C-Reactive Protein and Coronary Heart Disease: Predictive Test or Therapeutic Target?

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BACKGROUND: The hepatocyte-derived acute-phase reactant C-reactive protein (CRP) has been the subject of intense research over the last 2 decades for its possible role in the pathogenesis of cardiovascular diseases. This research has spawned interest in the use of the blood concentration of CRP for predicting a first coronary heart disease (CHD) event, which has been made possible with the development of high-sensitivity CRP (hsCRP) assays that can measure the typically low concentrations of CRP that circulate in the absence of an overt infective or inflammatory episode, and as a potential causal factor that might be targeted therapeutically. The research has encompassed observational and genetic epidemiology, basic science studies with cells and tissues, experiments with animal models and humans, and randomized trials (although not of specific CRP-lowering therapies as yet).

CONTENT: We focus on investigations of the potential role of small differences in basal hsCRP concentration seen in healthy individuals and the relationship of such differences to the long-term risk of a first CHD event, rather than on research devoted to the high acute-phase CRP concentrations, which occur after acute atherothrombotic events and can influence the severity of ischemic tissue damage and the subsequent prognosis. We concentrate mainly on research findings at the translational interface and draw on evidence from human observational and genetic epidemiology, as well as from randomized trials.

CONCLUSIONS: As the field matures from one of discovery to an evaluative science, the development of possible clinical applications requires a sharpening of focus on and a critical appraisal of the strengths and deficiencies of the accumulated evidence. Such assessments require attention to both the current state of affairs and the design of future research, so that the existing uncertainties about the utility of CRP in predicting CHD and its role in causing this disease can be resolved.

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Overview of the Sources of the Evidence on the Role of C-Reactive Protein in Coronary Heart Disease in Humans

OBSERVATIONAL STUDIES

Coronary heart disease (CHD), a major cause of morbidity and mortality, has environmental and genetic determinants and a long preclinical phase. The multifactorial nature of CHD complicates both the reliable identification of individuals more likely to experience clinical events and the elucidation of the disease’s causal pathways. This quality, which is shared by other common disorders, delays the development of new treatments.

Observational epidemiology provides a major source of information on possible risk factors for CHD. As early as 1981, more than 200 “exposures” or phenotypic differences documented in observational studies had been associated with a higher risk of CHD (1). The list is now much longer and is set to increase further with the emergence of “-omic” technologies (2). Risk factors for CHD include behavioral, dietary, and lifestyle factors (e.g., smoking, dietary fat, and physical activity); infections (exogenous exposures); alterations in endogenous blood constituents (“intermediate phenotypes,” “biomarkers,” “quantitative traits”), such as lipid and lipoprotein particles, inflammation and coagulation proteins, intermediary metabolites, and oxidant markers of stress; and more complex, higher-order interactions derived from the interaction among risk factors, as well as biological, psychosocial, and environmental factors.

Nonstandard abbreviations: CHD, coronary heart disease; BP, blood pressure; LDL-C, LDL cholesterol; CRP, C-reactive protein; hsCRP, high-sensitivity CRP; RCT, randomized controlled trial; SNP, single-nucleotide polymorphism; MR, mendelian randomization; NPHS-II, Northwick Park Heart Study II; EAS, Edinburgh Artery Study; NRI, net reclassification index; JUPITER, Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin; CVD, cardiovascular disease; NICE, National Institute for Health and Clinical Excellence; ERFC, Emerging Risk Factors Collaboration; NHANES, National Health and Nutrition Examination Survey; CCGC, CRP Coronary Heart Disease Genetics Collaboration.
order phenotypes and disorders, such as electrocardiographic changes (e.g., QT interval and dispersion), adiposity, blood pressure (BP), and diabetes mellitus (3).

Although the causal relevance of many of these risk factors is unknown (vide infra), one translational benefit of observational epidemiology has been the development of risk-assessment tools [e.g., the Framingham, PROCAM, EuroSCORE, and QRISK tools (4–7)] that use information on certain risk factors (e.g., smoking, BP, and lipids) and other variables (e.g., age, sex, and left ventricular hypertrophy) to estimate an individual’s risk of CHD and to help target preventive interventions. Tools of this type play an integral part in policies and guidelines for CHD prevention worldwide. For risk prediction, it is not necessary that a risk factor be causally involved in a disease, although we now recognize that both LDL cholesterol (LDL-C) and BP are. Indeed, some of the best predictive markers in medicine are those that index the presence of subclinical disease without necessarily being involved in the pathogenesis (8). Despite the widespread use of risk-assessment tools to guide preventive interventions, however, a substantial proportion of CHD events occur among the many individuals in a population with intermediate values of LDL-C and BP (or even Framingham risk) (9–11). This fact provides one motivation for evaluating new biomarkers for risk prediction, and of the large array of biomarkers that have been studied, C-reactive protein (CRP) has been the subject of the most attention.

Understanding which exogenous exposures and endogenous biomarkers are causally involved in CHD is a prerequisite for developing new treatments. Again, observational epidemiologic studies provide one source of evidence, and more than 30 prospective epidemiologic studies have consistently demonstrated an association between CRP concentrations measured in initially healthy middle-aged individuals and the risk of a first CHD event (12–51). These studies have used high-sensitivity CRP (hsCRP) assays to accurately quantify the lower concentrations of CRP that circulate in the steady state in the absence of acute infective or inflammatory episodes.

