Journal of the American College of Cardiology
© 2010 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 56, No. 10, 2010
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.02.066

QUARTERLY FOCUS ISSUE: HEART RHYTHM DISORDERS

Does Elevated C-Reactive Protein Increase Atrial Fibrillation Risk?

A Mendelian Randomization of 47,000 Individuals From the General Population

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Objectives The purpose of this study was to test whether the association of C-reactive protein (CRP) with increased risk of atrial fibrillation is a robust and perhaps even causal association.

Background Elevated levels of CRP previously have been associated with increased risk of atrial fibrillation.

Methods We studied 10,276 individuals from the prospective Copenhagen City Heart Study, including 771 individuals who had atrial fibrillation during follow-up, and another 36,600 persons from the cross-sectional Copenhagen General Population Study, including 1,340 cases with atrial fibrillation. Individuals were genotyped for 4 *CRP* gene polymorphisms and had high-sensitivity CRP levels measured.

Results A CRP level in the upper versus lower quintile associated with a 2.19-fold (95% confidence interval [CI]: 1.54- to 3.10-fold) increased risk of atrial fibrillation. Risk estimates attenuated slightly after multifactorial adjustment to 1.77 (95% CI: 1.22 to 2.55), and after additional adjustment for heart failure and plasma fibrinogen level to 1.47 (95% CI: 1.02 to 2.13) and 1.63 (95% CI: 1.21 to 2.20), respectively. Genotype combinations of the 4 *CRP* polymorphisms associated with up to a 63% increase in plasma CRP levels ($p < 0.001$), but not with increased risk of atrial fibrillation. The estimated causal odds ratio for atrial fibrillation by instrumental variable analysis for a doubling in genetically elevated CRP levels was lower than the odds ratio for atrial fibrillation observed for a doubling in plasma CRP on logistic regression (0.94 [95% CI: 0.70 to 1.27] vs. 1.36 [95% CI: 1.30 to 1.44]; $p < 0.001$).

Conclusions Elevated plasma CRP robustly associated with increased risk of atrial fibrillation; however, genetically elevated CRP levels did not. This suggests that elevated plasma CRP per se does not increase atrial fibrillation risk.
(J Am Coll Cardiol 2010;56:789–95) © 2010 by the American College of Cardiology Foundation

Elevated levels of C-reactive protein (CRP) have been suggested as a possible contributing factor to the initiation or maintenance of atrial fibrillation. However, whether increased plasma CRP levels are simply a marker for atrial fibrillation or whether elevated CRP actually contributes directly to causing the disorder presently is unknown (1,2). This question has clinical importance because several cardiovascular drugs, such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, modulate the inflammatory process in the heart (3,4), and because drugs that specifically lower CRP levels already are being developed for treatment of cardiovascular disease (5).

Cause-and-effect relationships such as the one suggested between plasma CRP levels and risk of atrial fibrillation can be studied using an approach called Mendelian randomization (6). This approach uses genetic variants randomly assorted during gamete formation and associated with levels of plasma CRP to test whether there could be a causal association between elevated plasma CRP levels and increased risk of atrial fibrillation. Thus, genetic variants that

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Manuscript received October 7, 2009; revised manuscript received February 11, 2010, accepted February 25, 2010.

Abbreviations and Acronyms

CI = confidence interval
CRP = C-reactive protein
OR = odds ratio
LDL = low-density lipoprotein

specifically increase plasma levels of CRP (7) can be used as instruments to assess the consequences of lifelong high CRP levels independently of other risk factors and not hampered by reverse causation (8).

We tested the hypothesis that there is a robust and potential causal association between elevated CRP levels and increased risk of atrial fibrillation. Robustness was tested by adjustment of risk estimates for age, sex, and statin use; age, sex, statin use, and *CRP* genotype; multifactorially for age, sex, statin use, low-density lipoprotein (LDL) cholesterol, body mass index, smoking, heavy drinking, diabetes mellitus, hypertension, and hyperthyroidism; multifactorially including heart failure, *AGT*-20A→C, *AGT* T174M, and *ACE* insertion or deletion genotypes; or finally, including plasma fibrinogen. Potential causality was tested by examining whether *CRP* genotype combinations associated with an increased risk of atrial fibrillation consistent with their life-long elevation of plasma CRP levels.

