus the observed probability). When testing these models a number of statistical issues have arisen. The first issue was if one can take the Framingham risk score or other function and transfer it to a different population without it losing its function? In other words, is the relative risk the same, is the discrimination as good, and is the level of calibration the same as in the original population? To address this question, the Framingham function was compared to one derived from the

Atherosclerosis Risk in Communities Study (ARIC) data set and showed that the relative risk for the Framingham Risk Score were comparable the those from the ARIC study in a subgroup of white men. On the other hand, the Framingham function dramatically over-predicts when used on the Honolulu Heart Study data set. Thus it is not calibrated correctly for the Honolulu Heart Study data and therefore must be recalibrated to provide a better fit. As an additional example, recalibration of the Framingham function also improved performance in the Chinese Multi-provincial Cohort Study (see Liu et al., *JAMA* 2004). Moreover work in this population also indicated that between 1984 and 1999 CHD risk in China increased significantly because increased total cholesterol, presumably due to dietary changes (see Critchley et al., *Circulation* 2004).

Another important statistical question is: how to deal with a new risk factor? When you add a variable or a genetic score to an existing risk model, what does that new variable add in terms of significance, discrimination, and calibration? Most variables are tremendously statistically significant, but do not add to the overall performance of the risk factor model. An alternative method is to re-estimate the probability with the new variable and count the individuals who move in the right direction in terms of risk.

A final question is how we should express risk, noting that someone's long-term risk will be much more dramatic than the 10-year risk. The long-term risk score was developed based on a 30-year risk score. One can have a good short-term CHD risk, but a dramatic 30-year CHD risk. Therefore scoring systems can be used in an inverted way and translate risk to something called "heart age" – telling a patient who is 30 years old, that based on the present risk factors, this person has the heart of a 36-year old. We have now developed a 30-year scoring system to calculate CHD risk based on the Framingham data (see Pencina et al., *Circulation* 2009).

In summary, risk predictions have evolved and risk models can be transported to different populations via calibration. Furthermore, there are now multiple new methods for handling new risk factors and incorporating them into the model for traditional risk prediction.

NOVEL RISK FACTORS HDL AS A RISK FACTOR

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It is still unclear if HDL is a causal risk factor, and if HDL can be used as a treatment target.

It should be noted that the Prospective Cardiovascular Muenster (PROCAM) Study has also generated a risk algorithm which uses the same parameters as the Reynolds Risk Score, except that it incorporates triglyceride values instead of CRP (see Assmann et al., *Eur J Clin Invest* 2007).

**PROCAM (Münster Heart Study):
Risk Factors Included in Standard PROCAM Algorithm
and Neural Network Analysis**

Standard PROCAM Algorithm

- Age
- Systolic Blood Pressure
- HDL-C and LDL-C
- Triglycerides
- Cigarette Smoking (yes/no)
- Diabetes (yes/no)
- Family History of MI

The PROCAM risk score can be accessed at www.chd-taskforce.com. While low HDL-C is an important CHD risk in Framingham, the Reynolds Risk Score, and PROCAM, the question is whether should it be a target for therapy? Since there are no intervention studies demonstrating that direct intervention into HDL protects against CHD (the effects of HDL intervention drugs are too broad for that purpose), it remains an open question if low HDL is a causal risk factor for CHD? Observational studies have shown that low HDL is associated with increased risk for CHD; however, in the high HDL-C groups there is not always reduced risk. It should be noted that people with low HDL tend to have one or more of the other risk factors and the properties of HDL as a risk factor are modulated by the absence or presence of other risk factors.

Thus, it is still unclear if HDL is a causal risk factor, and if HDL can be used as a treatment target. This is because the data from human and animal studies are conflicting or inconsistent. Furthermore it is difficult to draw any conclusions from the data derived from HDL drug and lifestyle intervention studies because these also tend to affect the other risk factors, especially triglycerides.

Another point of concern is that measuring HDL-cholesterol does not tell you anything about the functioning of the HDL particle itself. The composition of HDL is very heterogeneous and is associated with different functions and effects, creating a need for better tools to characterize the HDL subclasses for risk prediction. This is illustrated by a recent report which suggests that the HDL particle number, instead of HDL size, was the most predictive variable for CVD risk (see Van der Steeg et al., *J Am Coll Cardiol* 2008).