Understandably, the associations have stimulated substantial scientific and media interest, because the similar associations of BP and LDL-C with CHD, which were later shown to be causal, led eventually to the development of BP- and cholesterol-lowering treatments that now form the mainstay of CHD prevention; however, there are several reasons why observational studies of circulating biomarkers should not be used as the sole evidence for causation. Altered concentrations of biomarkers tend to cluster among individuals at higher CHD risk, making it difficult to ascertain the nature and direction of biological relationships between biomarkers or to determine the independent effect of a particular biomarker on disease risk. CRP is associated with an especially wide range of biomarkers and risk factors that are also associated with CHD (52–54). In observational studies, differences in hsCRP concentration among individuals at higher risk of CHD not only could be a marker of other risk factors (confounding) but also could simply be an indicator for subclinical disease (reverse causation), because atheromatous plaques, which become established decades before clinical events occur, are known to be a focus of inflammation. As an exquisitely sensitive marker of inflammation, CRP differences observed in individuals at higher risk of CHD may be reflecting the inflammatory activity of atheroma rather than contributing to its development or progression (see Fig. 1 in the Data Supplement that accompanies the online version of this review at http://www.clinchem.org/content/vol55/issue2).

The prospective design of most observational studies of CRP and CHD risk, in which hsCRP is measured years before the manifestation of clinical events, helps to limit (but not completely eliminate) the effect of reverse causation. In such studies, statistical adjustment for covariables can be used to address the problem of confounding; however, there may be residual confounding when a confounding factor is unknown, is unmeasured (which is a strong possibility given that several hundred risk factors have been associated with CHD), or is measured with some error (which is virtually inevitable). This possibility means that observational studies may overestimate the true causal association of CRP with CHD; however, other systematic sources of error in observational studies mean that the true causal association also can be underestimated. Biological variation and error in the measurement of CRP itself can lead to the phenomenon of regression dilution bias, which can be reduced by repeated CRP measurement and the application of an adjustment factor. The causal association can also be underestimated if a covariable is inaptly treated as a confounder, as would happen if the covariable mediated rather than confounded the association of a biomarker with CHD. Because the mechanisms by which CRP might alter the risk of CHD are uncertain, deciding which covariables should be treated as confounders and which as mediators has been a matter of subjective judgment.

**RANDOMIZED TRIALS**

Because of the difficulty in assigning a causal role for a risk factor from observational epidemiologic results alone, smoking, increased LDL-C, and increased BP have remained the only undisputed modifiable risk factors for CHD (55). For smoking, the large size of the
effect and the minimal effect of adjustments for potential confounders make the case compelling. For LDL-C, the association with CHD was controversial until the development of statin drugs that potently lower LDL-C and the demonstration of their efficacy in randomized controlled trials (RCTs) (56, 57). This evidence, along with the results of previous trials of nonstatin LDL-lowering drugs and dietary intervention as well as genetic evidence from patients with familial hypercholesterolemia, helped confirm a causal role for LDL in coronary disease. RCTs, which also confirmed the causal role of increased BP, currently provide the only large-scale source of experimental (as opposed to observational) evidence in humans. Randomization balances confounder effects, and the interventional design helps distinguish cause and effect, thereby overcoming the major limitations of observational studies. Because statins lower CRP as well as LDL-C, the results of RCTs of statin drugs have been proposed as evidence for the potential causal role of CRP in CHD (58–61); however, this evidence is imperfect, because the effects of statins are not restricted to CRP lowering (they were developed for their effect on LDL-C rather than on CRP). RCTs involving a selective CRP inhibitor would provide the necessary tool, but until the recent development of 1,6-bisphosphocholine hexane and related compounds, no specific inhibitor of CRP had existed (62). These agents are not orally bioavailable and have a short half-life; consequently, the first trials will be in the setting of acute atherothrombosis, in which CRP is hypothesized to play a causal role in ischemic tissue damage. It is likely, however, to be some time before a specific, orally available CRP inhibitor can be used to rigorously evaluate the role of CRP in the long, slow process of atherogenesis.

GENETIC EPIDEMIOLOGY AND MENDELIAN RANDOMIZATION (NATURAL RANDOMIZED TRIALS)
Until long-term randomized trials of a selective CRP inhibitor for preventing CHD become possible, genetic epidemiology can be used as an alternative source of randomized evidence for investigating the possible causal role of CRP in humans (63–65). CRP concentration is a heritable trait, and we and others have identified common single-nucleotide polymorphisms (SNPs) in the CRP gene (C-reactive protein, pentraxin-related) that influence the circulating concentration of the protein (66–73). These SNPs serve as an unbiased proxy for CRP and are useful as tools to help confirm or refute a causal role in CHD. This approach is possible because genotype is unique among naturally occurring interindividual differences in that it is determined by random allocation according to Mendel’s second law [mendelian randomization (MR)] (74). This property, which is shared with the treatment-allocation strategy in an RCT, means that confounding factors are balanced among genotypic groups (Fig. 1). Therefore, differences in phenotype, disease risk, or both between individuals of contrasting genotypes at a particular locus signify the causal actions of a gene variant (or a variant in linkage disequilibrium with it). All other exogenous exposures or phenotypic factors are balanced between genotypic groups. Moreover, unlike associations between phenotypes or the associations of phenotypes with CHD risk, genetic associations are protected from reverse causation because genotype is a nonmodifiable characteristic, so there is a unidirectional flow of information from common genome variation to mRNA to protein to complex phenotype and disease (64, 75). If CRP is causally involved in CHD, then individuals who carry CRP-increasing alleles should be at higher risk of CHD, and the difference in risk should be in proportion to the CRP increase. By contrast with the direct association of CRP itself with CHD, the genetic association should not be prone to confounding or reverse causation and should provide direct evidence on the causal role of CRP in CHD (65, 68, 76).

