Predicting the risk of coronary heart disease

with conventional, genetic and novel molecular biomarkers

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" *Prediction is very difficult,
especially about the future.* "

Niels Bohr

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Introduction

Coronary heart disease (CHD) is a major public health problem. A wide range of preventative interventions for individuals is available, involving either medication or lifestyle change. Both types of intervention are improved by accurate assessment of the individual's risk: the benefits of medication are proportional to the recipient's absolute risk of CHD, while lifestyle change is probably more likely in people who see themselves as at particularly high risk. Inaccurate risk assessment leads to failure to identify, treat and motivate high-risk people, to less cost-effective targeting of treatment to those at lower risk and to the potential for discouragement of all involved if the risk prediction model becomes devalued. Furthermore, if the model is biased, it may exacerbate health inequalities by, for example, systematically under-estimating the risk of CHD in socio-economically deprived people and those from ethnic minorities.

Given the public health importance of CHD, the enormous volume of epidemiological research into its aetiology and the interest of primary care practitioners in its prevention, it is not surprising that a number of tools for assessing individual risk have been developed. However, three problems remain for those seeking an evidence-based approach to choosing and using these tools:

1. How should risk be assessed? There is no consensus as to the most suitable risk prediction model, for use either internationally or in the United Kingdom.

2. Which biomarkers should be incorporated into risk assessment? There is uncertainty about the potential contribution of novel blood-borne molecular biomarkers to risk assessment, and about whether and how they should be used to identify those at higher risk.

3. Can genetic information improve risk prediction? All available risk prediction models leave an important proportion of individual variance in risk unexplained, and few integrate information on family history. Meanwhile, knowledge of the genetic contribution to risk is increasing.

This report aims to answer these questions. It goes on to explore the implications of its findings for the appraisal and use of biomarkers more generally.

1 Conventional risk factors

Summary

- Predicting the future occurrence of CHD is not possible, but the risk can be estimated with models based on cohort studies.

- Most existing models are based on long-standing research on the residents of Framingham, Massachusetts.

- The findings from Framingham yield inaccurate results when applied to contemporary British populations. In particular, they may exacerbate health inequalities.

- This is because the incidence of and mortality from CHD have fallen recently, the Framingham cohort differs from many groups to which findings from it have been applied, important risk factors such as ethnicity, socio-economic deprivation and family history are absent from the Framingham equations and susceptibility to risk factors varies between populations.

- Attempts to recalibrate or adjust the Framingham equations to improve their performance have not been shown to overcome these problems.

- SCORE, QRISK and ASSIGN are risk prediction models that have been developed based on different cohorts.

- The group developing NICE's guideline on lipid modification was uncertain about which risk prediction model to recommend for use in the NHS. Eventually they selected a modified version of the Framingham equation.

- However, QRISK appears to offer the best long-term promise.

Background

Clinicians and patients need reliable information about an individual's risk of developing CHD. Ideally, they would have entirely accurate data and would be able to use a perfect model to estimate risk. Such a model would be able to categorise people dichotomously into those who would develop CHD and those who would not. Indeed, the perfect model would even be able to predict the timing of the disease's onset. Those destined to develop CHD could receive intensive interventions to reduce their risk and postpone, if not prevent, the disease arising; those who would not develop CHD in the course of their lifetime could be reassured.

Of course, no such perfect model exists. Our knowledge of the disease's aetiology is too incomplete, in terms of both which risk factors are independently important and how they should each be weighted. In any case, many of the risk factors which are known to be important, such as blood pressure and serum cholesterol level, cannot be measured with sufficient accuracy to support risk assessment with this putative degree of certainty. They show considerable intra-individual variation, making repeated measurement necessary for an accurate assessment. This is good clinical practice before treatment decisions are taken, but difficult and expensive in a research setting.

Instead of dichotomising people in this way, the available risk prediction models estimate the probability of CHD arising in a specified future period, usually ten years. There is an obvious limitation to the value of information from such models, in that it falls far short of providing clarity for individuals about what will happen to them. Most people who go on to develop CHD have estimated risks that indicate that a CHD event is unlikely. More than half of the cardiovascular disease events in the next ten years among asymptomatic adults in the UK will occur in people below the current drug treatment threshold of 20% over ten years.[1]

Nevertheless, the outputs of these models can be used to categorise people according to their risk of CHD, and this can in turn be used to decide how intensively to intervene in order to reduce risk. This usefully aligns the inconvenience, risks and costs of intervention with the potential benefits of risk reduction. But, by the same token, risk prediction models which misclassify people can be damaging, leading to a misperception of risk, a misapplication of clinical effort and resources, and costs and harms not offset by commensurate benefits.

So the selection of which model to use is of critical importance. This chapter reviews how models are assessed, appraises those available and sets out to identify the most suitable for use in the United Kingdom.

The assessment of risk prediction models: calibration and discrimination

Risk prediction models have usually been assessed using two criteria, calibration and discrimination. The two are independent, meaning that whether a model has one characteristic is unrelated to whether it also has the other.[2]

A well-calibrated model will correctly estimate the average risk of a group of people. Poor calibration will lead to systematic inaccuracy in a model's performance; this might be universal, or might just occur in certain categories of subject. For example, people of south Asian ancestry living in western countries are at higher risk of CHD than white people. If a model omits ethnicity, it will systematically underestimate risk in south Asian people. The public health importance of this mis-calibration will depend on the proportion of south Asian people in the population in question; in an entirely white population it would not matter, but in modern British society it would be an important weakness.

A model that discriminates well ranks individuals' risk in the correct order, accurately labelling people as to how their degree of risk relates to that of the population as a whole. Such a model will have high sensitivity and specificity. Discrimination can be illustrated by receivor operator characteristic curves, which display models' discriminatory capacity over the range of possible thresholds. A model which ignored ethnicity could still discriminate well in a population made up entirely of south Asian people or of white people, since in both cases ethnicity is not relevant to their risk relative to one another. In a population of mixed ethnicity, it would discriminate less well the larger the minority group was.

So a model can discriminate well but be poorly calibrated if the population on which it is used is homogenous with respect to a variable which it incorrectly excludes. A well-calibrated but poorly discriminating model might have several faults contributing to its inability to rank individuals correctly; if these faults cancelled each other out, the model's prediction of average risk might (by chance) be correct, at least in some populations.

There are other dimensions to a model's performance that are worth considering, such as the extent to which it reclassifies people into risk groups more accurately than alternatives and how much of the variation in risk it explains. These are considered further in Section 4, but have seldom being used to assess existing CHD models.

Framingham model

The first research used to develop a risk prediction model was carried out in Framingham, a town in Massachusetts, USA. The Framingham Heart Study began in 1948, and is based on three generational cohorts of residents of the town, a total of 14,428 men and women. Members of the first cohort were all over the age of fifty years at inception. The Framingham Offspring Cohort, made up of children of the original cohort and their spouses, was recruited from 1971 and contained individuals recruited at a younger age than the original cohort, some as young as twelve years. In 2002, the Third Generation Study began, involving 4095 further people.

At the inception of the study, the Framingham researchers measured what have come to be known as conventional risk factors. These include blood pressure, smoking behaviour, blood lipids, and adiposity. The researchers then used multivariate analysis to develop an equation comprising those factors which influenced the risk of various cardiovascular outcomes, so that a prediction of risk could be made.[3] Risk factors were measured at the cohorts' inceptions and then the participants, who were initially all free from clinical CHD, were followed to see who developed a range of six outcomes, which included death from CHD and total CHD events, both fatal and non-fatal. Later, when up to twelve years' follow-up of the combined cohorts was available, the researchers were able to derive refined equations which, being based on more data, were expected to be more reliable.[4] Later still, a simplified model based on categorical rather than continuous variables was published.[5]

Models tend to perform best in populations which resemble closely those in which they were developed. Before a model can be used confidently in a novel population, particularly one that has *prima facie* differences from the one in which it was developed, its predictive accuracy needs to be tested in that population. The equations which have resulted from the Framingham study have been tested against various non-US cohorts for this purpose.