Methods

For full information on study populations, genotyping and biochemical analyses, covariates, and statistical analyses, please refer to Supplementary Methods. The CCHS (Copenhagen City Heart Study) is a prospective study of the Danish general population initiated from 1976 through 1978 with follow-up examinations in 1981 through 1983, 1991 through 1994, and 2001 through 2003 (9). The CGPS (Copenhagen General Population Study) is a cross-sectional study initiated in 2003 with ongoing inclusion. Participants were ascertained exactly as in the CCHS. All participants were white and of Danish

Table 1 Characteristics of Individuals in the 2 General Population Studies by Plasma CRP Quintiles

	Quintiles of Plasma CRP					
	1	2	3	4	5	p Value
Copenhagen City Heart Study						
CRP (mg/dl)	1.0 (0.1–1.2)	1.3 (1.2–1.5)	1.7 (1.5–2.1)	2.7 (2.1–3.6)	9.9 (3.6–103)	<0.001
No. of individuals	1,656	1,657	1,654	1,654	1,655	
Women	47	56	58	55	56	<0.001
Age (yrs)	50 (37–63)	57 (44–68)	61 (50–70)	64 (53–72)	64 (55–72)	<0.001
Statin use	0.5	0.8	0.9	1.2	0.2	<0.001
LDL cholesterol (mmol/l)	3.3 (2.7–4.1)	3.6 (2.9–4.3)	3.8 (3.1–4.5)	3.8 (3.1–4.7)	3.7 (3.0–4.5)	<0.001
Body mass index (kg/m^2)	23 (21–25)	24 (22–27)	25 (23–28)	26 (24–29)	27 (24–30)	<0.001
Smoking, active and former	51	56	60	66	69	<0.001
Heavy drinkers	10	10	10	9	10	0.52
Diabetes mellitus	2	3	3	5	8	<0.001
Hypertension	38	48	57	65	65	<0.001
Hyperthyroidism	2	2	2	2	3	0.06
Heart failure	4	7	10	15	19	<0.001
Fibrinogen (mg/l)	2.5 (2.1–2.9)	2.7 (2.3–3.1)	2.9 (2.5–3.4)	3.2 (2.8–3.7)	3.8 (3.2–4.5)	<0.001
AGT-29 A→C (AA/AC/CC)	70/27/3	70/27/3	72/26/2	72/25/3	70/28/2	0.71
AGT T174M (TT/TM/MM)	77/22/2	76/23/2	79/19/1	77/21/2	77/21/1	0.29
ACE ins/del (dd/id/ii)	26/50/24	26/49/24	26/50/24	26/48/25	25/51/24	0.56
Copenhagen General Population Study						
CRP (mg/dl)	0.5 (0.01–0.8)	1.1 (0.8–1.3)	1.5 (1.3–1.9)	2.5 (1.9–3.4)	8.7 (3.4–330)	<0.001
No. of individuals	7,331	7,320	7,316	7,315	7,318	
Women	53	52	53	52	57	<0.001
Age (yrs)	53 (45–63)	58 (48–66)	59 (49–67)	60 (50–69)	62 (51–71)	<0.001
Statin use	6	10	11	11	11	<0.001
LDL cholesterol (mmol/l)	3.1 (2.2–3.7)	3.1 (2.5–3.8)	3.2 (2.6–3.9)	3.3 (2.7–4.0)	3.3 (2.6–3.9)	<0.001
Body mass index (kg/m^2)	24 (24–26)	25 (23–27)	26 (23–28)	27 (24–30)	28 (25–31)	<0.001
Smoking, active and former	55	45	39	49	58	0.12
Heavy drinkers	2	3	3	3	3	0.02
Diabetes mellitus	2	3	3	4	6	<0.001
Hypertension	55	63	67	72	77	<0.001
Hyperthyroidism	1	1	1	1	2	0.02
Heart failure	1	1	1	2	4	<0.001
Fibrinogen (mg/l)	1.0 (0.9–1.2)	1.1 (1.0–1.2)	1.2 (1.0–1.3)	1.3 (1.1–1.4)	1.4 (1.2–1.7)	<0.001

Continuous values are summarized as median (interquartile range); p value is for trend among quintiles by a nonparametric test by Cuzick. Categorical values are summarized in percent; p values for trend by Cuzick's extension of a Wilcoxon rank-sum test.
CRP = C-reactive protein; LDL = low-density lipoprotein.