

## Perspective

# More Evidence Against a Causal Association between C-Reactive Protein and Diabetes

Bernard Keavney

## The Association between CRP and Diabetes

In this issue of *PLoS Medicine*, Eric Brunner and colleagues address the causal nature of the association between C-reactive protein (CRP), the classical acute phase reactant, and the presence of insulin resistance or diabetes [1], using a genetic approach known as “Mendelian randomisation.”

Previous studies, including a literature-based meta-analysis of ten studies involving 2,675 cases of diabetes, have shown a significant association between the risk of diabetes and higher plasma levels of CRP [2]. However, it remains uncertain whether CRP is causally involved in the pathogenesis of diabetes. An alternative possibility to explain the association is that CRP is a confounder, since CRP is strongly associated with known causal risk factors for diabetes, such as obesity. A third possibility is reverse causality (i.e., diabetes causes a raised CRP), since the pathological processes leading to insulin resistance are known to operate from an early stage in life and could therefore be well established at the time of enrolment in prospective studies.

## Mendelian Randomisation

In the Mendelian randomisation approach, investigators first identify genetic polymorphisms that affect levels of the risk factor whose causal significance is queried (in this case CRP). They then quantify the relationships between polymorphisms and risk factor, risk factor and disease, and polymorphisms and disease. If the hypothesised risk factor causes disease, it is anticipated that the association between the polymorphisms and disease risk will be at least

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## Linked Research Article

This Perspective discusses the following new study published in *PLoS Medicine*:

Brunner EJ, Kivimäki M, Witte DR, Lawlor DA, Davey Smith G, et al. (2008) Inflammation, insulin resistance, and diabetes—Mendelian randomization using *CRP* haplotypes points upstream. *PLoS Med* 5(8): e155. doi:10.1371/journal.pmed.0050155

Using a Mendelian randomization approach, Eric Brunner and colleagues show that the associations between serum C-reactive protein and insulin resistance, glycemia, and diabetes are likely to be noncausal.

commensurate with what would be “expected,” given the association between the polymorphisms and the risk factor, and the association between the risk factor and disease.

Genotypes are randomly allocated at conception; therefore, if their effect is specific to the risk factor of interest, confounding ought not to influence any association between genotypes and disease. Since “randomisation” to a particular genotype takes place well before the onset of the disease of interest, reverse causality similarly should not explain any association observed. Mendelian randomisation therefore provides a potential method to assess the causality of associations described in epidemiological studies where suitably specific drugs to undertake randomised controlled trials are not yet available [3,4].

## The New Study

Brunner and colleagues genotyped three single nucleotide polymorphisms (SNPs) in the *CRP* gene that together “tag” the common genetic variation present at the locus. They measured serum CRP in 5,274 men and women enrolled in the Whitehall II prospective study, and measured the incidence

of diabetes, insulin resistance (using HOMA-IR, the homeostasis model assessment of insulin resistance), and haemoglobin A1c (HbA1c) thirteen years later. Serum CRP at mean age 49 years was significantly associated with incident type II diabetes (270 cases) during thirteen years of follow-up. Serum CRP was also associated with HOMA-IR and HbA1c. The strength of the associations with diabetes or with HOMA-IR and HbA1c diminished substantially upon adjustment for potential confounders.

As in previous studies, the authors found evidence of a significant association between *CRP* haplotypes (combinations of alleles along chromosomes) and serum CRP. There was, however, no association between the *CRP* haplotypes associated with higher serum CRP and either HOMA-IR or HbA1c levels among 4,357 and 5,266 individuals, respectively. The authors conducted an “instrumental variable” analysis using the *CRP* haplotypes, which is equivalent to the

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**Abbreviations:** CHD, coronary heart disease; CRP, C-reactive protein; Hb, haemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; LFIP, low-frequency, intermediate-penetrance; SNP, single nucleotide polymorphism; WTCCC, Wellcome Trust Case-Control Consortium

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Mendelian randomisation approach. This analysis showed that there was no significant evidence of association between CRP and either HOMA-IR or HbA1c when genotypes were used as instruments. These findings suggest that the association observed in the analyses not incorporating genotype may have been due to residual confounding. However, the upper 95% confidence intervals for the “instrumental variable” analyses were generally similar to the upper 95% confidence intervals for the analyses not incorporating genotype: thus, the genetic analyses could not, for the most part, rule out effects of the size suggested by the analyses not incorporating genotypes.

Finally, Brunner and colleagues tested for an association between *CRP* haplotypes and diabetes. Primary data were generated among 522 cases of diabetes and 6,534 controls from the Whitehall II study and a second prospective study, the Northwick Park Heart Study II. No association between any of the four most frequent haplotypes and incident diabetes was observed, although increases in diabetes risk between 30% and 60% for the different haplotypes could not be excluded. The publicly available data from the Wellcome Trust Case-Control Consortium (WTCCC) genome-wide association study in 1,923 cases of type II diabetes and 2,932 controls were also obtained. SNPs typed by the WTCCC that were good proxies for the three SNPs typed to generate Brunner and colleagues’ primary data were identified. There was no significant association ( $p > 0.05$ ) with diabetes for any of the proxy SNPs in the WTCCC data; differences in diabetes risk of greater than about 10% were ruled out. The authors conclude that serum CRP levels do not lie in the causal pathway leading to insulin resistance, hyperglycemia, or diabetes, and speculate that more proximal

mediators in the inflammatory cascade (such as interleukin-6, the principal regulator of CRP production) may be important.

### Implications of the Study

This study provides significant additional evidence against major causal effects of CRP on quantitative phenotypes related to diabetes risk. By inclusion of the WTCCC data, the study provides the strongest argument yet against any material association of common SNPs in CRP that affect plasma CRP levels with type II diabetes.

However, it also illustrates an important issue for the Mendelian randomisation approach—the difficulty of identifying common polymorphisms or haplotypes that have sufficiently large effects on intermediate traits that they can be used to draw definite conclusions. Although very large meta-analyses using a Mendelian randomisation framework are a potential solution [5], focusing on an alternative class of genetic variants for this type of analysis may also be a fruitful approach. “Low-frequency, intermediate-penetrance” (LFIP) variants with substantial effects on quantitative phenotypes have now been identified in a number of genes. For example, premature stop codons (in populations of African origin) or hypomorphic alleles (in white populations) in the *PCSK9* gene, which have around a 3% prevalence in each population, are associated with substantially lower levels of low-density lipoprotein (LDL) cholesterol (28% and 15%, respectively) and lower levels of coronary heart disease (CHD) risk (88% and 47%, respectively) [6]. If we did not already know that raised LDL cholesterol causes CHD, the association between the *PCSK9* variants, low LDL cholesterol, and low levels of CHD risk would have been compelling evidence in favour of a causal relationship

between raised LDL cholesterol and CHD. Counterfactually, if the LDL cholesterol/CHD relationship observed in epidemiological studies had all along been due to confounding, the large effects of these *PCSK9* variants on LDL cholesterol could well have been large enough to identify a discrepancy in a Mendelian randomisation analysis.

Recent technological developments have transformed the speed and cost of genetic sequencing [7]. As a consequence, the deep resequencing of large numbers of individuals necessary to discover such LFIP variants in candidate genes can now be undertaken on a systematic basis. Although LFIP variants causing large effects on potentially causal risk factors of interest may not be present for all such factors, they should, when present, considerably facilitate future analyses of the type undertaken by Brunner and colleagues. ■

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