Having summarized the various research designs and some of their strengths and limitations, we now discuss in more detail the evidence from the different categories of investigation on the role of CRP in the prediction and pathogenesis of CHD. We highlight some of the more recent and forthcoming developments that may help resolve the existing uncertainties. We have recently reviewed these areas and the basic science findings and have provided a detailed, systematic, and comprehensive appraisal of the research on the role of CRP in both acute atherothrombotic events, which could influence the severity of ischemic tissue damage and the subsequent prognosis, and the long-term risk in CHD (77).

Evaluating the Performance of CRP as a Predictor of CHD

In the late 1980s and the 1990s, concern was expressed that a large proportion of CHD events occurred among individuals without apparent increases in the concentrations of known risk factors (i.e., among nonsmoking, nondiabetic individuals with average or below-average BPs and concentrations of total cholesterol or LDL-C) (78). Such observations seemed counterintuitive, particularly as randomized intervention trials began to show unequivocally that BP and LDL-C were causally linked to CHD. It seemed likely that other causal factors for CHD must exist, with some commentators variously suggesting that half of all CHD events were not explained by established risk factors or that half of all CHD events occurred in people with "nor-
mal” or below-average cholesterol concentrations. This interpretation fueled research aimed at identifying novel risk factors, one translational aim being to improve the prediction of CHD events. Additional risk factors for CHD are very likely to exist, with recent genome-wide association studies perhaps providing the most direct evidence for this hypothesis, but the high proportion of CHD events observed among individuals with below-average BPs and LDL-C concentrations is perhaps partly explained in terms of Rose’s prevention paradox (79), which we discuss in more detail later. CRP emerged as preeminent among the “novel” risk factors, partly because of the growing interest in local inflammation within the atherosclerotic plaque and systemic inflammation (from low-grade infection or noninfective inflammatory disorders such as rheumatoid arthritis) as contributory factors in the development, progression, and complications of atherogenesis. Certain characteristics of CRP as an analyte also marked its measurement as a promising test for CHD. These characteristics include its stability in stored serum and plasma, the existence of a WHO standard material for calibration of new assays, the ability to perform large-scale, high-throughput analyses with the nephelometers widely used in clinical practice, and the development of high-sensitivity assays that accurately measure the trace concentrations of circulating CRP seen in healthy individuals (80). Other biological attributes of CRP also seemed valuable. These attributes include the limited biological variation in the circulating concentration (excluding the acute increases in CRP that occur during infections), which is comparable to that of LDL-C. The CRP concentration is also associated with many of the established and novel risk factors for CHD (81); therefore, CRP concentration might be considered as a barometer for exposure to cardiovascular risk factors (although, as we discuss later, the catholic list of associations and correlations can be a double-edged sword, both when evaluating the causal relevance of CRP and when assessing its predictive utility).

Observational studies of CRP, which form the major basis of the evidence for its role as a predictor of CHD, have tended to report the association of CRP with CHD events through the use of a relative measure of risk (e.g., the use of odds or hazard ratios). A recent systematic review and critical appraisal of CRP in predicting CHD events that included new data from the Northwick Park Heart Study II (NPHS-II) and the Edinburgh Artery Study (EAS), as well as from 31 reports of 28 cohorts including 84 063 individuals and 11 252 incident CHD events, noted that the reporting of these measures was almost universal (81). Typically, some measure of relative risk of a CHD event was evaluated in individuals with high CRP values by comparison with individuals with low values (e.g., between individuals in the top quartile vs the bottom quartile of the CRP distribution) (81). Although useful when considering the potential etiologic role of CRP in CHD, in the context of which odds ratios of 1.5–3 (depending on the degree of adjustment) can be regarded as very substantial, these metrics provide limited and only indirect information on the performance of a marker in the prediction of disease. The shape of the association of a marker value with disease risk and the distribution of marker values in a population are additional important determinants of how well or how poorly a marker performs in predicting a CHD event. Moreover, some authors have considered odds ratios

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**Fig. 1. Parallels between genetic studies and randomized trials.**

The expected outcome from a hypothetical RCT of a selective CRP-lowering intervention and from a mendelian-randomization analysis, if CRP were causal in the development of cardiovascular (CV) events and the effect were reversible. Alleles of the CRP gene associated with low and high CRP concentrations are indicated as aa and AA, respectively. For simplicity, only homozygous individuals are shown [Hingorani et al. (65)]. Reproduced with permission.
of the size observed in observational studies of CRP to be insufficiently large for good prediction (82, 83).

DISCRIMINATION

More direct measures of the predictive performance of a marker exist. "Discrimination" refers to the ability of a marker to distinguish individuals who will develop an event from those who will remain disease free. The metrics used to evaluate discrimination include sensitivity, specificity, and predictive values (84). For a marker such as CRP that can take on a range of continuous values, one way of summarizing discrimination is the area under the ROC curve, the values of which can range from 0.5 (no discrimination) to 1 (perfect discrimination). The discrimination achieved by jointly measuring a panel of markers can also be assessed (e.g., the discrimination offered by a model with variables present in the Framingham risk equation with or without the addition of CRP). Our systematic review (81) revealed only one study that reported the ROC value for CRP alone. We were able, however, to derive the area under the ROC curve, as well as the sensitivity, specificity, and predictive values for different CRP cutpoints, from 25 published reports.

Fig. 2. Study-specific estimates of (a) sensitivity (for a 10% false-positive rate) and (b) area under the ROC curve for CRP in the discrimination of CHD, both obtained directly in NPHS-II and EAS and inferred from data extracted from 25 published reports.