In summary, the cholesterol content in HDL is only a rough estimation of particle size and number. HDL can vary in size and composition and this can make a big difference for the functioning for HDL. New data suggest that HDL particle number is more important than HDL-C and HDL size in CVD risk prediction, because it is not confounded by the components of the metabolic syndrome. Furthermore, there is a direct need for better biomarkers which truly reflect the functioning of the HDL particles and which can be used in risk prediction models.

BIOMARKERS FOR RISK ASSESSMENT IN THE ARIC STUDY

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The total/HDL cholesterol ratio is difficult to beat as a risk marker.

In the Atherosclerosis Risk in Communities (ARIC) study, 98% of the women in the age category 45-64 years had a low risk (i.e. less than 10%) according to the Framingham Risk Score. Although women get disease later, from a clinical point of view it is difficult not to look at these individuals and try to identify who will develop CHD in the future. Impaired fasting glucose, for instance, is useful for risk assessment and management. Furthermore the data on diabetes prevention with lifestyle modification is very strong, and therefore guidelines should look at both CHD and diabetes risk. Diabetes via lifestyle modification is perhaps the most preventable healthcare challenges at the moment. In addition to subclinical atherosclerosis, which has proven to be a strong risk marker for cardiovascular disease, the question is how strong is the data for novel risk markers like Lp(a), non-HDL, apoB, total LDL particle number, lipoprotein associated phospholipase A2 (LpPLA2), and CRP for the use in risk management and risk assessment.

With regard to Lp(a), levels are determined by the number of kringle 4-like repeats within the apo(a) protein on the surface of the Lp(a) particle. Those who have a lower number of these repeats have higher Lp(a) concentrations. A high Lp(a) concentration has been associated with an increased risk for CHD events. The data for Lp(a) is quite strong indicating that Lp(a) is a risk factor and a risk marker for CHD and stroke.

The next question is which to use, non-HDL, apoB, or LDL-particles. Historically, LDL cholesterol has been measured as a surrogate for the amount of LDL lipoprotein particle in the circulation. Since HDL cholesterol is "good" and the rest is "bad," the Helsinki Heart Study mentioned 30 years ago the need to look at HDL-C versus non-HDL-C as markers of good and bad cholesterol. There is much enthusiasm about measuring apoB; however, in the ARIC study this was not supported, thus questioning if an apoB measure is necessary. Univariate analysis showed an association for apoB, but apoB was not that impressive as a marker in the fully adjusted model. Furthermore, questions are raised on the direct LDL measure. In general non-HDL makes more sense than direct LDL and overall, the total/HDL cholesterol ratio is difficult to beat as a risk marker because this ratio is driven by the non-HDL cholesterol versus the HDL cholesterol. Clinically it is important to note which variable has the strongest associations with the outcomes and in that respect, both non-HDL and apoB showed a strong association with the outcome. However, note that while statins reduce LDL-cholesterol, they do not affect apoB levels as much. It is currently unknown what the apoB

target value should be for on treatment on statins to get the greatest risk reduction. The overall standpoint right now is that non-HDL, apoB, or LDL-particles are better than LDL cholesterol for risk assessment and for assessing optimal lipid therapy. The question remains whether to measure apoB and LDL particles, or is non-HDL cholesterol sufficient?

Finally, LpPLA2 is an enzyme associated with LDL. Information on LpPLA2 and CHD risk has been well summarized (see Davidson et al, *Am J Cardiol* 2008). In the ARIC study there was an association between LpPLA2 with stroke in conjunction with CRP. These findings have been validated in prospective studies showing strong associations of LpPLA2 with disease outcome. The next question however is what do you do when a patient has a high value of LpPLA2? The only way to address this question is to conduct a clinical trial and currently there is a large outcome study being conducted investigating whether an LpPLA2 inhibitor actually reduces cardiovascular events.

