C-Reactive Protein and the Risk of Cancer: A Mendelian Randomization Study

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Elevated plasma levels of C-reactive protein (CRP), a marker of inflammation, are associated with an increased risk of cancer, but it is unclear whether this association is causal. We examined whether four common single-nucleotide polymorphisms (SNPs) in the CRP gene that are associated with altered plasma CRP levels are causally associated with an increased risk of cancer. The study population included participants in a prospective study (n = 10,215) and a cross-sectional study (n = 36,403) of the adult general population in Denmark, all of whom were genotyped for the CRP SNPs. The association between plasma CRP levels measured by a high-sensitivity turbidimetry assay and the risk of cancer was examined for 8,224 participants in the prospective study. The hazard ratio of cancer for a doubling of the plasma CRP level was 1.09 (95% confidence interval [CI] = 1.03 to 1.14). The nine most common genotype combinations of the four CRP SNPs were associated with up to a 72% increase (95% CI = 58% to 87%) in CRP levels but not with an increased risk of cancer. The estimated causal odds ratio for cancer associated with a genetically induced doubling in CRP level was 0.94 (95% CI = 0.81 to 1.08). This finding suggests that elevated CRP levels do not cause cancer.


Elevated plasma levels of C-reactive protein (CRP), an acute-phase marker of inflammation, are associated with an increased risk of cancer (1–7). However, this association may be explained by other factors that increase both CRP levels and the risk of cancer (ie, confounding) or by occult cancer that could itself increase CRP levels (ie, reverse causality). Studies that use a Mendelian randomization approach take advantage of the random assortment of alleles from parents to offspring at the time of gamete formation to provide a relatively unbiased assessment of whether modifiable risk factors are causally related to disease (8–12). With this approach, genetic variants that specifically increase the plasma level of CRP can be used to assess the consequences of high CRP levels on the risk of cancer and thereby provide a risk estimate for the causal association between elevated CRP levels and the risk of cancer.

We used a Mendelian randomization design to test the hypothesis that genetically elevated levels of CRP because of polymorphisms in the CRP gene cause an increased risk of cancer in the general population. To test this hypothesis, we first examined whether elevated plasma CRP levels were associated with an increased risk of cancer; second, whether CRP polymorphisms were associated with plasma CRP levels; and third, whether the polymorphisms that were associated with altered CRP levels were associated with the risk of cancer. Finally, on the basis of the associations between CRP polymorphisms and plasma CRP levels and between CRP polymorphisms and the risk of cancer, we provide a risk estimate for the association between genetically elevated CRP levels and the risk of cancer and compare this estimated causal effect of CRP on the risk of cancer with the observed association between plasma CRP levels and the risk of cancer.

The study population consisted of participants in two studies of the adult general population in Denmark: the prospective Copenhagen City Heart Study (13,14) and the cross-sectional Copenhagen General Population Study (9,12) (n = 36,403). All participants in these cohorts were white and of Danish descent, and no individuals appeared in both cohorts. Herlev Hospital and the Copenhagen and Frederiksberg (KF) and the Capital Region of Denmark (H) ethical committees approved the Copenhagen City Heart and Copenhagen General Population Studies (KF-100.2039/91, KF-01-144/01, and H-KF-01-144/01), and all participants gave written informed consent.

The primary endpoint in this study was the diagnosis of any cancer. Diagnoses of cancer from 1947 through July 2007 were obtained from the Danish Cancer Registry (15–17), which identifies 97.8% of all cancers in Denmark (15), and from the Danish National Patient Registry. Diagnoses were classified according to the World Health Organization International Classification of Diseases, Seventh Revision (ICD-7 codes 140–205) or Tenth Revision (ICD-10 codes C00–D09) (18,19). Dates of deaths were obtained from the Danish Civil Registration System, which is 100% complete. Follow-up time for each participant in the Copenhagen City Heart Study began at blood sampling and ended at occurrence of cancer, death, emigration, or August 11, 2007, whichever came first. We excluded participants who had a diagnosis of cancer before study entry from the prospective analyses. During the study period, we had 100% follow-up.

We determined the plasma CRP levels by using high-sensitivity turbidimetry (Dako, Glostrup, Denmark) or nephelometry (Dade Behring, Deerfield, IL) assays according to the manufacturers’ protocols. Levels of CRP were measured in plasma stored at −80°C for 12–15 years from 8,224 Copenhagen City Heart Study participants who were examined during 1991–1994, in fresh plasma samples from 36,94 Copenhagen City Heart Study participants who had a follow-up examination in 2001–2003, and in fresh plasma samples from 36,403 participants in the Copenhagen General
Population Study, CRP measurements were assessed daily for precision (coefficient of variation ranged from 3% to 8%) and monthly for accuracy through a Scandinavian quality control program. For statistical analysis, we divided CRP levels into quartiles and also used CRP as a continuous predictor variable. We also determined levels of fibrinogen in fresh plasma samples from 792 participants in the Copenhagen City Heart Study (1991–1994 examination) using a colorimetric method (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s protocol. For statistical analysis, we used fibrinogen levels as a continuous predictor variable.