A sensitivity analysis by duration of follow-up is also shown. The pooled values were calculated with the inverse variance-weighted method under random-effects models [Shah et al. (81)]. CIs are presented as the lower and upper confidence limits. Reproduced with permission.
sible to detect approximately 60% of cases in NPHS-II, but at the expense of a much higher false-positive rate (approximately 40%); evaluation of the other studies produced similar findings. Pooled inferred values for the area under the ROC curve for CRP alone were 0.59 (95% CI, 0.57–0.61), 0.59 (95% CI, 0.57–0.61), and 0.57 (95% CI, 0.54–0.61) for studies of <5 years, 5–10 years, and >10 years of follow-up, respectively (Fig. 2).

Why should CRP, a sensitive marker of the inflammatory processes linked to atherogenesis and possibly even contributing to atherogenesis itself, perform only modestly well in discriminating future CHD cases? The reasons could lie in the fundamental epidemiologic relationships that constrain the predictive performance of a marker. Wald et al. pointed out that in the case of BP or cholesterol, the relationship with CHD risk is log-linear with no threshold value, whereas BP and cholesterol values in a population are normally distributed (10). Thus, a large proportion of CHD events are to be expected among individuals with near-average BP or cholesterol concentrations, who are at intermediate risk; this is a reiteration of Rose’s prevention paradox (79). This situation creates wide overlaps in the distributions of BP and cholesterol values among individuals who experience CHD events and those who remain disease free, which make it difficult to identify a cutpoint value that discriminates the cases (11). It also contributes to the finding that about 50% of CHD cases occur among people with normal or below-average BP or LDL-C concentrations. The distribution of CRP values in populations is lognormal (see Fig. 2 in the online Data Supplement), and the relationship with risk also appears log-linear (Fig. 3). Therefore, many CHD cases are also expected to occur among individuals with near-average CRP values. This factor is likely to help explain why the overlap in CRP values between cases and controls found in prior prospective studies was wide, accounting for the difficulty in identifying a CRP value that distinguishes cases (Fig. 2) (81). Thus, as a predictive marker for CHD risk, CRP has some of the same limitations as BP and cholesterol.

In the UK and Europe, individual risk factors tend not to be used for risk assessment in isolation but instead tend to be assessed jointly as part of risk scores; however, Wald et al. showed that panels composed of modestly discriminating risk markers perform only slightly better than the individual risk factors alone in distinguishing disease cases (83). This finding is likely to explain why the addition of CRP to models based on variables used in the Framingham risk equation has provided limited incremental ability to discriminate cases (Fig. 4). There may be other reasons, however. CRP is associated not only with BP, HDL-C, age, and sex, but also with diabetes, smoking, left ventricular hypertrophy, and atrial fibrillation (42, 51, 70, 85–88), all of which already contribute to the Framingham risk model. Thus, the incremental information provided in a CRP measurement above that provided by the panel of variables included in the Framingham risk equation may be limited.

**ESTIMATED ABSOLUTE RISK**

A separate approach to risk prediction, one that is perhaps more relevant to the current approach to CHD prevention, is the estimation of an individual’s absolute risk of a CHD event over a given period by means of a multivariable model (such as the Framingham risk equation) that incorporates several risk factors (4–7, 89). Such an approach is usually coupled with an...
agreed threshold risk, above which an individual is offered a preventive intervention. For example, in clinical practice such estimation could trigger the decision to prescribe cholesterol- or BP-lowering treatment. The basis of this approach rests on observations that preventive pharmacologic interventions produce fairly constant proportional reductions in risk over a wide range of baseline risks of disease. Thus, the absolute reduction in risk is greater (and the number to treat is lower) if treatments are targeted to high-risk individuals. Moreover, the precise constellation of risk-factor abnormalities in any individual seems to be less important than the overall risk, however constituted. Under this model, a 65-year old male smoker with a high BP but an average or below-average LDL-C concentration is expected to receive proportional benefits from statin treatment similar to someone with the same absolute risk but that is largely attributable to a high LDL-C concentration. This approach to primary prevention by targeting high risk was particularly appropriate for directing statin use at the time of the drugs’ arrival on the market, when they not only were expensive but also had an uncertain long-term safety profile. As we discuss below, the considerations may now be different after the passage of a decade or two, in an era of cheap off-patent statins with reassuring long-term safety profiles.

The performance of a multivariable risk model used to estimate the individual absolute risk of disease is evaluated via calibration, which is a means of comparing the predicted and observed risks of events in a population, with the difference being small in a model that is well calibrated. More recently, other approaches to the evaluation of a marker’s performance have been proposed. One new approach (termed “reclassification”) involves quantifying the extent to which a new marker shifts individuals between the categories of CHD risk initially determined with established risk models. The net reclassification index (NRI) provides a further refinement of the approach that distinguishes appropriate reclassifications (i.e., shifts of eventual cases to higher-risk categories and those remaining disease free to lower-risk categories) from inappropriate reclassifications. In the US and the UK, the

Fig. 4. Observed values for area under the ROC curve (AUC) or C statistic for Framingham-based models with and without the addition of CRP in NPHS-II, EAS, and 6 studies that published these data with measures of dispersion available.

Shown in a footnote to the figure are AUCs from 7 additional studies [Shah et al. (81)]. CIs are presented as the lower and upper confidence limits. Reproduced with permission.
Framingham risk equation is the most commonly used multivariable risk model (4).

Several studies have assessed the effect of adding CRP to the calibration of risk models based on Framingham variables (34, 43, 47–49). Assessments were made in different ways in the 5 published studies that evaluated the effect of adding CRP to the calibration of the Framingham risk equation or similar models, but none of the studies identified a quantitatively large improvement in model fit, although the authors sometimes interpreted statistically significant differences as being of potential clinical importance (81). In our own analysis that used the NRI to assess the effect of adding CRP to a risk model based on Framingham variables in NPHS-II and EAS, we found no evidence for a major impact of CRP measurement on improving risk prediction. Although CRP measurement led to the reclassification of a substantial proportion of the individuals, we found that the proportion of appropriate reclassifications was almost matched by inappropriate ones, producing a low NRI value (81).