Brindle *et al*. compared the results of predictions from the 1991 version of the Framingham equation with the outcomes from the British regional heart study.[6] They found that the overall risk of death predicted for the cohort over ten years by the Framingham equation (4.1%) was much higher than that actually experienced by the cohort (2.8%, 95% confidence interval 2.4% to 3.2%). CHD mortality was over-predicted by 47% and fatal and non-fatal CHD events by 57% (p < 0.0001 in both cases). The degree of overestimation of risk was even across quintiles of risk, so the authors recalibrated the Framingham equation downwards to produce an adjusted equation which better fitted the outcomes in each quintile of risk. They did not report the receiver operator characteristic curves for either the original or recalibrated equation; such curves are an important measure of the discriminatory power of a risk model.[7]

The main reason for the inaccuracy of the Framingham equation is thought to be the large secular fall in population risk of CHD since the cohort was established.[8] This has only been partly due to reductions in the prevalence of risk factors, especially smoking, which would be reflected in the outputs of the model. Better treatment of risk factors and of CHD itself have reduced the incidence and mortality of the disease, but equations based on earlier mortality experience will not take account of this. There are also specific influences of ethnicity on risk which are absent from the Framingham equations because the cohort was largely white. Furthermore, the complex effect of socio-economic deprivation on CHD risk is missing from the Framingham model because of the cohort's relative economic security and prosperity.

There are also potentially important risk factors which are not included in the Framingham equations. These include family history, body mass index, the metabolic syndrome, socio-economic status and lack of physical activity. The importance of these risk factors is better understood now, and some, such as obesity, have become more common.

A systematic review compared the predicted risk according to the Framingham equations with that observed in other cohorts.[9] The review included 27 studies with data from 71,727 participants on predicted and observed risk of either CHD or cardiovascular disease more generally. Pooled risks were not calculated because of the heterogeneity of the results from individual studies, itself indicative of a problem with the generalisability of the Framingham model. For CHD, the ratios of predicted to observed risk over ten years ranged from an under-prediction ratio of 0.43 (95% confidence interval 0.27 to 0.67) in a high-risk population, to an over-prediction ratio of 2.87 (95% confidence interval 1.91 to 4.31) in a lower-risk population. Under-prediction of risk was particularly likely in patients with diabetes and a family history of premature CHD, and in a higher-risk UK primary care population. Over-prediction of risk occurred in lower risk populations, mainly in Germany and France. This type of error is harder to correct by simple recalibration of the equation and suggests that the Framingham model is now unsuitable for use in populations which differ significantly from that in which it was developed.

A more recent systematic review of validation studies shed light on a possible reason for the variable performance of the Framingham equations.[10] It found that the Framingham score performed well in North American and Australasian cohorts, but consistently worse in European cohorts, where it tended to overestimate risk. This may reflect lower underlying risks in European populations, along with the impact of ethnic, other genetic, and environmental differences which the Framingham model does not take into account. Susceptibility to risk factors – the extent to which a given risk factor affects outcome – may vary systematically by ethnicity and socio-economic status.

These possible reasons for the disappointing results of Framingham-based approaches in Europe were explored in a systematic review of cohort studies of CHD.[11] It showed once again that the Framingham equation tends to overestimate risk in low-risk populations and underestimate it in high-risk populations. Furthermore, the systematic review reported that the estimated relative risks associated with major risk factors, such as age, systolic blood pressure, serum total cholesterol, smoking and diabetes, varied significantly between populations. For example, the association with smoking varied from 1.33 in a North American cohort to 2.44 in a Norwegian one. These variations severely compromise the suitability of risk models for international or global use.

The inaccuracies which result from use of the Framingham equation can lead to biases with pernicious effects on public health. In one study, the equation systematically underestimated risk when applied to a cohort of people from areas of high deprivation and from manual social classes, relative to more affluent individuals, so that fewer people in deprived areas reached thresholds for treatment.[12] This kind of error could lead to clinical decisions which exacerbate socio-economic health inequalities.

Augmented Framingham models

One response to the deficiencies of the Framingham equations is to add new variables to them, in an effort to improve their performance. Studies taking this approach are mostly confined to evaluating whether the addition of a single biomarker, such as C-reactive protein, adequately corrects the Framingham equations. C-reactive protein has been studied with particular interest because a number of epidemiological studies have reported positive associations between elevated levels and CHD.[13] Yet a systematic review found no definitive evidence that, for most individuals, C-reactive protein adds substantial predictive value beyond that provided by risk estimation using conventional risk factors for CHD.[14] Use of C-reactive protein may however add to risk estimation in a small number of individuals at intermediate predicted risk according to the Framingham risk score.

Another study examined whether including the presence of the metabolic syndrome improved CHD risk prediction beyond that achieved by the Framingham equations.[15] Analysis using receiver operator characteristic curves showed that it did not. No other additions to the Framingham equations have shown a substantial impact on their accuracy; given the reasons for their poor calibration, it is not surprising that this approach has not generally been effective.

There are several risk prediction models based on Framingham equations, but with a different presentation to aid use. Examples include the New Zealand risk tables[16], the joint European Societies' charts[17] and the second Joint British Societies' recommendations[18]. In some cases, modest adjustments were made to improve accuracy, such as in the Sheffield tables.[19] However, this approach is based on a belief in the appropriateness of using the Framingham equations, and is neither intended, nor likely, to overcome the more deep-rooted flaws that limit the Framingham model's value. Two validation studies used risk predicted by Framingham as a gold standard, which does not provide any information on the underlying validity of the Sheffield tables.[20] [21]

In a more thorough-going approach to dealing with the inaccuracies of the Framingham model in British ethnic minority populations, the equation was recalibrated to reflect the higher incidence of cardiovascular disease in ethnic minorities, even when differences in conventional risk factors are accounted for.[22] The results had face validity, with the recalibrated equation, termed ETHRISK, showing higher risks in people of South Asian origin than the original equation did. However, the study did not report whether the risks which ETHRISK predicted were those experienced by the populations in question, nor has the use of ETHRISK been validated in a prospective cohort study.

Alternatives to Framingham

SCORE

The SCORE project represented a break from the Framingham approach.[23] The researchers set out to develop a risk scoring system for use in the clinical management of cardiovascular risk in Europe, based on a pool of datasets from twelve European cohort studies, most of which were in general population settings. The pooled dataset was large, including 205,178 persons and representing 2.7 million person years of observation. The researchers calculated separate estimation equations for CHD and for non-coronary cardiovascular disease, for high-risk and low-risk regions of Europe and for risk based on total cholesterol and on total cholesterol/HDL cholesterol ratio. The equations are based on only five variables: sex, age, smoking, systolic blood pressure and either total cholesterol or cholesterol/HDL ratio. They predict the risk of fatal events only.

SCORE therefore has several advantages over Framingham, being based on a more recent, larger and more diverse cohort, potentially (though not necessarily) more similar to the contemporary British population ethnically and genetically. The authors gauged the predictive value of the risk charts by applying them to people in separate cohort studies; areas under the receiver operator characteristic curves ranged from 0.71 to 0.84, which suggests the equations discriminate well.

The SCORE risk equation was applied to a separate cohort of residents of Vorarlberg, in western Austria.[24] The SCORE equation substantially over-estimated the risk of CHD death. The observed CHD death-rate over ten years was 0.7% (95% confidence interval 0.6% to 0.8%) whereas SCORE predicted a death rate of 1.0%. The predicted rate of CHD deaths fell outside the observed rates' 95% confidence intervals for both men and women. The same over-estimation occurred with death rates from any cardiovascular cause. The receiver operator characteristic curves for both sexes and both causes were more supportive of SCORE's validity, with areas under the curve between 0.75 and 0.84. These results indicate that, in this population at least, SCORE discriminated well but was poorly calibrated. Good discrimination does not compensate for overestimation of risk, because decisions about the use of interventions need to be based on estimated absolute event rates, particularly for more costly interventions.

Another validation study compared predicted rates of fatal CHD and cardiovascular disease according to the appropriate SCORE equation with rates according to the Framingham equations corrected for German mortality experience, and with German national mortality statistics.[25] SCORE predicted CHD death rates 1.3 times higher than Framingham, and cardiovascular disease rates 1.4 times higher. Recorded mortality outcomes lay between the two. The authors concluded that SCORE overestimated the risks of CHD and cardiovascular disease in Germany.