In conclusion, with regard to novel biomarkers, the only biomarker we have data available for is CRP: patients with high CRP will benefit from statin therapy, even if LDL is not elevated – this was proven in a clinical trial (see Ridker et al. *Lancet* 2009). There are no comparable data available for the other markers. Lp(a) is emerging a risk marker, but more data are needed. LpPLA2 is not clear if it can be used in risk assessment, because no clinical trials have targeted this specific question (see Davidson et al., *Am J Cardiol* 2008).

APPROACHES FOR DISCOVERY OF NEW BIOMARKERS WHICH MAY ASSIST IN THE PREVENTION OF CARDIOVASCULAR DISEASE

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The future of biomarker discovery will be technology driven...

What is a biomarker? A biomarker is something you measure in relation to disease outcome or in response to treatment. What is personalized medicine? Personalized medicine is the ability to give the right drug, to the right patient, in the right dose at the right time, using the information from that patient to achieve a better outcome. Traditional approaches to biomarker discovery were to first understand the risk factor contribution to the biology of CHD. This requires a combination of research from basic science, observational population science, and large scale clinical trials, before any new strategy for prevention could be initiated on a population scale. The downside of this approach is that historically it has been slow and there has been poor integration between the different scientific fields.

The future of biomarker discovery will be technology driven, including genetics and genomics, as well as proteomic, metabolomics, and lipomics. It will become very important to develop new ways of dealing with the information generated by these technologies. In other words, bioinformatics and computational biology approaches must be developed to make use of the enormous amounts of data being generated by these new technologies. Future strategies will be to integrate all the data generated by these new technologies into risk prediction models.

As an example of such a strategy is the NHLBI's SABRe Cardiovascular (CVD) Initiative. The SABRe CVD initiative's aim is to characterize the molecular signatures of clinically important diseases, focusing on risk factors for atherosclerosis and the metabolic syndrome, using high throughput technology to measure large numbers of biomarkers. Participants are derived from the Framingham Offspring Cohort, the Third Generation Cohort, and the Omni Minority Cohort. The project consists of four different components. The first component is the so-called "omic-discovery." The aim is to discover new biomarkers via case-control studies, followed by a validation of the promising biomarkers and replication of the results in other observational settings. The second component is the development of immunoassay methods that are able to measure 200 different proteins in about 1 mL of serum. The proteins will be selected based on the results of proteomic, GWAS, and gene expression studies. The third component is gene expression profiling which will look at gene expression patterns which can be related to gene products (i.e proteins) and disease. The last

component is to characterize expression of microRNAs, which have important regulatory properties. The overall goal of SABRe CVD is to integrate genetic variation data with gene expression data and relate them to circulating biomarkers (proteomics and metabolomics) and to disease outcome. As mentioned before, a central issue will be to integrate all the information generated by the different platforms and relate them to the risk factors and disease outcomes. Optimal approaches necessary to analyze these data do not fully exist yet and the future challenge will be to develop new analytical techniques to analyze the vast amounts of data generated by these new high throughput technologies.

In summary, studies of biomarker of atherosclerosis and the metabolic syndrome will provide insights into the pathogenesis of cardiovascular disease. This will not only promote the development of new diagnostic tests that will identify high risk individuals early on when treatment approaches are likely to be most successful – which falls into the category of personalized medicine – but these approaches will also help us understand the biology of health and disease and will speed up the identification of new therapeutic targets that can be tested and entered into the pipeline for new drug development for treatment and prevention.

HOW DO YOU INTRODUCE A NOVEL BIOMARKER INTO CLINICAL PRACTICE?

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The biomarker should be cost effective and the overall accumulated data should be compelling enough to overcome traditional clinical thinking.

In his landmark paper in 1961, Dr. Kannel changed medical history by identifying hypertension, diabetes, elevated cholesterol, smoking, and male gender as risk factors for CHD (see Kannel et al., *Ann Intern Med* 1961). The question with regard to biomarkers, however, has always been how to keep moving forward? Being an independent predictor is one thing, but to change reclassification and offer something about risk is a more complex story.