Previous reports from the Women’s Health Study and the Physician’s Health Study (47, 89, 91) assessed the effect of adding CRP to risk models that were based on established risk factors and the use of reclassification. These reports presented tables in which the observed risk (or actual event rate) was reported for the various categories of predicted risk for individuals who did or did not shift category on the addition of CRP to the base model. The addition of CRP appeared to place individuals more accurately into risk strata; however, when estimates of the observed (actual) risk in each category of predicted risk were compared for models that included or omitted CRP (i.e., comparisons that included all of the individuals in each risk category, not simply those who shifted category), the observed and predicted risk estimates in NPHS-II and EAS were very similar for each model (Table 1) and were concordant with a similar analysis with calibration. It would be interesting to reanalyze the reclassification tables from prior and more recent reports in the same way (47, 89, 91).

### Table 1. Observed event rates in each category of predicted risk for models based on established risk factors including or omitting CRP in the NPHS-II and EAS studies.

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<th>Category of predicted risk</th>
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<td>1036</td>
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<td>910</td>
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<td>6.6%</td>
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*See Shah et al. (81) for further details.

Risk is 10-year CHD risk in NPHS-II and EAS. Values in cells are the total number of individuals in each predicted-risk category and the particular 10-year event rate.

**INDIVIDUALS AT INTERMEDIATE RISK**

Because management decisions for patients at high and low risk for CHD are more straightforward, some have argued that one strategy for CRP measurement for risk prediction could involve focusing on individuals who are at intermediate risk of CHD, as assessed by evaluating established risk factors. CRP measurement has been suggested to possibly be of help in reassigning a proportion of such individuals to higher- or lower-risk categories, with the relevant impact being on the decision on whether to prescribe statins (47, 89). This approach has some limitations, however. First, the different available risk models (Framingham, QRISK, PROCAM, and Reynolds Risk Score) use slightly different repertoires of risk factors; thus, occupancy of the intermediate-risk category is likely to vary, depending on the model used. Second, conversion of a continuous risk score into categories will produce some information loss. Third, our own analysis of the effect of adding CRP to a Framingham-based model indicated that limiting CRP measurement to individuals at intermediate
risk might simply artificially deplete this group, because CRP measurement of the entire population actually produces “in-filling” of the intermediate-risk category by individuals reclassified from high- and low-risk groups (81).

IMPACT OF THE RECENT JUPITER TRIAL ON PRIMARY PREVENTION OF CARDIOVASCULAR DISEASE

JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) randomized 17,802 people (62% men, 16% smokers) with no prior evidence of cardiovascular disease (CVD), a high baseline hsCRP concentration (≥2 mg/L, median, 4.3 mg/L; interquartile range, 2.8–7.1 mg/L), and a low LDL-C concentration (≤3.36 mmol/L; median, 2.8 mmol/L; interquartile range, 2.4–3.1 mmol/L) to 20 mg/day rosvastatin or placebo (60, 61, 92). The aim of the trial was to “assess the effect of rosvastatin on first ever cardiovascular events in apparently healthy men and women who do not qualify for statin therapy due to low concentrations of LDL-C, but who are at increased cardiovascular risk due to increased levels of hsCRP.” The Data Safety Monitoring Board halted the trial early (after a median of 1.9 years of follow-up) for reasons of efficacy because of a 44% relative-risk reduction (95% CI, 31%–54%) in the primary endpoint of myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes. The results of the JUPITER trial pose several questions: (a) Does the general approach of targeting statin treatment based on assessment of baseline risk with a multivariable model need to be changed, (b) is part of the statin effect actually mediated through CRP lowering, and (c) do individuals with a high hsCRP concentration receive disproportionately greater benefits from statins? We now focus on question (a) but will return to the other 2 questions in a later section. First, of the 90,000 people screened for eligibility, only 17,802 were enrolled (92), with 90% being excluded either because their hsCRP concentration was too low or because their LDL-C concentration was too high. In the UK at least, clinical guidelines from the National Institute for Health and Clinical Excellence (NICE) advocate the use of statins for the primary prevention of CVD in people whose estimated 10-year risk of CVD exceeds 20%, as calculated with the Framingham risk equation (or a similar risk equation) (http://www.nice.org.uk/Guidance/CGr67). From the baseline characteristics of the participants in the JUPITER trial at entry (mean age, 66 years; mean BP, 134/80 mmHg; median total cholesterol, 4.8 mmol/L; median HDL-C, 1.3 mmol/L) (92) and assuming the same mean age of the men and women, we estimated the overall weighted-mean 10-year risk of CVD as 19.5% and the 10-year risk of CHD as 12.1%. The reported mean Framingham 10-year risk of “hard” CHD events in JUPITER was 10%, but the 10-year Framingham risk of CVD was not reported. Thus, despite the low mean LDL-C concentration for the participants in the study, the 10-year risk of CVD was not insubstantial, particularly among the men (who constituted 62% of the trial participants). See Table 2 for a comparison with other statin trials for which baseline hsCRP concentrations have been reported. The JUPITER findings are important, however, because they extend the evidence base for statins to individuals who are at lower risk than those targeted by current CVD-risk thresholds and because they confirm that LDL-C lowering is effective in CVD prevention, even at low starting LDL-C concentrations. The findings will motivate a reevaluation of current guidelines on primary prevention to consider whether individuals who have a lower absolute risk of CVD than those currently targeted should receive statins. One very important question for guideline developers is whether people with a baseline risk of CVD similar to that of the JUPITER participants are just as readily identified and targeted with established methods for risk assessment (with a lowering of the threshold of CHD risk for intervention) without measurement of hsCRP.