It is surprising that a Framingham model underestimated risk in this study. A possible explanation is that the cohort to which the equations were applied excluded high-risk people, such as those who already had cardiovascular disease, those living in residential care and those with a particularly adverse risk factor profile, whereas the national mortality statistics were comprehensive. This suggests that the over-estimation of risk by SCORE is greater than this study indicates.

ASSIGN

ASSIGN is a risk score developed in Scotland.[26] It is of note because it was the first risk score to incorporate socio-economic deprivation and family history; earlier research had indicated the importance of these factors, but they had not been measured and used systematically to assess risk. However, ASSIGN has yet to be validated in an independent cohort.

QRISK

QRISK is a new British risk assessment tool.[27] It is based on a database representative of British general practice containing the health records of 10 million people over 17 years. The cohort from which the model was derived contained 1.3 million adults aged 35 to 74 years, and the validation cohort contained 610,000 people. It is the largest cardiovascular disease prediction study to date. Because it is from a routine clinical information system, rather than a specially assembled cohort of volunteers, it is likely to be less affected by selection and volunteer biases.

The model in its first form (QRISK1) included age, sex, smoking status, systolic blood pressure, ratio of total serum cholesterol to high density lipoprotein cholesterol, body mass index, family history of coronary heart disease in a first degree relative aged less than 60 years, an area measure of deprivation and existing treatment with an anti-hypertensive agent. The model's predictions were compared with those of the Framingham equation and of ASSIGN and with the outcomes recorded for the cohort in Office for National Statistics mortality data.

In the evaluation, the Framingham equation over-predicted mortality by 35% and ASSIGN over-predicted by 36%, whereas QRISK1 over-predicted by only 0.4%. The areas under the receiver operator characteristic curves for QRISK were 0.77 for men and 0.79 for women, slightly higher than those for the ASSIGN and Framingham equations. QRISK1 also had somewhat higher D statistics, a more useful measure which indicates it discriminated better between people with differing levels of risk, and R^2 statistics, indicating that it explained more of the variation in risk. Perhaps because it included deprivation status, the QRISK equation was better calibrated to the UK than the other two models, showing less propensity to overestimate risk in affluent areas and underestimate it in deprived ones.

The authors subsequently refined QRISK1, publishing a revised risk prediction algorithm (QRISK2) which incorporated self-assigned ethnicity, type 2 diabetes, treated hypertension, rheumatoid arthritis, renal disease and atrial fibrillation.[28] They compared the performance of this model with the original QRISK equation, and the modified version of the Framingham equation recommended by NICE (see below), in which, for south Asian men, the risk according to the Framingham equation is multiplied by 1.4. QRISK2 performed slightly better than QRISK1 and substantially better than the modified Framingham equation.

A study applying the QRISK1 and Framingham equations to a separate primary care database of 1.07 million British patients confirmed QRISK1's superior performance.[29] An independent validation and verification analysis also found that QRISK1 performed better than the Framingham equation in a contemporary British population.[30]

NICE's clinical guideline

In December 2003, the National Institute for Health and Clinical Excellence (NICE) was asked to prepare a clinical guideline on lipid modification, covering the estimation of cardiovascular risk and the use of interventions to modify blood lipids in the primary and secondary prevention of cardiovascular disease. A draft guideline was published for consultation in June 2007, recommending risk estimation using the Framingham equations. The final version of the guideline was expected to be published in January 2008.

During the consultation period, the first QRISK1 paper was published.[27] In October 2007, NICE announced a delay to the process and asked the group developing the guideline to assess QRISK1 and reconsider their recommendations on risk estimation, seeking advice on technical issues from independent experts.

The guideline development group received recommendations of QRISK1 from Professors Doug Altman, Rod Jackson and Sir Richard Peto. In January 2008, the group unanimously agreed that QRISK1 should be recommended instead of the Framingham equations, and accordingly issued for consultation a revised draft of the section of the guideline dealing with risk assessment.

However, the final version of the guideline recommended use of the Framingham equation, with the estimated risk adjusted in South Asian men and in people with a family history. The choice about which risk assessment method to recommend was "one of the most difficult decisions that the guideline development group faced".[31] The main reasons for the decision to prefer a Framingham-based approach were:

- Ascertainment and accuracy of outcome data: because the QRISK outcomes data were ascertained via routine datasets rather than via formal research, they may be less accurate.

- Independent validation of QRISK: the details of the QRISK1 equation had not yet been made available, so independent validation and comparison with other scores was not possible.

- Use in practice: the novelty of QRISK raised questions about how readily it could be used in clinical general practice.

- Comparisons with ASSIGN: the differences between Framingham, ASSIGN and QRISK1 were small in terms of discrimination. The guideline development group did not believe that they had enough evidence to decide that QRISK1 was definitively the better score for the UK, and superior to ASSIGN.

- Overestimation versus underestimation: The group believed that Framingham's overestimation errors were more acceptable than QRISK's underestimation, although the former are much larger than the latter.

The guideline development group could not make a decision that one risk assessment equation was clearly superior in the UK population. They recognised a strong case for the use of a risk equation which has been developed and validated on a UK population and which takes account of deprivation, but recommending a new score required a higher level of certainty than they had with regard to QRISK1. After a vote, the group decided to return to their original recommendation of Framingham, despite its known limitations, because it was currently in use and its limitations were understood.

Appraisal

The four leading candidate risk scores are Framingham, SCORE, ASSIGN and QRISK2. Table 1 compares them.

Table 1: Comparison of CHD risk estimation models

	Framingham	**SCORE**	**ASSIGN**	**QRISK2**
Calibration (predicted risk equals observed risk)	Overestimates risk when it is low and underestimates it when it is high	Overestimates risk	Not yet adequately tested	Apparently good
Discrimination (individuals ranked correctly)	Adequate	Adequate	Not yet adequately tested	Apparently good
Geographical origin	United States	Europe	Scotland	United Kingdom
Recency	Outcomes predate fall in CHD mortality	More recent than Framingham	Recent	Recent
Validation in the UK	Shows poor calibration, though partial adjustment possible	None apparent	Scotland only	Yes
Ethnicity	Not included	Not included	Not included	Included
Socio-economic deprivation	Not included	Not included	Included	Included
Standardisation of outcome measurement	Yes	Yes	Yes	No
Capacity for continuous updating	No	No	No	Yes

The last two rows of Table 1 show specific differences between QRISK2 and the other three risk measurement tools.

- *Standardisation of outcome measurement* draws attention to the fact that QRISK is based on outcome data gathered from routine clinical datasets, while the other risk estimation models are constructed from formal cohort studies with scientifically adjudicated outcome measures. The former are likely to be less accurate. If, for example, incident cases of CHD were under-recorded in the clinical dataset, then the true risk in individuals would be higher than that predicted by the model.

    Validation of deaths against Office for National Statistics (ONS) data showed that 93% of deaths from cardiovascular disease recorded by the ONS were also in the general practice dataset.[28] The under-ascertainment of non-fatal incident events was not assessed.

    This issue could reduce both the calibration and discrimination of QRISK when used in practice, but its importance cannot be easily gauged at present. This limits the confidence we can have in QRISK, and can only be resolved by more research showing the predictive accuracy of QRISK in populations with more reliably ascertained outcomes.

- While the origins of QRISK in an open clinical cohort, rather than a closed research one, raise questions with regard to ascertainment of outcomes, they are an advantage in terms of *capacity for continuous updating*. This means that as more data accrues from the practices supplying data for QRISK, the accuracy of the model improves; if the overall risk of CHD changes over time, or the importance of a risk factor alters, that too can be readily detected and incorporated into the risk model.

    This is an important advantage. Many of the problems with Framingham-based approaches stem from the difficulty of adapting them to change, be it geographical, ethnic or secular. If QRISK proves capable of continuous semi-automatic modification, it will have an attractive durability.

QRISK emerges from Table 1 with the greatest potential. It is most likely to be sensitive to the equity issues of great current concern, it reflects best the contemporary British population and its initial results are encouraging. The uncertain approach of NICE's guideline development group illustrates how difficult it can be to judge how much evidence is needed before a change in practice can be recommended, but if QRISK lives up to its promise, it will in time become established as the risk assessment method of choice.

2 The role of novel molecular biomarkers in estimating risk

Summary

- About three quarters of coronary heart disease (CHD) risk is explained by conventional risk factors.