We used an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Inc, Foster City, CA) to genotype four noncoding single-nucleotide polymorphisms (SNPs) in the CRP gene that are associated with an altered plasma CRP level but not with protein function: rs1205, rs3091244, rs1130864, and rs3093077 (12,20,21). Genotyping was verified by DNA sequencing in more than 30 individuals with each genotype. Because we performed runrems, 99.9% of all available participants were genotyped for all four SNPs, and all participants in this study have genotype information about all four SNPs (details on genotyping assays including polymerase chain reaction primer and probe sequences and reaction conditions are given in Supplementary Material, available online). From the four CRP SNPs, we generated all possible genotype combinations and ranked the nine most frequent genotype combinations in the study population according to increasing levels of CRP.

The data were analyzed using STATA statistical software (version 10.1; StataCorp, College Station, TX), and a two-sided \( P \) value less than 0.05 was considered statistically significant. We used Cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of cancer as a function of elevated plasma CRP levels. To automatically adjust for age, we used left truncation and age as the time scale. We used a model that included age and sex only and a multifactor-adjusted model that included age, sex, smoking status (never, former, or current smoker), smoking dose (cigarettes per day), alcohol consumption (women: \( \leq 168 \) or \( >168 \) g/wk; men: \( \leq 252 \) or \( >252 \) g/wk), and body mass index (<18.5, 18.5–24.9, 25–29.9, or \( \geq 30.0 \) kg/m\(^2\)). Hazard ratios were corrected for regression dilution bias (22). We used one-way analysis of variance to test for trend of mean log(CRP) levels among different genotypes or genotype combinations. Because the distribution of the plasma CRP levels was skewed, with most subjects having lower plasma CRP levels, we used natural logarithmic transformation of CRP levels to approach a normal distribution. To estimate hazard ratios or odds ratios of cancer as a function of CRP genotypes or genotype combinations, we used Cox proportional hazards regression models with left truncation or logistic regression models that were adjusted for age and sex only or for multiple covariates as described above. To estimate the ratio of cancer as a function of genetically elevated CRP levels (ie, the risk of cancer as a function of elevated CRP levels because of polymorphisms in the CRP gene), we used instrumental variable analysis in which we plotted the odds ratios of cancer for the nine CRP genotype combinations as a function of the observed differences in CRP levels among the nine CRP genotype combinations and used generalized least squares regression (23) to calculate an odds ratio of cancer for a doubling in genetically elevated CRP levels.

For Cox proportional hazards regression analyses, we tested the assumption of proportional hazards graphically by plotting log(cumulative hazard) as a function of age. Any indication of nonconformity to proportional hazards assumptions led to further examination with a test based on Schoenfeld residuals. We detected no major violations of the proportional hazards assumption. For the association between CRP levels as a continuous predictor variable and risk of cancer, we used natural logarithmic transformation of CRP and assumed that there was a linear relationship between log(CRP) and the log hazards of cancer. We tested this assumption by computing a likelihood ratio test comparing a model that contained a second- and a first-order term of log(CRP) (a quadratic model) with one containing only the first-order term (linear model). We detected no signs of nonlinearity.

**Context and Caveats**

**Prior knowledge**

Elevated circulating levels of C-reactive protein (CRP), a marker of inflammation, are associated with an increased risk of cancer, but whether this association is causal remains unclear.

**Study design**

A Mendelian randomization study that examined whether four common single-nucleotide polymorphisms in the CRP gene that are associated with altered plasma CRP levels are causally linked to an increased risk of cancer among participants in a prospective study and a cross-sectional study of the adult general population in Denmark.

**Contribution**

Polymorphisms in the CRP gene that are associated with increased circulating levels of CRP were not associated with an increased risk of cancer.

**Implications**

Elevated circulating levels of CRP do not cause cancer. It remains a possibility that inflammation could lead to cancer.

**Limitations**

Combining a heterogeneous group of cancers with diverse etiologies into a single outcome limits applicability of the results to specific cancer types.

**From the Editors**

Characteristics of the participants at DNA sampling are shown in Supplementary Tables 1 and 2 (available online), and the distribution of cancer types observed in the two studies is shown in Supplementary Table 3 (available online). Except for the levels of CRP, the covariates did not differ among the nine most frequent CRP genotype combinations. In the prospective Copenhagen City Heart Study, the median time from blood collection to cancer onset was 6 years (range = 0–15 years).