What steps are required for the broad clinical acceptance of a novel biomarker? The first important step or criterion is that there is an accepted, reliable, and reproducible method to measure the biomarker. This methodology should be widely available and accessible to everyone. The second criterion is that the novel biomarker should consistently predict risk in multiple and diverse cohorts, preferably with multiple ethnicities. If there is a strong association of the novel biomarker with disease outcome, the third step is to determine that the novel biomarker is largely independent of the established risk factors and to determine whether the novel biomarker reclassifies a substantial number of individuals who were classified differently according to the classical risk factors. If this is the case, then the final or fourth step should be the documentation in clinical trials that individuals identified by the biomarker benefit from treatment which they otherwise wouldn't have received. Finally, for successfully introducing a new biomarker in clinical practice, the screening strategy of the biomarker should be cost effective and the overall accumulated data should be compelling enough to overcome traditional clinical thinking (i.e. convince clinicians to routinely use the biomarker in clinical practice). So why work on biomarkers? What is the application of a biomarker, separate from their use in a biological study? Do we treat on the basis of the Framingham/Reynolds Risk Score or do we treat on the basis of biomarkers?

To address this question, one can look at role of CRP as a biomarker in the JUPITER trial. The JUPITER trial was designed to include patients with no diabetes, no CVD, and LDL cholesterol below 130 mg/dl (which is below the treatment target, so not treated with a statin); however, they had a CRP above 2 mg/L, which is indicative of an enhanced inflammatory response. These patients were treated with an aggressive statin and followed over time for hard clinical endpoints. Overall, the use of rosuvastatin was associated with a 44% reduction in the trial primary endpoint of

all vascularevents, a 55% reduction in myocardial infarction, a 48% reduction in stroke, and a 46% reduction in need for angioplasty or bypass surgery (see Ridker et al., *New Eng J Med* 2008). The JUPITER trial also showed the greatest reduction in events in those who achieved an LDL-C < 70 mg/dl and a CRP value of < 1.0 mg/L (see Ridker et al., *Lancet* 2009). These data are consistent with prior work suggesting that statin therapy is effective by both reducing LDL-C and reducing inflammation (see Ridker et al, *Lancet* 2009). Most relevant for primary prevention, JUPITER prospectively confirms data from the earlier AFCAPS/TexCAPS trial that those with elevated CRP but low LDL-C have substantive vascular risk and benefit markedly from statin therapy, an issue of public health importance.

In conclusion, biomarkers can be used to define the right population to study in clinical trials and get a better understanding of the underlying biology.

GENETICS OF CVD RISK FACTORS THE GENETICS OF HYPERTENSION

Patricia Monroe, PhD

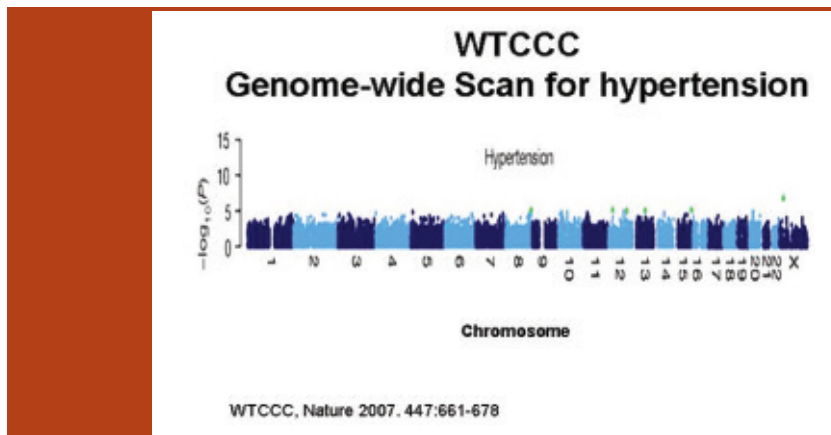
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The Wellcome Trust Case Control Consortium genotyped 2.5 million SNP's in 2,000 cases with high blood pressure and 3,000 controls.