- Novel biomarkers present in blood are also associated with increases in risk.

- To contribute substantially to an improvement in the performance of existing models, novel biomarkers would need to have a close association with CHD, to exhibit statistical independence from conventional risk factors and to be prevalent, sensitive and specific in diagnostic performance, and easy and inexpensive to measure reliably.

- None of the novel biomarkers satisfy these criteria at present.

- Several are independently associated with CHD. However, more stringent analysis of their discriminatory performance is either absent (and unlikely to be positive) or shows that they bring little advantage.

- The novel biomarkers may provide insights into the pathogenesis of CHD and into how to monitor and treat patients. They may also have a role in risk assessment in specific clinical situations. However, they seem unlikely to have major public health importance.

Background

Section 1 described how knowledge of conventional risk factors can be used to estimate the risk of CHD in individuals. Although the calibration of models based on those risk factors is good, they do not discriminate perfectly, and leave about a quarter of the risk of CHD unexplained, prompting researchers to look for other risk factors which may be important.[32]

A range of novel molecular biomarkers have been identified which may be independently associated with CHD risk. Hitherto, researchers' interest in these novel biomarkers has been largely driven by the insights into the mechanism of vascular disease that they might provide, rather than the development of improved prediction models for clinical use.

Those most frequently investigated are listed in Table 2.

Table 2: Novel molecular biomarkers of coronary heart disease

Biomarker	Description
C-reactive protein	A plasma protein synthesised mainly by the liver in response to inflammation. Its function is unknown.
Fibrinogen	The principal protein in blood coagulation, during which it is converted into fibrin by thrombin. Fibrinogen is an important determinant of blood viscosity and platelet aggregation.
Homocysteine	An amino acid. People with homocystinuria, a rare inborn error of metabolism, have high levels of circulating homocysteine and premature vascular disease. Homocysteine may be raised for other reasons, such as diet and medication. High circulating levels of homocysteine can affect blood coagulation and endothelial resistance to thrombosis, and interfere with the vasodilator and antithrombotic functions of nitric oxide.
N-terminal fragment brain natriuretic peptide (NT-pro-BNP)	Brain natriuretic peptide (BNP) is a polypeptide secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells. It is co-secreted with an N-terminal fragment (NT-pro-BNP) which is biologically inactive but has a longer half-life, making it more suitable for diagnostic blood testing. BNP is a marker of congestive cardiac failure. It reduces systemic vascular resistance and central venous pressure and causes natriuresis.
Small dense lipoproteins	The lipoproteins which carry cholesterol in the blood are categorised according to their density, with the most commonly measured being low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. Low density lipoprotein molecules are not homogenous; those which are smaller and denser may have independent effects on risk of atherosclerosis.
Apolipoproteins	Apolipoproteins are proteins that bind to lipids to form the water-soluble lipoproteins by which cholesterol is transported in blood. They are also enzyme co-factors, receptor ligands and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in tissues. There are many genetic polymorphisms which affect apolipoproteins' structure and function. There are six classes of apolipoprotein, A, B, C, D, E and H.

Lipoprotein-associated phospholipase A2	Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme of 441 amino acids. It is produced by inflammatory cells and hydrolyzes oxidized phospholipids in low-density lipoprotein. The products of this reaction are believed to cause inflammation in the arterial lining.
Lipoprotein(a)	Lipoprotein(a) is a low-density lipoprotein-like particle synthesised by the liver. It consists of an apolipoprotein molecule covalently linked to a very large glycoprotein known as apolipoprotein(a). Its physiological and pathological role are uncertain, but *in-vitro* and animal studies suggest that lipoprotein(a) can promote thrombosis and inflammation.

There are several important questions about these novel biomarkers:

- Are they independently associated with CHD?
- If so, how strong is the association?
- Do they lie on the pathogenetic pathway for the development of atherosclerosis, plaque rupture or plaque-associated thrombosis?
- Would we be better able to estimate the risk of CHD in individuals if we took any of these biomarkers into account?

This chapter reviews the relationship between measures of association and predictive value, briefly summarises the strength of evidence linking these biomarkers to CHD and considers whether they are yet suitable for inclusion in risk prediction models.

Measures of association and of predictive value

Most studies of novel biomarkers measure the strength of the association between the biomarker and an outcome of interest, such as diagnosis of or death from CHD. These associations are expressed as odds ratios, relative risks or hazard ratios, usually after adjustment to eliminate as far as possible the confounding which can arise from the separate association of the biomarker and conventional risk factors, especially smoking, raised total or low-density lipoprotein cholesterol and diabetes.

This approach is suitable for exploring the connection between the biomarker and CHD, and especially for identifying biomarkers which may be indicators of, or even participants in, the process by which CHD occurs. It may also provide insights into how to monitor and treat patients. However, for a biomarker to be generally useful in classifying individuals as to their level of risk, it must have more than a statistically significant association with disease occurrence. The prevalence of the risk factor, its positive and negative predictive values, the magnitude of the risk estimate, selection of thresholds and the co-variation among risk factors and among their combinations are all of importance.

Theoretical analysis, supported by empirical work, has shown that, once a model has fairly good discrimination, adding further risk factors which have significant associations with the disease usually does not make much difference to the area under the receiver operator characteristic curve. Even epidemiologically impressive relative risks of two or three will not increase the area under the receiver operator characteristic curve to a clinically important extent; the association must be unusually strong for the addition of the risk factor to materially affect the curve.[33] The lack of change in the receiver operator characteristic curve may be a sign of the irrelevance of the added risk factor or of the inappropriate insensitivity of the assessment method, but it has prompted a search for better ways of measuring discrimination, such as reclassification. These are discussed later; a recent article by Pencina *et al.* describes two newer and potentially important measures of discrimination.[34]

C-reactive protein

A systematic review reported an overall odds ratio of 1.58 (95% confidence interval 1.48 to 1.68) derived from comparison of participants in the top third of the group with respect to base-line C-reactive protein values with those in the bottom third.[35] The review included 7068 participants in twenty-two nested case-control studies, twenty of which adjusted their results for smoking and other established risk factors. To limit publication bias, the authors also reported an analysis restricted to the four studies involving more than five hundred people; this yielded an odds ratio of 1.49 (95% confidence interval, 1.37 to 1.62). The authors also reported results from the Reykjavik Study, a nested case-control study of CHD based in the Icelandic capital. Addition of C-reactive protein levels only improved the area under the receiver operator characteristic curve to 0.65 from 0.64, its level based on knowledge of total cholesterol, smoking status and systolic blood pressure.

A study based on 4446 people from the Framingham cohorts supported these results.[36] The discriminatory capacity of the risk model for cardiovascular disease and CHD was unchanged by the addition of C-reactive protein to the multivariable model. The authors concluded "Elevated [C-reactive protein] level provided no further prognostic information beyond traditional office risk factor assessment to predict future major CVD and major coronary heart disease in this population sample."

Furthermore, a systematic review included 31 studies of 28 prospective cohorts, involving a total of 84,063 individuals and 11,252 incident CHD events.[37] Improvements in calibration from the addition of C-reactive protein to the Framingham risk score were either absent or very small. Evidence from 13 studies (7201 cases) indicated that C-reactive protein did not consistently improve the discrimination of the Framingham risk score, with area under the receiver operator characteristic curve increments in the range 0 to 0.15. There was wide overlap of C-reactive protein values among people who later suffered events and those who did not.

Finally, a study using Mendelian randomisation cast further doubt on the causal role of C-reactive protein.[38] It reported that the risk of CHD was unrelated to the presence or absence of single nucleotide polymorphisms which cause increased levels of circulating C-reactive protein. This suggests strongly that the association between C-reactive protein and CHD not causal, but is instead the result of confounding by other factors, such as inflammation, causing both CHD and, separately, an increase in the biomarker. However, this does not in itself mean that the biomarker could not be of value in risk prediction.

Fibrinogen

A systematic review examining the association of fibrinogen with CHD included records of 154,211 participants in 31 prospective studies.[39] After adjustment for conventional risk factors, the hazard ratio per 1 g/l increase in fibrinogen was 1.82 (95% confidence interval 1.60 to 2.06). This result may be substantially inflated by residual confounding. In any case, the study did not report areas under the receiver operator characteristic curve, but it is unlikely that a hazard ratio of this level would add usefully to a model's discriminatory ability.