Is CRP a Potential Therapeutic Target for CHD Prevention?

The experience with BP and cholesterol as risk factors indicates that a risk factor may be an important contributory cause of CHD while being only a modest predictor. Therefore, the issue of whether CRP is a novel causal factor for CHD is an immensely important one, irrespective of its performance as a risk predictor.

EXISTING AND FORTHCOMING EVIDENCE FROM POPULATION-BASED OBSERVATIONAL STUDIES

Observational studies can both over- and underestimate the true causal relevance of a biomarker for CHD and thereby compromise the ability to be certain about the biomarker’s potential as a therapeutic target. Prospective observational studies of CRP and CHD currently have made the largest contribution to the evidence base for this issue. It is therefore essential that such data be used optimally when evaluating this issue.

An important component of this optimization of the observational data set involves metaanalysis. The most recent overview of the prospective studies of hsCRP and CHD involved aggregate (summary) and tabular data from 22 studies of a total of 7068 incident CHD cases, with a mean follow-up of 12 years (39). A comparison of the risk among individuals in the top tertile of the hsCRP distribution with those in the bottom tertile (corresponding to mean hsCRP values of
Table 2. Summary of RCTs of statins (including JUPITER) for which CRP values were reported at baseline.

<table>
<thead>
<tr>
<th>Trial</th>
<th>1°/2°</th>
<th>Mean age, years</th>
<th>Men, %</th>
<th>Smokers, %</th>
<th>TC, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>SBP, mmHg</th>
<th>LDL-C, mmol/L</th>
<th>CRP, mg/L</th>
<th>CVD risk</th>
<th>CHD risk</th>
<th>CHD-event rate</th>
<th>Treatment</th>
<th>ΔLDL-C, mmol/L</th>
<th>Relative-risk reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFCAPS/TexCAPS</td>
<td>1°</td>
<td>57.9</td>
<td>85</td>
<td>12.5</td>
<td>5.71</td>
<td>0.94</td>
<td>138</td>
<td>3.89</td>
<td>1.5</td>
<td>2.34</td>
<td>1.73</td>
<td>1.06</td>
<td>L20–40</td>
<td>−0.94</td>
<td>0.37 (0.21–0.50)</td>
</tr>
<tr>
<td>WOSCOPS</td>
<td>1°</td>
<td>55.1</td>
<td>100</td>
<td>44</td>
<td>7.03</td>
<td>1.14</td>
<td>136</td>
<td>5.0</td>
<td>1.88</td>
<td>2.47</td>
<td>1.88</td>
<td>1.94</td>
<td>P40</td>
<td>−1.07</td>
<td>0.29 (0.12–0.40)</td>
</tr>
<tr>
<td>LIPID</td>
<td>2°</td>
<td>62</td>
<td>83</td>
<td>9.5</td>
<td>5.64</td>
<td>0.93</td>
<td>134</td>
<td>3.88</td>
<td>2.72</td>
<td>2.84</td>
<td>2.47</td>
<td>1.94</td>
<td>P40</td>
<td>−1.03</td>
<td>0.24 (0.15–0.32)</td>
</tr>
<tr>
<td>PROSPER</td>
<td>1°/2°</td>
<td>75.3</td>
<td>48</td>
<td>26.8</td>
<td>5.7</td>
<td>1.3</td>
<td>154.6</td>
<td>3.8</td>
<td>3.01</td>
<td>3.47</td>
<td>1.97</td>
<td>3.81</td>
<td>P40</td>
<td>−1.04</td>
<td>0.19 (0.06–0.31)</td>
</tr>
<tr>
<td>CARE</td>
<td>2°</td>
<td>59</td>
<td>86</td>
<td>21</td>
<td>5.4</td>
<td>1.27</td>
<td>134</td>
<td>2.79</td>
<td>3.6</td>
<td>2.75</td>
<td>2.47</td>
<td>1.94</td>
<td>P40</td>
<td>−1.03</td>
<td>0.24 (0.09–0.36)</td>
</tr>
<tr>
<td>JUPITER</td>
<td>1°</td>
<td>66.3</td>
<td>61.8</td>
<td>15.8</td>
<td>4.78</td>
<td>1.27</td>
<td>134</td>
<td>2.79</td>
<td>3.6</td>
<td>2.75</td>
<td>2.47</td>
<td>1.94</td>
<td>P40</td>
<td>−1.03</td>
<td>0.24 (0.09–0.36)</td>
</tr>
</tbody>
</table>

AFCAPS/TexCAPS, Air Force/Texas Coronary Atherosclerosis Prevention Study; WOSCOPS, West of Scotland Coronary Prevention Study; LIPID, Long Term Intervention with Pravastatin in Ischaemic Disease; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; CARE, Cholesterol and Recurrent Events.

1° and 2° indicate primary and secondary prevention, respectively.

TC, total cholesterol; SBP, systolic BP; NE, not estimable, because data were not reported for an endpoint of nonfatal myocardial infarction and CHD death.

CVD and CHD risk are estimated with summary data and the Framingham risk equation and are converted to an annualized risk.

CHD event rate (for an endpoint of nonfatal myocardial infarction and CHD death) was that observed in the control arms of the trials and was annualized to allow comparison.

L20–40, 20–40 mg lovastatin per day; P40, 40 mg pravastatin per day; R20, 20 mg rosuvastatin per day.

ΔLDL-C concentration between treatment arms 12 months after randomization.