Hypertension is an important risk factor for CHD, stroke, and renal failure, and is the cause of a significant global disease burden. Hypertension prevalence is increasing, and of the patients who have their hypertension treated, 60% stay hypertensive despite the fact that they are usually on more than one hypertension-reducing medication. Therefore there is great need to understand the etiology of hypertension. Hypertension is a multi-factorial disease of which 50% is thought to be inherited. There are two genetic approaches to unravel the underlying genetics of this disease (and multi-factorial diseases in general): the first approach is to study candidate genes at selected gene loci, while the second approach is to conduct a genome-wide scan study (GWAS), having no *a priori* hypothesis and looking at millions of single nucleotide polymorphisms (SNP's) across the genome.

One of the promising candidate genes to study in relation to blood pressure is the WNK1 gene. In the BRIGHT Study, 28 tag SNP's (these are single SNP's which are representative for a haplotype block in the gene of interest) were typed for the WNK1 gene. The SNP's were considered individual and as a haplotype. The results of the BRIGHT Study showed that one individual SNP in the WNK1 gene showed to affect blood pressure and this was replicated in a number of other study populations. In addition, the haplotype analysis of this gene revealed a few rare haplotypes which affected blood pressure, lowering both systolic and diastolic blood pressure; the effects were highly significant.

The second approach is the GWAS, which has been highly successful for the investigations of complex diseases. In 2007 the Wellcome Trust Case Control Consortium genotyped 2.5 million SNP's in 2,000 cases with high blood pressure and 3,000 controls from the BRIGHT Study to see if any of them were associated with high blood pressure. The results however showed that none of the SNP's from the GWAS passed the significance threshold.



The lack of finding an association perhaps contributed to the possibility that the relative risks for blood pressure are much lower than those of other complex diseases. Additionally, blood pressure is an imprecise phenotype as blood pressure is highly variable and there is potential observer bias in the measurement itself. As a follow-up to these negative findings a few steps were taken. The first step was to conduct a blood pressure meta-analysis, followed by more precisely defining the blood pressure phenotype. The meta-analysis was conducted in the form of the Global Blood Pressure Consortium with a total sample size of 34,433 individuals, harboring eight candidate SNP's. One very good candidate was a SNP in the CYP17A gene, a key enzyme in the metabolism of glucocorticoids. The results were replicated in the LOLIPOP study and the CYP17A SNP remained significant.

In summary, eight novel blood pressure loci were identified; however, each individual variant only explains a very small proportion of the variation in blood pressure. The aggregate effect of having one or more of these alleles may be very meaningful at the population level to the risk of strokes and CHD. In addition, these studies come up with new biological mechanisms which can control blood pressure and all represent therapeutic targets.

GENETIC ANALYSIS OF PLASMA LIPID LEVELS

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The strongest associations were still found with the well-known loci, such as the APOE locus with the LDL-based fractions and the CETP locus with the HDL-based fractions.

A genome-wide association study was conducted among over 17,000 women from the Women's Genome Health Study, focusing on the genetic analysis of 22 plasma lipoprotein measures derived from a combination of conventional assays and nuclear magnetic resonance (NMR)-based measurements. The Illumina Human Hap 300 dual + platform was used to generate genotype data for about 317,000 SNP's covering common variation at the 5% level in samples from European origin, as well as an additional approximately 30,000 SNP's with prior demonstrated function or relevance for cardiovascular disease.

Supplementing conventional assays for plasma lipid fractions, the NMR methodology measures the concentration of lipoprotein fractions according to class (i.e. LDL, HDL, or VLDL) and size within each lipoprotein class. This NMR sub-phenotypic gives a better representation of the metabolic pathways and therefore a better resolution to the genetics and thus potential to more precise understanding of the disease phenotype.

The results showed 30 hits across the genome (with the level of significance threshold being set at p -value $< 5.10^{-8}$) many of which have previously been reported in genome-wide scan studies (GWAS) or other genetic analysis. Eight of the primary associations mapped to novel loci not previously described in other genome-wide association studies. The validity of these novel candidate genes was tested by replication in different study populations being the PROCARDIS Study and the NHLBI's Framingham Heart Study. These studies had the same NMR lipoprotein measurements available as in the Women's Genome Health Study. Each of the previously know loci could be validated in the PROCARDIS and Framingham study. Of the eight novel loci, six have been validated and the other two are being confirmed in ongoing studies.