Homocysteine

Several systematic reviews have meta-analysed prospective studies of the relationship between homocysteine and CHD. One included 72 case-control studies of the prevalence of a mutation in the methylenetetrahydrofolate reductase gene, which causes elevated blood levels of homocysteine, and twenty prospective studies of serum homocysteine and disease risk. The odds ratios for CHD for a 5 micromol/l increase in serum homocysteine concentration were 1.42 (95% confidence interval 1.11 to 1.84) in the genetic studies and 1.32 (95% confidence interval 1.19 to 1.45) in the prospective studies.[40]

A second meta-analysis used data from thirty prospective or retrospective studies involving a total of 5073 CHD events.[41] After adjustment for known cardiovascular risk factors and regression-dilution bias in the prospective studies, a 25% higher usual homocysteine level was associated with an odds ratio of 1.12 (95% confidence interval 1.04 to 1.20).

As with fibrinogen, the substantial correlations between homocysteine levels and conventional risk factors, along with error in the measurement of the latter, mean that substantial residual confounding is probably present in all these analyses.

N-terminal fragment brain natriuretic peptide

There are only two published population-based cohort studies of the relationship between N-terminal fragment brain natriuretic peptide and CHD, including 225 cardiovascular events.[42 43] Both these studies reported an association between the biomarker and CHD, but considerable further work is required before a secure evidence-base will exist.

Small dense lipoproteins

There are no published population-based cohort studies of the relationship between concentrations of small, dense lipoproteins and CHD. However, a number of non-systematic reviews have cast doubt on the existence of an independent relationship between the two. For example, Sacks and Campos concluded "In summary, the picture that is emerging from epidemiology is that small LDL [low density lipoprotein] does not have a special relationship to CHD beyond its contribution to LDL concentration. ... We think that it is likely that confounding by triglycerides and other lipid risk factors is severe since most studies reported that the risk initially associated with small LDL becomes null or inverse after adjustment."[44] Another review article concluded "To date, the magnitude and independence of the association of LDL size with cardiovascular diseases has been tested in more than 50 studies, including cross-sectional and prospective epidemiologic as well as clinical intervention trials. The vast majority, but not all, of these trials demonstrate a significant univariate association of small, dense LDL with increased coronary heart disease (CHD) risk. However, LDL size is rarely a significant and independent predictor of CHD risk after multivariate adjustments for confounding variables, in particular plasma triglyceride levels and HDL cholesterol concentrations."[45] The same authors observed elsewhere "it may be that the increased risk associated with smaller LDL size in univariate analyses is a consequence of the broader pathophysiology of which small, dense LDL is a part (e.g. high triglycerides, low HDL cholesterol, increased LDL particle number, obesity, insulin resistance, diabetes, metabolic syndrome), rather than a reflection of an intrinsic increased atherogenic potential. A clear causal relationship between small dense LDL and increased cardiovascular risk cannot be proven, based on our present knowledge."[46]

Apolipoprotein A1

Apolipoprotein A1 is the major lipoprotein of high-density lipoprotein cholesterol. Variants of the gene that codes for apolipoprotein A1 lead to lower levels of high-density lipoprotein cholesterol. A meta-analysis of 21 studies involving 6333 CHD cases reported a relative risk for CHD of 1.62 (95% confidence interval 1.43 to 1.83) in a comparison of those in the bottom third of baseline values of apolipoprotein A1 with those in the top third; following correction for within-person variation in apolipoprotein A1 levels, the relative risk was 2.03 (95% confidence interval 1.69 to 2.42).[47] There was evidence of considerable heterogeneity among these studies, limiting the reliance which can be placed on the result. Furthermore, many studies did not adjust fully for confounders, including lipid levels, some of which are highly correlated with apolipoprotein levels. It is therefore likely that much of the apparent inverse association between apolipoprotein A1 and CHD is in fact attributable to CHD's well-studied association with high-density lipoprotein cholesterol.

Apolipoprotein B

Apolipoprotein B fulfils the same role with respect to low-density lipoprotein cholesterol that apolipoprotein A1 does with respect to high-density lipoprotein cholesterol. Correspondingly, higher levels of this apolipoprotein are associated with higher rates of CHD. A meta-analysis of 19 studies involving 6320 CHD cases reported a relative risk for CHD of 1.99 (95% confidence interval 1.65 to 2.39) comparing those in the top third of baseline values of apolipoprotein B with those in the bottom third.[47] Following correction for regression dilution bias, the relative risk was 2.50 (95% confidence interval 1.96 to 3.19). Again, there was evidence of considerable heterogeneity amongst the published studies. The same issue of lack of adjustment for confounders limits the reliability of these studies, as it did those of apolipoprotein A1.

Apolipoprotein E

The structure of circulating apolipoprotein E is determined by a genotype made up from two genes drawn from three common alleles, *ε2, ε3* (the commonest) and *ε4.* Differences in the structure of apolipoprotein isoforms influence metabolic handling of cholesterol; people carrying *ε3* have a lower total cholesterol than those with the *ε4* allele, but higher than those with the *ε2* allele. In the case of this biomarker, researchers have therefore concentrated on investigating the relationship between CHD and genotype, rather than phenotype. There are no published systematic reviews of the relationship between CHD and apolipoprotein E phenotype, but one meta-analysis of the relationship with genotype reported that, compared with people with *ε3, ε2* carriers had an odds ratio of CHD of 0.80 (95% CI, 0.70 to 0.90) and *ε4* carriers had an odds ratio of 1.06 (95% CI, 0.99 to 1.13).[48] Odds ratios increased progressively from those with the *ε2/ε2* genotype (0.83, 95% confidence interval 0.55 to 1.25), through *ε2/ε3* (0.82, 95% confidence interval 0.72 to 0.92), *ε2/ε4* (0.93, 95% confidence interval 0.81 to 1.07), *ε3/ε4* (1.05, 95% confidence interval 0.99 to 1.12) and *ε4/ε4* (1.22, 95% confidence interval 1.08 to 1.38). As with the other apolipoproteins, there was heterogeneity between studies.

Lipoprotein-associated phospholipase A2

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme that hydrolyses phospholipids, specifically LDL, in the artery wall. This reaction produces pro-inflammatory, atherogenic by-products which attract monocytes, impair endothelial function, cause cell death by disrupting plasma membranes and induce apoptosis in smooth muscle cells and macrophages. Other inflammatory markers are raised in the presence of non-vascular inflammation, reducing the specificity of testing for them. The status of Lp-PLA2 as a more specific marker of vascular inflammation and atheroma has stimulated particular interest.

A systematic review of the relationship between Lp-PLA2 and CHD reported an odds ratio adjusted for conventional CVD risk factors of 1.60 (95% confidence interval 1.36 to 1.89).[49] The risk estimate compared the highest quantile (in different studies tertiles, quartiles or quintiles) versus the bottom quantile or the difference in risk associated with a one standard deviation change in Lp-PLA2 levels. There will be an important interaction between how many quantiles a study used to calculate the measure of association and the size of the measure of association, but the authors did not adjust for this.

Lipoprotein(a)

There appear to be no published systematic reviews of the relationship between lipoprotein(a) and CHD. An analysis of the Reykjavik Study reported an odds ratio of 1.60 for this association (95% confidence interval 1.38 to 1.85), in a comparison of extreme thirds of baseline Lp(a) levels.[50] Again, this is not a strong enough association to make it likely that the addition of lipoprotein(a) will improve models' discriminatory power, though that has yet to be investigated.

Discussion

There are clear and consistent independent associations reported between several novel biomarkers and risk of CHD. However, the association is probably less than that reported, because of residual confounding for which adjustment cannot be made.

It has been claimed that enough is known about C-reactive protein in particular to secure its place in clinical risk prediction.[51] However, these arguments are based on the demonstration of higher risks of CHD in people with higher levels of the biomarker, not on analysis of improved risk estimation if the biomarker is incorporated. Analyses adopting the latter approach undermine the argument; for example, in the Atherosclerosis Risk in Communities study, conventional risk markers predicted CHD with good discrimination, with an area under the characteristic curve of about 0.8.[52] Nineteen novel biomarkers were investigated; none individually made a useful contribution to risk prediction. The addition of C-reactive protein only improved the area under the receiver operator characteristic curve by 0.003, Lp-PLA2 by 0.006 and homocysteine by 0.001.