For an endpoint of nonfatal myocardial infarction and CHD death. Data are presented as the mean (95% CI).
more borderline or abnormal levels of a number of factors (smoking, total cholesterol concentration, HDL-C concentration, triglyceride concentration, BP, fasting glucose concentration, and body mass index) in up to 78% of men and 67% of women (101).

It is uncertain which of these associated factors are correctly considered confounders and which might actually be mediators of any CRP effect on CHD risk. As an illustration of the difficulty, higher hsCRP concentrations in middle-aged individuals have been reported to be independently associated with the development of diabetes and hypertension later in life (54, 102). It has been usual, however, to adjust for hypertension and diabetes in observational studies of CRP and CHD. If the hypothesized causal effect of CRP on CHD risk were mediated through BP or diabetes, adjustment for these variables would actually be controlling for part of the causal effect. Similarly, associations of CRP with CHD are substantially attenuated by adjustment for the inflammation marker and the coagulant protein fibrinogen (42). Studies of CRP in vitro, however, have prompted the proposal that CRP may exert proatherogenic effects through activation of tissue factor (103), which, if correct, could lead to low-grade activation of the coagulation system, which means that adjustment for fibrinogen might underestimate the true causal effect of CRP on CHD risk.

An RCT of a selective CRP inhibitor not only would provide the most direct evidence on the causal role of CRP in CHD but also would help distinguish confounding factors from mediators. Confounders should be balanced among the randomized groups, whereas factors altered by CRP should differ. Because genetic studies of common CRP gene variants can take on the role of a natural randomized trial (65, 76), they can also be used to help distinguish mediators from confounders to help rationalize the process of adjustment in observational studies (vide infra).

EXISTING AND FORTHCOMING EVIDENCE FROM GENETIC STUDIES

CRP is not stored but is released as it is synthesized, with a constant plasma half-life for clearance. The major point of regulation of plasma concentrations is likely to be at the level of gene transcription (104). In 2003, we reported that common polymorphisms in the CRP gene influence the basal hsCRP concentration and the increase in hsCRP with inflammation (66). These results provided a new research opportunity, because studying the effect of CRP indirectly through common polymorphisms in the gene provides a less biased and less confounded assessment of its influence on disease risk than studying CRP itself (65, 68, 76). This conclusion derives from the fact that the randomized allocation of alleles at conception leads to a balanced distribution of confounders among the genotypic groups (64, 75). In this respect, allocation to a low-concentration CRP genotype (or haplotype) at conception is analogous to being randomized to a specific CRP-lowering drug in a clinical trial (Fig. 2). Moreover, because the genotype is fixed and unaffected by disease, genetic associations are protected from reverse causation. We used an SNP in the 3′ region of the CRP gene to conduct an MR study for evaluating the causal role of CRP in CHD (68). Others have used a similar approach to assess the causal relevance of CRP for high BP and the metabolic syndrome (70, 105). Importantly, these analyses confirmed on a larger scale that SNPs in the CRP gene affect the basal CRP concentration and that a wide range of covariables associated with CRP itself are distributed evenly among the genetic groups.

The fact that at least 12 common SNPs are in the immediate vicinity of the CRP gene in populations of European ancestry means that typing of a wider range of SNPs is required to capture comprehensively the spectrum of genetic variation at the CRP locus. To this end, we have conducted further studies of the effect of CRP gene variation on hsCRP concentration that use haplotype-tagging SNPs, i.e., a parsimonious subset of 3 or 4 SNPs in the CRP gene that capture information on untyped SNPs by linkage disequilibrium. This approach became possible because of the resequencing of the CRP gene by the National Heart, Lung, and Blood Institute Program for Genomic Applications, which provides open-access information on all common variants in the gene (http://pga.gs.washington.edu/data/crp). Realizing that MR analyses of CRP in CHD would also require more precise estimation of the effect of CRP SNPs on hsCRP concentration, we conducted a large-scale meta-analysis of 26 genetic-association studies involving a total of 32 802 individuals to examine the relationship of CRP genotype to hsCRP concentration (73). This analysis initially presented a problem, because the studies had typed variable and partially overlapping sets of SNPs in the gene, which meant that orthodox meta-analysis would require partitioning the data into subsets of studies that had typed the same SNP, with a consequent loss of information. Moreover, the interpretation of these analyses would not be straightforward, because they would not account for association between markers due to linkage disequilibrium. To resolve these issues, we developed a new Bayesian approach to meta-analysis of genetic-association studies that allows pooling of all genetic data irrespective of the SNP typed by incorporating prior information on the allelic association between SNPs. Because the analysis for each SNP takes into account...
information on all others (whether typed or untyped), each SNP association in effect is adjusted for all others, thus allowing inference on the location of causal SNPs. With this approach, we found evidence for 4 CRP-modifying alleles distributed over the common haplotypes of the CRP gene, each of which could be captured by typing haplotype-tagging SNPs. These SNPs could be causal themselves or, alternatively, they could mark the presence of another causal variant in tight linkage disequilibrium with the tag SNPs, which could be within a region of strong allelic association that extends 100–300 kb upstream and downstream of the CRP gene (Fig. 5). In our metaanalysis, these variants caused differences in CRP concentration of 0.19–0.58 mg/L (depending on the SNP studied), which are equivalent to approximately 0.3–0.8 SD of the CRP concentration distribution (73) (Fig. 6).