In addition, at a sub-genome-wide level of statistical stringency, associations linked additional candidate genes to the NMR-based and conventional lipoprotein measures. Among the genome-wide loci, some of them associated with one specific lipid trait, but most of them showed associations with a variety of lipoprotein measures. The strongest associations were still found with the well-known loci, such as the APOE locus with the LDL-based fractions and the CETP locus with the HDL-based fractions. Combining all the candidate loci could explain up to 17% of the variance in the traditional and NMR-based lipoprotein fractions.

NEW LESSONS FROM OBSERVATIONAL STUDIES AND THE FUTURE OF BIOMARKERS

LIFE STYLE AND HEART DISEASE RISK - RESULTS FROM THE INTER HEART STUDY

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**A population strategy of prevention is likely to
be most effective in preventing cardiovascular
disease and the global burden.**

The greatest increase in cardiovascular disease is predicted to occur in developing and low income countries. The INTER HEART Study (IHS) was set up to understand why different ethnic groups have different CVD risk factors. For this case-control study, a total of 27,000 people were recruited from 262 sites from 52 countries around the world, including countries where little or no risk factor research has been previously conducted.

The first interesting observation from the IHS is that women develop a myocardial infarction (MI) 10 years later than men and this pattern is seen around the world. Some populations, however, develop MI earlier than other populations. The risk factors are the same, but the number of risk factors is more frequent and present at younger ages.

In the IHS, the greatest risk factor for acute MI was non-fasting apoB/ ApoA1 ratio (with a population-attributable risk (PAR) of 50%). Other risk factors included smoking (PAR 36%), abdominal obesity (PAR 20%), and hemoglobin A1C (which was predictive of MI over self-reported diabetes and hypertension). In addition, fruit and vegetable intake, exercise, and alcohol use were associated with lower risk for acute MI. The PAR from the multivariate model was around 90%.

Body mass index (BMI) was not predictive for MI when adjusted for abdominal obesity. The waist-hip ratio (WHR), a measure for central adiposity, on the other hand was a predictor of MI (even when adjusted for BMI). Interestingly, although a higher waist circumference was associated with a greater risk for MI, a larger hip circumference was protective factor against MI. There are many theories for this observation but none of them have been confirmed.

In general, the risk factors are similar in men and women with the exception of hypertension and diabetes, which is slightly greater in women than in men. Looking across the age groups, the risk factors give greater odds ratios in younger people than in older, i.e. there is a greater association with MI when the risk factors are present in young people (see Yusuf et al., *Lancet* 2004.)

One of the aspects of the IHS was the ability to compare different regions and different ethnic groups in terms of the variations in the PAR. Variations in the PAR are expected because the frequency of the risk factors also varies per region or ethnic group. The IHS, however, demonstrated that the risk factors for MI are the same across age, groups, gender, and ethnic populations. There are variations in the PAR for certain variables, but in general the risk factors for MI are the same for all regions and ethnic groups.

Thus, if the risk factors are ubiquitous within the population, this suggests that a population strategy of prevention is likely to be most effective in preventing cardiovascular disease and the global burden. Although we have been well informed by randomized controlled trials how to manage patients with an established event, the greatest challenge, however, is to have a societal approach to MI prevention, i.e. how can we change our physical environment as well as the policy environment to prevent risk factors from ever developing (see Yusuf et al., *Lancet* 2004).

In summary, CHD is the leading cause death in the world and increasing in low and middle income populations. Risk factors for CHD, however, are common throughout the populations. Societal and individual changes with drug treatments, perhaps the Polypill (see next topic), are needed to suppress the epidemic of CHD. In addition, the IHS data has the potential to come up with region-specific strategies for CHD prevention.

THE POLYPILL: FROM CONCEPT TO REALITY

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The Polypill concept means adopting a simple population-based approach to preventing CVD.