Similar findings were reported from the Framingham cohort itself.[53] The authors measured the contribution to CHD risk of ten biomarkers, including C-reactive protein, NT-pro-BNP, fibrinogen and homocysteine. The results were disappointing: areas uner the receiver operator characteristic curve for major cardiovascular events were 0.68 with age and sex as predictors, 0.70 with age, sex, and a score based on the novel biomarkers as predictors, 0.76 with age, sex and conventional risk factors as predictors and 0.77 with all predictors. The receiver operator characteristic curves with and without the novel biomarkers crossed several times, with no clear advantage from the latter's inclusion.

These results were reinforced by a study from Sweden.[54] C-reactive protein and N-terminal fragment brain natriuretic peptide made only trivial differences to a risk prediction model based on conventional risk factors, increasing the area under the receiver operator curve by 0.007 (p = 0.04) and 0.009 (p = 0.08), respectively. The proportion of participants reclassified was small (8% for cardiovascular risk, 5% for coronary risk), and the net reclassification improvement was not significant for either cardiovascular events or coronary events.

Another issue of importance is the feasibility of routine laboratory analysis. Before these biomarkers could be used in primary care settings, we would need reliable and affordable means of measuring them in district hospital chemical pathology laboratories.

Conclusion

While the associations of novel biomarkers with CHD shed useful light on the pathogenesis of the disease and may be valuable in developing ways of monitoring and treating patients, statistical considerations coupled with available empirical studies make it unlikely that the associations are strong enough to have an important general influence on the discriminatory ability of risk models.

Whether there are specific circumstances in which they may be of more benefit is considered in the final chapter.

3   Genetics and coronary heart disease

Summary

- Genetic factors influence CHD risk, partly through their effects on conventional risk factors.

- There are important theoretical advantages to risk prediction based on genomic information.

- However, the influence of genes on CHD is harder to unravel than in other cardiac and some non-cardiac disorders.

- Existing research has revealed a modest number of loci definitely associated with CHD.

- Single alleles with an individual influence on risk large enough to improve the performance of risk models probably do not exist.

- In breast cancer, there is potential value in a polygenic approach to risk estimation, in which the multiplicative effect of several genes is modelled.

- This approach has been attempted in CHD. However, specific features of the epidemiology of CHD make it less fruitful. Merging genetic and phenotypic inputs in a risk prediction model is far from straightforward, and the proportion of people in whom genetic risk makes an important difference may be small.

- Simulations show that gene frequencies must be fairly high for risk estimation using genes alone to be viable.

- Hypothetical and empirical studies confirm the potential of this approach, but also its dependence on genes which confer substantial increases in risk and are fairly prevalent.

- One empirical study indicates that combining conventional and genomic risk factors may produce a larger area under the receiver operator characteristic curve than either alone.

- It is likely to be some time before genetic information is of value in improving the estimation of individuals' risk of CHD.

Background

Section 2 showed that, although about a quarter of the risk of coronary heart disease (CHD) is unexplained by conventional risk factors, novel biomarker molecules present in blood are unlikely to improve our ability to detect prospectively those likely to suffer from CHD. Are the prospects for genetic information any better? This chapter explores what is known about the contribution of genetic knowledge to estimating CHD risk, looking at single gene and polygenic studies.

Advantages of genomic prediction

In principle, using genetic biomarkers to estimate risk is more straightforward than using non-genetic ones, because the former can be measured almost without error and do not vary in an individual over time. Also, they need only be ascertained once, and this can be early in life, whereas other risk factors may not manifest until later in life. This allows lifestyle and other interventions to begin earlier, potentially increasing their effects. A further advantage of genotype information is that multiple genetic markers can be measured simultaneously, in the same assay; this is not true for the other biomarkers.

Although there would be substantial difficulties in integrating genetic and non-genetic biomarkers to produce a single risk model, these could be circumvented if genetic risk modelling alone was capable of generating risk estimation of equivalent accuracy. Measurement of non-genetic risk factors would still be necessary for clinical management, but this approach would capitalise on the theoretical advantages of genetic risk factors.

Another potential role for genetic information is in identifying individuals who may gain particularly from risk factor changes, either specific or general. For example, control of hypertension might be of high value in someone whose genes made his or her arteries especially prone to atheroma in the presence of hypertension.

The role of genes in CHD

Heritable effects on CHD operate in two ways. Some conventional risk factors are in part genetically determined traits; for example, both plasma cholesterol concentration and hypertension are heritable.[55][56] However these effects are not fully explanatory. Family history is an important independent risk factor for CHD; in Framingham, a family history of premature atherosclerotic vascular disease increased CHD risk, even after adjustment for conventional risk factors, by a factor of 2.0 in men and 1.7 in women.[57] So, it is likely that some of the unexplained risk is attributable to genes operating other than via effects of conventional risk factors.

Recent years have seen great advances in understanding the genetic basis of less common cardiac disorders. Causative mutations have been found in about two-thirds of cases of hypertrophic cardiomyopathy, nearly as high a proportion of cases of dilated cardiomyopathy and most cases of familial cardiac arrhythmias.[58] CHD is proving a much less tractable problem. It is a multi-factorial disease, not attributable to any single genetic or environmental cause. Risk alleles are incompletely penetrant and do not co-segregate with the disease phenotype.

Existing knowledge

Existing research into the genetic basis of CHD falls into two categories. Firstly, earlier studies investigated candidate genes on which suspicion fell as a result of evidence that the gene influenced one of the mechanisms by which CHD arose, such as lipoprotein metabolism or inflammation. More recently, genome-wide association studies have investigated many variants across the genome, without any underlying hypotheses. These empirical studies are not dependent on prior knowledge of gene function. The studies in both categories are often relatively small and many may be biased, for example due to the population studied or to publication bias.

The overwhelming majority of this research has failed to identify convincing associations between genetic factors and CHD. However, as studies become more numerous and larger, statistically significant positive findings are emerging. However, any list of genes identified promptly becomes obsolete, so none will be provided here.

This research is far from mature. Because of the limited scope of existing genetic testing equipment, studies of the whole genome have been at low density; less than a tenth of the genome has been evaluated at high density. The small sample size of many studies further impairs their power. In time, this research is likely to narrow the confidence intervals around some chromosomal locations so that they become statistically significant, and to identify the responsible alleles in the suspect chromosomal locations.

However, if there were individual genotypes which conferred a high risk of CHD, it is likely that their effect would by now be detectable and their existence known, even if their location in the genome was not. The odds ratios for most implicated genes, and certainly for the more common ones, are therefore likely to be no more than 1.3, too low for a single gene usefully to improve the discriminatory power of risk models. These odds ratios are lower than those associated with many conventional risk factors; for example, in the derivation cohort of QRISK2, the hazard ratio in men associated with treated hypertension was 1.68 (95% confidence interval 1.60 to 1.77), for current smoking 1.65 (95% confidence interval 1.60 to 1.70) and for type 2 diabetes 2.20 (95% confidence interval 2.06 to 2.35).[28] Rare homozygotes will have higher risks.

Polygenic approaches

In breast cancer, more progress has been made in understanding the contribution of genes to disease. Genome-wide association studies have so far reported seven breast cancer susceptibility loci at high levels of statistical significance, each of which confers a small increase in risk. However, they are each fairly common, independently inherited and appear to act multiplicatively. Pharoah *et al*. showed that knowledge of a woman's genotype can be used to adjust the baseline risk of cancer implied by her age and thus improve the targeting of screening and other risk reduction measures.[59]

Could the same approach work in CHD? A proof-of-principle study was based on a model of ten genes which earlier research suggested might confer higher risk.[60] The genes had odds ratios of between 1.13 and 1.42 and frequencies between 11% and 80%. The modal number of risk alleles per individual was three. Compared with those carrying no risk alleles (only 0.8% of the population), those with all ten had an odds ratio for CHD of 8.25. Compared with the mean risk of the population, probably a more useful comparison, those with six or more alleles had a significantly higher risk of CHD. They constituted only 4.9% of the population but had a combined odds ratio of 3.73.