In the prospective Copenhagen City Heart Study, increasing levels of CRP were associated with an increasing risk of cancer (Supplementary Figure 1, available online). The multifactor-adjusted hazard ratio of cancer for the highest vs the lowest quartile of CRP was 1.24 (95% CI = 1.04 to 1.48). To test whether the association between plasma CRP level and the risk of cancer was specific to CRP or was with inflammation in general, we adjusted for levels of the...
acute-phase reactant fibrinogen as a proxy for inflammation. This adjustment resulted in a hazard ratio of 1.19 (95% CI = 0.98 to 1.44), suggesting that the association was not specific to CRP (Supplementary Figure 1, available online). Additional adjustment for use of anti-inflammatory drugs, hormone therapy, and statins gave similar results (data not shown).

The observed genotype frequencies for the four CRP SNPs were in Hardy-Weinberg equilibrium. For the four CRP SNPs, the difference in CRP level between subjects with the rare genotypes and those with the common homozygous genotype ranged from a 31% decrease (95% CI = 28% to 34%) to a 98% increase (95% CI = 50% to 162%) (Figure 1; Supplementary Figure 1, available online). For the nine most frequent genotype combinations, there was a 72% increase (95% CI = 58% to 87%) in CRP level between the genotype combination with the lowest CRP level and the genotype combination with the highest CRP level.

Among subjects who participated in the Copenhagen City Heart Study, none of the CRP SNPs was associated with an increased risk of cancer; on the contrary, rs3093077 and rs3091244 were associated with a decreased risk of cancer (Figure 1). Among subjects who participated in the Copenhagen General Population Study, none of the CRP SNPs was associated with the risk of cancer (Figure 1). Additional adjustment for use of anti-inflammatory drugs, hormone therapy, and statins gave similar results (data not shown).

To achieve maximal statistical power, we combined the data from the Copenhagen City Heart Study and the Copenhagen General Population Study and calculated the odds ratios for cancer as a function of genotype combination. On the basis of these odds ratios and the associations between genotype combination and CRP level, we estimated a causal odds ratio for cancer for a genetically induced doubling of CRP levels of 0.94 (95% CI = 0.81 to 1.08) (Figure 2). By contrast, the hazard ratio of cancer for a doubling of the plasma CRP level was 1.09 (95% CI = 1.03 to 1.14) (Figure 2). The 95% confidence interval for the estimated causal effect did not include the point estimate for the observed association between plasma CRP level and the risk of cancer, suggesting that the two estimates are truly different and that elevated CRP levels per se are unlikely to increase the risk of cancer. In agreement with these findings, a prospective cohort study on the association between CRP SNPs and four common non–skin-related malignancies found no association between CRP SNPs and the risk of any malignancy.

**Figure 1.** Risk of cancer by CRP genotype and genotype combination for participants in the Copenhagen City Heart Study and the Copenhagen General Population Study. Hazard ratios (HRs) and odds ratios (ORs) were adjusted for age, sex, smoking status and dose, alcohol consumption, and body mass index. P values (two sided) are from a test for trend of hazard ratios or odds ratios across genotypes or genotype combinations with increasing C-reactive protein (CRP) levels. Black circles indicate hazard ratios or odds ratios, and error bars indicate 95% confidence intervals (CIs). *Change in plasma CRP levels is based on data from the Copenhagen General Population Study (Supplementary Figure 2, available online) and is the percentage difference in CRP level between subjects with the rare genotypes and those with the common homozygous genotype. †The nine CRP genotype combinations were generated from all possible CRP genotype combinations from the four CRP single-nucleotide polymorphisms and represent the nine most frequent genotype combinations in the study population ranked according to increasing CRP levels. Thus, the nine CRP genotype combinations do not include all participants.
cancer among 5956 individuals who were genotyped (6).

A limitation of this study involves our combination of all cancer types as a single outcome. Although the combined endpoint yielded a reasonable statistical power, it comprises a heterogeneous group of cancers with diverse etiologies, which limits the generalizability of these results to specific types of cancer. For example, inflammation is known to play a role in the pathogenesis of colorectal cancer (24,25) and, possibly, lung cancer (26) but does not play a role in the pathogenesis of breast cancer (27). In this study, a doubling of the plasma level of CRP was associated with a statistically significant increased risk of lung cancer (multifactor-adjusted HR = 1.30, 95% CI = 1.17 to 1.45), whereas a genetically induced doubling of CRP level was not (multifactor-adjusted HR = 1.15, 95% CI = 0.67 to 1.98) (Supplementary Table 4, available online).

In conclusion, we have demonstrated that polymorphisms in the CRP gene that are associated with increased circulating levels of CRP are not associated with an increased risk of cancer. However, although we may be able to exclude CRP per se as a cause of cancer, we cannot exclude the possibility that inflammation could lead to cancer. Also, our results do not invalidate the potential clinical use of slightly increased plasma CRP levels to predict the risk of certain cancer subtypes.

References


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