The Polypill is a single pill that can modify several causal cardiovascular (CVD) risk factors simultaneously (see Wald and Law, *Br Med J* 2003). The indication for preventive treatment should be influenced by a person's overall risk of CVD, not the level of individual risk factors such as serum cholesterol and blood pressure. If a decision to offer preventive treatment is made, all reversible risk factors should be treated where this can be done effectively and safely, not just those judged to be high. Drugs have side effects and, with most blood pressure lowering drugs, halving the dose of the blood pressure drug more than halves the reduction in the side effects, but efficacy is only reduced by about 20%. Using half doses of several drugs from different classes therefore increases the beneficial effects and decreases the prevalence of side effects. Thus in the prevention of cardiovascular disease there is benefit in using combination low dose blood pressure lowering drugs together with a statin at standard dose. There is a therapeutic robustness in having many drugs in one pill: if one is not effective (for, say, genetic reasons), only a small amount of the risk reduction is lost, but with mono-therapy this is not the case.

Components of the Polypill				
Risk Factor	Component	Approximate risk factor reduction	Reduction in risk	
			IHD	Stroke
LDL cholesterol	Statin e.g. simvastatin 40mg/day atorvastatin 10mg/day	1.8 mmol/L	61%	17%
Blood pressure	Half standard dose of 3 from:	10.7 mm Hg diastolic	46%	63%
	- Thiazide - β -blocker - ACE-inhibitor or ARB - Calcium channel blocker (CCB)			
Serum homocysteine	Folic acid (0.8mg/day)	3 μ mol/L	16%	24%
Platelet aggregation	Aspirin (75mg/day)	-	32%	16%
Combined effect			88%	80%

Starting blood pressure 120/80

The Figure shows the expected effect of a Polypill with 6 components. All components have been shown in trials to reduce CVD risk except for folic acid, for which the effect is based on epidemiological results and is therefore less certain. A recent trial of a Polypill documented significant reductions in total cholesterol, LDL cholesterol, and blood pressure (see The Indian Polycap Study, *Lancet* 2009).

How should people be selected for the Polypill? In many countries about 95% of IHD and stroke deaths occur over the age of 55, so this is a reasonable starting point, or possibly about age 50 if events are to be caught rather than just deaths. Important causal risk factors such as serum cholesterol and blood pressure are poor predictors of who will develop CHD events or strokes. They add little extra discrimination to the use of age alone, which is the most powerful predictor. It is much simpler to use age alone in screening: the small loss in discrimination is of little consequence given the efficacy, safety, and potential low cost of the Polypill. The Polypill concept is more than formulating a tablet or capsule with several components because it encompasses the strategy of offering it to all healthy people above a certain age to prevent first CHD events and strokes. The Polypill is unlikely to make people less health conscious and adopt unhealthy behaviors; the well informed and health conscious are likely to be those who use the Polypill more than others, and they will not abandon their attitudes to diet, weight, and general health. The public health challenge is that those in most need, that is people who are overweight, smoke, and eat a poor diet, will be less likely to take the Polypill. Different Polypills will be needed for primary and secondary prevention (the former without aspirin because the hazard of bleeding may not be adequately offset by the anti-clotting benefits in people who have not had an ischemic event. For the present folic acid may need to be omitted because of its uncertain efficacy). A primary prevention Polypill consisting of 4 components (three blood pressure lowering drugs and a statin) would be affordable and, if offered to everyone from aged 55, on average 1 in 3 people without a history of CVD or a stroke who took the Polypill regularly would benefit and each would gain about 11 years of life without a heart attack or stroke. The Polypill concept means adopting a simple population-based approach to preventing CVD, similar to a vaccination policy in preventing the complications from infectious diseases.

WHAT ARE THE NEXT STEPS FOR BIOMARKERS?