The authors went on to apply their results to the baseline risk of CHD, and so to estimate how knowledge of risk alleles might change estimations of absolute risk. For a man of 55 years, the average baseline risk was assumed to be 16% over ten years; this fell to 8.1% if he carried no risk alleles and rose to 42% with all ten present.

How does this model compare with Pharoah *et al*.'s findings? In the breast cancer model, the odds ratio was 2.36 times higher in a woman on the ninety-fifth centile compared with one on the fifth centile. This is similar to the odds ratio of 3.05 in Drenos *et al*.'s model, comparing average risk in the first and tenth deciles.

There are however some important problems with the polygenic approach to CHD.

1. The odds ratios used in Drenos *et al*.'s model were unadjusted for conventional risk factors. They would need to be adjusted before they could be added to existing risk models, to avoid violation of the assumption of independence of effects which underlies the models. Since the alleles are known to be associated with phenotypic risk factors and have modest odds ratios to begin with (only four had a lower 95% confidence limit above 1.1), their apparent effect might be entirely the result of residual confounding.

2. The goal of this research is to create a model in which genotypic and phenotypic information is merged to improve CHD risk estimation. However, to some extent the genes operate via modifying the effect of behaviour and environment on phenotype; for example, cholesterol concentration is influenced by genes and diet.

    Substantial research would be required to generate adequate data on how the risk associated with each genotype is influenced by environment and behaviour. Yet without such research, the modelling of risk from genotypic and phenotypic information cannot be merged. If the genes' effects are dependent on carrier-specific environment and behaviour, the problem becomes even less tractable, since risk estimates from genes will be less generalisable over time and between populations.

3. As Drenos *et al*. acknowledge, it is unlikely that so many genes would interact in a simple multiplicative way. Given the pathogenic pathway which they influence, they probably have overlapping effects, by which one modifies the effect of others, or other forms of gene-gene interaction occur. This increases the complexity of the problem considerably. The way the risks are treated arithmetically is central to the outputs of the model, yet gene-gene interaction is a poorly understood area of genomics, there are many genes involved here, more will be identified in future and the way they interact may well be conditioned by the environment.

4. A final issue is the limited population value of this approach. In Drenos *et al*.'s model, more than 95% of people have five or fewer risk alleles. The most at-risk members of this group – those with five alleles, constituting 12% of the population – did not have an odds ratio which differed significantly from that of the population as a whole. This suggests that only the rare individuals with many hazardous alleles will benefit from a genetic approach. In breast cancer, there is value in identifying low-risk women, because of the risks and costs of mammography that could be avoided by not screening them. By contrast, there are few substantial benefits from transferring people from a moderate CHD risk category to a low risk one, if no specific action to reduce risk is indicated in either situation. If it becomes accepted that people at moderate risk should receive interventions to reduce CHD risk, this would change.

    We cannot rely on more data identifying more alleles and resolving this difficulty. The odds ratios in Drenos *et al*.'s model were derived from meta-analysis, but meta-analyses which take into account factors such as the publication bias, ethnicity and heterogeneity can undermine confidence in previously promising genes.

Other researchers have investigated the potential of a polygenic approach to CHD risk prediction. Janssens *et al*. published a simulation study showing the relative importance of allele frequency, population disease prevalence and odds ratios in a polygenic model.[61] The simulation showed that, since the effects of susceptibility genes are generally modest, the value of the approach depended on the frequency of the genes in the population. In particular, for plausible risk associations, the areas under the receiver operator characteristic curve were low with gene frequencies below 30%.

Single nucleotide polymorphisms (SNPs) on chromosome 9p21.3 have been consistently associated with CHD. Talmud *et al.* analysed the effect of inclusion of two alleles associated with increased CHD risk in the estimation of risk of the disease among 2742 white male participants in the second Northwick Park Heart Study.[62] One of the SNPs (rs10757274 A>G) was associated with CHD in this cohort, with a hazard ratio independent of conventional risk factors including family history of 1.60 (95% confidence interval 1.12 to 2.28, p = 0.03). However, the area under the receiver operator characteristic curve was not significantly improved by its inclusion in risk estimation (0.62 became 0.64, p = 0.14).

The authors went on to model the impact on risk of up to ten hypothetical, randomly assigned gene variants, with allele frequencies and risk similar to those of rs10757274 A>G. The addition of only one of these SNPs increased the area under the receiver operator characteristic curve significantly (p<0.03), with the inclusion of two or more SNPs having a greater effect (p<0.001), and further SNPs having smaller incremental effects. With all ten SNPs included, the area under the curve was 0.76. The classification of participants was improved by the additional SNPs; specifically the addition of the rs10757274 A>G SNP to the Framingham Risk Score meant that fifty-five men (2% of the cohort) were moved from below to above the 20% ten-year risk threshold for statin treatment. The observed risk in these men was 24%, indicating the appropriateness of the reclassification. The authors do not state how many were reclassified in the opposite direction.

This paper shows once again the potential of the polygenic approach, but deals with alleles not yet known to exist. Arguably, if there were several other alleles with as large an effect as the one they studied, some evidence of their existence would by now be apparent.

The potential of genetic information, alone and in combination with conventional risk factors, to predict risk was explored in another paper from the same group.[63] For this paper, members of the same cohort study were tested for twelve genes known to influence plasma lipids, haemostasis and vascular cell biology. Only four genes remained in the model in stepwise multivariate analysis; their combined area under the receiver operator characteristic curve was 0.62 (95% confidence interval 0.58 to 0.66). By comparison, the area under the receiver operator characteristic curve for age, triglyceride and cholesterol concentration, systolic blood pressure and smoking was 0.66 (95% confidence interval 0.61 to 0.70), which was not significantly different from the result based on genotype (p = 0.20). Combining the conventional risk factors and the genotypes significantly improved discrimination compared with the former alone (p < 0.001), the area under the receiver operator characteristic curve increasing to 0.70 (95% confidence interval 0.66 to 0.74).

This study encourages belief that CHD prediction will be enhanced by the inclusion of genotype information, and is one of very few studies showing that effect for real, as opposed to hypothetical, genes. The authors sound a note of caution by pointing out that only two of the four included genes (*APOE* and *LPL*) have been shown by meta-analysis to be associated with CHD. Furthermore, they identified the genes and tested the impact of their assessment in the same cohort, which will tend to exaggerate their apparent impact.

Conclusion

In principle, estimating risk using genetic biomarkers has important advantages. A polygenic approach is a powerful way to use genetic information about diseases where many low-penetrance genes confer small alterations in risk, with breast cancer providing a good example of its potential. By contrast, CHD is characterised by better knowledge of aetiological epidemiology, more uncertainty about the influence of genes and severe difficulties in bringing together genetic and environmental information.

The available studies reinforce the potential of this approach, but often rely on hypothetical genes with prevalences or strengths of association that may be implausible. Although continued investigation of the role of polygenic models is important, it is likely to be some time before genetic information is of value in improving the estimation of individuals' risk of CHD.

4   Next steps: towards better models, better assessment methods and better translation into practice

Summary

- Great progress has been made in using knowledge of the aetiology of CHD to predict risk in individuals, though our present methods are far from perfect.

- Risk prediction systems for CHD and other diseases are likely to grow in importance.

- However, our knowledge of how to appraise their performance and manage their translation into practice is poorly developed.

- Various methods for gauging the value of risk models have been developed, but none is obviously superior, and there is uncertainty about how to integrate the information they provide in order to make clinical and wider policy decisions.

- We therefore need to specify what needs to be known before a risk prediction system can be recommended for general use.

- Wider use of risk prediction systems has important implications for the education of practitioners, policy-makers and the public.

Background

At first sight, this report's findings may appear disheartening. The evidence indicates that novel molecular biomarkers are unlikely to improve substantially our ability to predict the risk of CHD in individuals, and also that single alleles with a strong enough association with CHD to improve the performance of risk models probably do not exist.

There are grounds for optimism however. Good progress has been made in predicting CHD risk; indeed, there are few other diseases with a multi-factorial aetiology which we can predict with similar accuracy. The available models give considerable insight into individuals' risk of developing the disease and can be used to target interventions.

The fact that such a variety of risk scores exists, and that at least one of them appears to perform well in the contemporary British population, is encouraging, although clearly a substantial amount of variation in risk is still unexplained. Also of note is the developing knowledge of how to measure the performance of risk prediction systems in a way which is more relevant to their practical application.