Because these tag SNPs comprehensively capture information on causal variation at the CRP gene, they provide robust tools for MR analyses of the causal role of CRP in CHD and related phenotypes. We have used CRP tag SNPs to investigate any potential causal effect of CRP on diabetes and the development of carotid atheroma (106, 107), and others have extended our initial MR analysis of CHD (108). Whereas these MR studies have provided evidence against a causal effect of CRP on these outcomes, a conclusion that was supported by a very extensive and comprehensive MR analysis in the Copenhagen City Heart Study (109), a prior study by Lange et al. (110) found genetic evidence for an effect of CRP on CVD mortality and stroke in whites, and on stroke and myocardial infarction in African Americans. Therefore, to generate a data set of sufficient power to confirm or refute a modest but po-
tentially important effect of CRP on fatal and nonfatal CHD events, the CRP Coronary Heart Disease Genetics Collaboration (CCGC) is currently coordinating a large-scale MR analysis of 35 studies that includes 37,207 CHD cases and 119,524 controls (111). The collaboration will collate participant-level information on CRP genotype, CRP concentration, fatal and nonfatal CHD, and a range of other variables (where available) to compare the consistency between the following 3 risk estimates: (a) the association of CRP concentration with CHD (through the efforts of the ERFC); (b) the association of CRP genotype with CRP concentration (achieved through our prior metaanalysis and further expanded through the efforts of the CCGC); and (c) the association of CRP genotype with CHD risk. Consistency in the risk estimates would confirm a causal role for CRP, whereas a null genetic estimate would indicate that the observational association of CRP with CHD is overestimated in observational studies by residual confounding effects, reverse causation, or both. The breadth and depth of the analysis also will allow the application of CRP genotype to distinguish likely mediators of the effect of CRP on CHD risk from founders and will permit the assessment of modification of the genetic effect by nongenetic exposures.

In parallel with these efforts, whole-genome analyses of the genetic determinants of CRP concentration have recently been published. These studies not only have confirmed that CRP concentration is influenced by common SNPs in the CRP gene but also have provided new evidence that SNPs in a variety of other genes, including those that encode apolipoprotein E, hepatocyte nuclear factor 1a/HNF4, interleukin-6 receptor, glucokinase regulator protein, and the leptin receptor, also influence CRP concentration (112–114). These associations are unlikely to be attributed to linkage disequilibrium with CRP. Instead, the findings provide important insight into the biological pathways regulating CRP in trans, and it is notable that the protein products are all expressed in the hepatocyte.

Of the available genetic tools for MR analysis, however, SNPs in the CRP gene remain the most specific genetic tools for determining the causal role of CRP in CHD, because SNPs in the other genes that influence CRP are likely to do so indirectly through the downstream actions of the proteins they encode. If MR analyses that used CRP SNPs were able to show CRP to be causal in CHD, how-

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**Fig. 6.** Summary effect from traditional metaanalysis and Bayesian multiple-SNP hierarchical linear model of the 8 SNPs in the CRP gene.

Values shown are additive genetic effects on log CRP concentrations with 95% CIs or 95% credible intervals for traditional and Bayesian analyses, respectively. For the Bayesian analysis, results are shown only for those markers that appear to be strongly associated after variable selection. Asterisk indicates the dominant model; negative values indicate that the variant allele is associated with a lower CRP concentration. N/A, SNPs excluded from the model [Verzilli et al. (73)]. Reproduced with permission.
ever, the findings from the whole-genome analyses would then provide a potentially important source of novel drug targets for modifying CRP concentration.

Evidence from Randomized Trials of Statins

Because statin drugs lower CRP as well as LDL-C, some have argued that part of their salutary effect can be ascribed to CRP lowering, thereby providing indirect evidence on a causal role for CRP in CHD (115, 116). As we discussed previously, however, it is difficult to distinguish this proposed mechanism of action from the recognized effects of statins on LDL-C lowering. Although the effectiveness of statins has been associated in individual studies with the concentration of CRP attained during treatment, apparently independently of LDL-C, a recent systematic review and metaanalysis of 65 statin-intervention studies that included 16,260 patients for whom changes in LDL-C and CRP were both reported indicated that the reductions in LDL-C and CRP concentrations were correlated, particularly after accounting for baseline values, the type of statin used, and dosage (118). It therefore remains uncertain whether changes in CRP concentration with statin treatment contribute to the protective action of these agents or are a marker of the LDL-C–lowering effect.

The effectiveness of statins in the participants of the JUPITER trial, who were selected because of a high CRP concentration but a low LDL-C concentration, could also be interpreted as indicating that the effect of statins depends in part on CRP lowering or that people with high CRP concentrations derive a disproportionately greater benefit from statins, whatever the particular mechanism (60, 61). There are alternative explanations, however. A participant-level metaanalysis of observational studies has indicated that the relationship between LDL-C and the risk of CHD extends without threshold over the entire range of usual LDL-C concentrations (119). Moreover, the Cholesterol Treatment Triallists Collaboration showed that statin drugs exert similar proportional risk reductions over a wide range of baseline risks and at high and low levels of individual risk factors, including the LDL-C concentration (56). The benefit from statin treatment in a placebo-controlled trial of individuals at risk of CHD, even those with a low LDL-C concentration, might be regarded as concordant with this result. The question of whether individuals with a high CRP concentration derive a disproportionate benefit from statins will therefore require further evaluation of trial evidence on the effectiveness of statins in individuals with a wider range of CRP values.

Conclusions

Like LDL-C, CRP distinguishes individuals who will experience CHD events only modestly well, and once established risk factors have been evaluated to estimate absolute risk, CRP concentration appears to provide very modest incremental risk information, regardless of whether risk is assessed by discrimination, calibration, or reclassification. More detailed analysis of the utility of CRP in predicting CHD, however, will be possible through the efforts of the ERFC. Until specific CRP inhibitors that can be administered orally become available, MR analysis with SNPs in the CRP gene offers one of the best immediate prospects for answering the important question of whether CRP contributes causally to CHD.

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