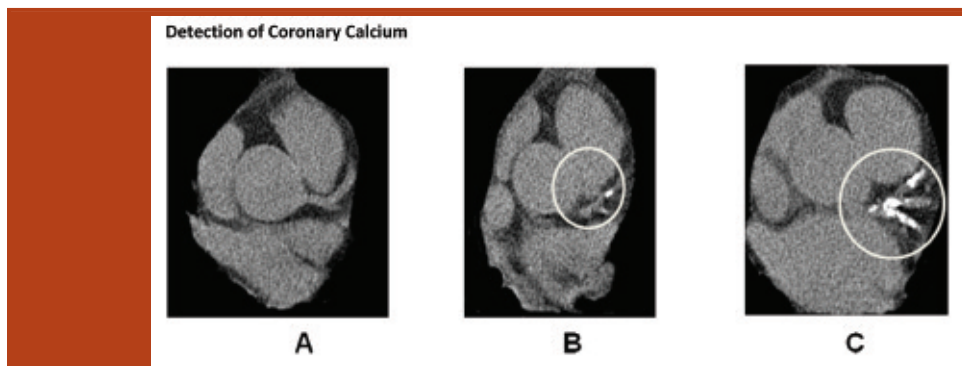
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Among the several issues that accompany the explosion of genetic data generated by the genome-wide association studies is the need to discover the biology underlying significant SNP associations and the need for bioinformatic capacity and tools to make sense of the vast amounts of data.

Population studies have led to the identification of risk factors for CHD. Such information has served as the basis for randomized controlled trials and later on for intervention strategies. The question is what the next steps will be for serum-, imaging-, and genetic-biomarkers and where this may be going in the future.

Studies using electron beam computed tomography (CT) or rapid 64-slice CT (takes 30 seconds) have shown that the amount of calcium in the heart is a very powerful predictor of future CHD events and mortality (see studies by Greenfield et al., *JAMA* 2004, Vliegenthart et al., *Circulation* 2005, Detrano et al., *New Engl J Med* 2008, Folsom et al., *Arch Int Med* 2008, Budoff et al., *J Am Coll Cardiol* 2007, Boyar, *J Thorac Imaging* 2006, Budoff et al., *J Am Coll Card* 2009).



Biomarker discovery and clinical validation is a dynamic field and the biomarker evaluation criteria have been previously reviewed (see Vasan, *Circulation* 2006). Before moving a novel biomarker forward into larger studies and clinical practice, there are various criteria to determine whether confounding has had a role in any of the associations between biomarkers and CVD. In addition, it

is also essential to investigate whether multi-marker strategies are superior over single marker strategies. These validations are necessary to determine whether the novel biomarker adds to the established risk factor algorithm and ultimately changes clinical practice.

Genomics is a new important area in the biomarker field. Parental history is a strong risk factor for CVD in the NHLBI's Framingham Heart Study population and the heritability for a number of risk factors and biomarkers has been estimated to be between 20-50%. Thus genetics is driving a significant portion of the inter-individual variability in risk.

There has been a true explosion of gene association studies, with one of the more interesting findings being the strong association of the 9p21 locus with MI, replicated in a number of GWAS studies. The great variability in the results from candidate gene studies, however, have created skepticism towards gene association studies and have caused many people not to believe the candidate gene study data. This has ultimately lead to strong recommendations on replicating findings from genetic association studies.

Among the several issues that accompany the explosion of genetic data generated by the genome-wide association studies is the need to discover the biology underlying significant SNP associations and the need for bioinformatic capacity and tools to make sense of the vast amounts of data. Furthermore, the need for large sample sizes has led to the formation of multicenter consortia to combine all the genetic data, because it has been found that one study alone cannot provide complete data about most biomarkers and risk factors underlying CVD.

The final question is: will genotype data eventually add to the risk factor algorithm? Currently the genotype risk score does not add information to the standard Framingham risk score and it is not yet known how the genotypes are going to affect current medical practice. As more data are gathered, SNP's may predict as well, or even better than traditional risk factors. At that point, it is critical to determine how to use these genetic biomarkers for prediction, prevention, and personalization of medicine.



CONCLUSIONS

Today, many studies are conducted to identify novel biomarkers and the field is moving quickly in identifying and evaluating those new biomarkers that will have relevance for prevention of disease in the future.

Biomarker discovery will be driven even more by technology than now is the case and in the future it will be very important to develop new ways of dealing with the information generated by these technologies and develop new strategies to integrate all the data generated by these new technologies for risk prediction models and personalized medicine.

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RISK FACTORS FOR ATHEROSCLEROSIS

Highlights

Supported by in part by
educational grants from

AstraZeneca 

 **Schering-Plough**