Nevertheless, there is still an important amount of unexplained variation in risk, and all the available models have limited discrimination. This is being addressed by research on several fronts. There is continuing investigation of novel molecular biomarkers, stimulated by the hope that these may either provide insight into the pathogenic mechanism of the disease or enhance the performance of existing risk scores. There are genome-wide association studies, searching for SNPs linked to an increased likelihood of CHD. And there are new models, either making better use of molecular and lifestyle biomarkers or exploring the potential of a polygenic approach.

Which of these approaches will prove most fruitful is uncertain, and all are worth pursuing. Given what we know about the importance of both inherited and lifestyle factors in the aetiology of CHD, there is particular interest in the potential of risk models which combine the two, although there are significant barriers to their development.

Future challenges

Risk prediction systems for CHD and other diseases are likely to grow in importance. More candidate risk factors are being identified and more interventions are available to reduce risk, both via primary and secondary prevention. There is increasing pressure, partially fuelled by recent advances in human genetics, for a shift in both medicine and public health from detection and cure to prediction and prevention of disease. Furthermore, there is rising societal and professional interest in personalised medicine, the tailoring of care to the specific characteristics of individuals; this approach is as relevant to prevention as it is to treatment, and will increase interest in risk prediction systems. The identification of more risk factors, and perhaps new statistical techniques, will mean that the models themselves will become more complex.

The growing availability, variety, complexity and potential value of risk prediction models for many diseases including CHD have important implications for clinical medicine, public health and the wider community. Physicians, scientists, policy-makers and consumers will need to assess the validity, utility and wider implications of approaches to risk prediction, and to choose which models to use. But at present they lack the means to do so in a systematic manner, for two reasons:

- Risk prediction systems give different results on different metrics, but it is not clear how to respond to this. Which metrics are more useful in indicating suitability for general use? Can we identify which metrics are more important in particular clinical and policy situations?

- It is not clear how to interpret differences in the metrics: for example, small improvements in the area under a receiver-operator characteristic curve may be statistically significant, but does this imply an important improvement in discrimination? How do improvements in the accuracy of a risk score affect its clinical utility?

A way forward

An earlier generation was confronted with similar issues when population screening tests and programmes began to emerge in the 1960s. In 1968, Wilson and Jungner published criteria by which to appraise approaches to screening and to decide which were suitable for implementation. Their work has been highly influential; although the standards have been refined and developed since their promulgation, Wilson and Jungner focussed attention on the key attributes of the disease, the programme and the test that still merit the most attention.

We now need to establish criteria against which risk prediction systems can be appraised. To the extent that such systems are used in healthy populations, the Wilson and Jungner criteria for population screening programmes will still be of relevance to their appraisal, though their use for prognostication in people who are already symptomatic raises different issues. Moreover, existing criteria for screening tests describe the characteristics of suitable tests in ways which cannot be readily applied to risk prediction models. For example, the UK National Screening Committee criteria include a requirement that "There should be a simple, safe, precise and validated screening test",[64] but do not explore the trade-off between simplicity and precision, or describe how validity is to be assessed. If the complexity of a risk prediction model is handled reliably by software invisible to the model's users, then we might willingly forego a great deal of simplicity in order to achieve improved validity. Furthermore, risk prediction models are no longer solely confined to use within national screening programmes, and therefore may require somewhat different methods of appraisal. For example, general practitioners are increasingly using risk models opportunistically, while numerous disease risk models are also available directly to the consumer via the internet, and may therefore be used outside formal healthcare altogether.

By specifying what needs to be known before a risk prediction system can be recommended for general use in a way which reflects its context, we can provide a basis for the appraisal of such systems and for a more rational process of policy development and implementation.

Questions of particular importance include

- What is meant by validity in statistically complex risk prediction models?
- How should validity be assessed?
- How should utility be assessed?
- What principles should guide decisions about the translation of risk prediction models into general use?
- What knowledge do practitioners, policy-makers and the public need to make best use of risk prediction systems?

The uncertainty about these questions made decision-making harder for those developing NICE's clinical guideline on lipid modification. By addressing these questions, we can build on previous success in CHD risk prediction and develop an even sounder understanding of how to gauge reliably the risk of many other diseases, and prevent them where possible.

Techniques for assessing models

Most assessments of novel biomarkers have reported a measure of the association between the biomarker in question and CHD. This provides little insight into the extent to which use of the biomarker would improve risk estimation, or the particular circumstances in which it might have value. Some studies have also reported the areas under the receiver operator characteristic curve, with and without the biomarker included. This is more useful in assessing whether the biomarker improves the discrimination of risk modelling. In some cases, for example QRISK, the accuracy of models' calibration have been assessed; this has seldom been done with models that include novel molecular biomarkers.

However, there are other dimensions to a model's performance. One is the proportion of variance which it explains, an indication of its comprehensiveness. But arguably of greater importance is the extent to which a model improves the accuracy of classification. This is especially so with CHD, where the estimated risk is used to classify people into those who will and will not be offered specific interventions, such as statins. For example, a risk model may generally perform well, with the addition of a biomarker making little overall difference to measures of its calibration and discrimination. However, the new model might have particular effects close to the threshold for intervention, currently in the UK a twenty percent risk of CHD over the next ten years. The model's use might lead to the transfer of a group of people, previously classified as below the intervention threshold, to a high-risk group, and therefore, to them being offered treatment. If the risk in this transferred group was indeed more than 20%, then the reclassification was appropriate and the new model has benefits not readily detectable without the assessment of its reclassification effects. Correct reclassification downwards is also of benefit, in that less cost-effective and potentially harmful treatment is avoided in people in whom the benefits are less than they would otherwise have seemed.

Reclassification is emerging as a metric for assessing models.[34] It is of particular interest because it responds to an important criticism of other forms of model assessment – that they do not take into account the clinical consequences of what they measure. The value to patients and clinicians of a small improvement in a model's calibration or discrimination is far from clear, but the advantages of generally correct reclassification are tangible and can even be valued in an economic evaluation.

We need to know more about the reclassification effects of different models and biomarkers. This could shed light on whether the addition of a biomarker may be of more value than its effects on discrimination and calibration would suggest, either generally or in specific clinical situations. These might either be in people close to an important intervention threshold, or people with a particular pattern of risk, such as "normal" conventional risk factors but an ominous family history. Articles promoting use of novel biomarkers often specifically advocate their use in people at intermediate risk of CHD (10% to 20% over ten years).[65 66] To what extent is this justified, either by theoretical considerations or by the evidence? Can novel biomarkers individualise risk estimations in people in whom the correction of a mis-estimate from conventional risk factors is especially important?

Towards successful translation

Risk prediction can have several benefits. For some risk scores, there are interventions available for those diagnosed as at higher risk, that are known to mitigate their risk of either developing the disease or experiencing a worse long-term outcome. Examples include weight loss to prevent type 2 diabetes and mammography to detect breast cancer, where earlier definitive diagnosis and treatment reduces mortality. However, in other cases there is no intervention which alters the disease's trajectory – for example, Alzheimer's disease. Here, the only benefit of treatment is to provide the individual with an indication on their risk relative to the population as a whole.

We need further research into the clinical and cost effectiveness of the interventions available to those who are shown to be at higher risk by a prediction system, and more understanding of the threshold at which these interventions are appropriate. For example, should an asymptomatic person be offered a coronary angiogram if his risk of CHD was the same as someone with angina?

Risk prediction models are often complex, and are likely to become more so. Their successful translation into practice requires not only better understanding of how to appraise their performance, but also more widespread knowledge of how to use their outputs in practice. The implications of this for the education of practitioners, policy-makers and the public are substantial.

Conclusions

The potential of risk predication models in health is far from fulfilled. Their capacity to incorporate advancing knowledge of biomarkers and to individualise health care give them great potential to integrate epidemiological, molecular and genetic information into a modern, personalised form of healthcare. Although further scientific advances will strengthen existing models and lead to the emergence of new ones, what is most urgently needed is a better understanding of how to manage their translation into practice. This involves the identification of better metrics, a more systematic approach to the appraisal of models, and the education of practitioners, policy-makers and the public in the use of the models.

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