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Pleiotropy of C-reactive Protein Gene Polymorphisms with C-reactive Protein Levels and Heart Rate Variability in Healthy Male Twins

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Abstract

Reduced heart rate variability (HRV) and increased C-reactive protein (CRP) levels are both predictors of coronary artery disease (CAD), and are correlated with each other. We examined whether these two phenotypes share a common genetic substrate and investigated the relations of the *CRP* gene polymorphisms with both CRP levels and HRV indices. We examined 236 male twins free of symptomatic CAD, with mean age ($\pm$ SD) of 54 years ($\pm$ 2.9). Plasma CRP levels were measured and frequency domain measures of HRV were assessed using a 24-hour ECG recording, including ultra-low, very-low, low and high frequency power (ULF, VLF, LF, and HF). Three SNPs in the *CRP* gene were genotyped. Generalized estimating equations were used to examine the association between CRP and HRV, as well as the genotype-phenotype association. Bivariate structural equation modeling was performed to estimate the genetic and environmental correlations between CRP and HRV, and the explanatory effect of *CRP* gene polymorphisms on the CRP-HRV association. Both CRP ($h^2=0.76$) and HRV indices ($h^2=0.56-0.64$) showed high heritability. Higher CRP levels were significantly associated with lower HRV. A robust genetic correlation was found between CRP and ULF ($r_G=-0.3$, $P=0.001$). One *CRP* SNP (rs1205) was significantly associated with both CRP ($P=0.003$) and ULF ($P=0.005$) and explained 11% of the genetic covariance between them. In conclusion, reduced HRV is significantly correlated with increased CRP plasma levels and this correlation is due, in large part, to common genetic influences. A polymorphism in the *CRP* gene contributes to both CRP levels and HRV.

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Keywords

C-reactive protein; heart rate variability; common genes; genetic polymorphisms

Several studies have reported a relation between impaired cardiac autonomic function as measured by heart rate variability (HRV) and increased inflammatory markers such as C-reactive protein (CRP) in patients with coronary artery disease (CAD),¹ as well as in apparently healthy subjects.² However, the causal direction of this association remains unclear and may actually be bidirectional.^{3–5} It is also plausible that a common genetic factor accounts for the observed correlation between autonomic dysfunction and inflammation, since both phenotypes are heritable.^{6–8} Discovery of a common genetic substrate may point towards a common etiological pathway and improve our understanding of the mechanisms underlying autonomic-immune interactions. In the present study, we examined whether shared genetic vulnerability plays a role in the phenotypic correlation between plasma CRP levels and HRV frequency domains in middle-aged male twins. We further investigated whether *CRP* gene polymorphisms explain a portion of the genetic correlation between CRP levels and HRV indices.

METHODS

Twins included in the Twins Heart Study (THS) were selected from the Vietnam Era Twin (VET) Registry, which includes 7369 middle-aged male-male twin pairs both of whom served in the United States military during the time of the Vietnam War.⁹

The THS included 360 twins from the VET Registry all born between 1946 and 1956 (>90% of the twins in the VET registry fall into this range). The methods of construction of this sample were shown in Figure 1 and were also described before.⁸ The twins were free of a self-reported previous diagnosis of cardiovascular disease based on survey data collected in 1990.¹⁰ From this group, we randomly sampled two groups of twin pairs: one group included depression-discordant twins, where one member of the pair had a lifetime history of major depressive disorder (MDD) and the other did not; the second group of twins included pairs where neither had a history of MDD. Once selected, twin pairs came together but were examined separately at the Emory University General Clinical Research Center between March 2002 and March 2006, where the twins had a comprehensive physical examination and were queried again about previous diagnoses of cardiovascular diseases that might have occurred since the initial screen in 1990. We also administered the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition to classify subjects based on a lifetime history of MDD which may occur since 1990. To avoid potential influences of CAD on CRP and HRV, twins who reported a history of myocardial infarction, angina pectoris, coronary angioplasty or coronary bypass surgery since 1990 ($N = 35$) were excluded. No subjects had recent immunologic disorders or acute illnesses. We further excluded 61 subjects with unavailable HRV data, 4 subjects with outliers of inflammation (>3SD from the mean) and 24 subjects with missing genotypes. The present analyses, therefore, included 236 twins (90 pairs and 56 singletons). The singletons were included because they contributed to estimates of means and variances. This protocol was approved by the Institutional Review Board at Emory University and informed consent was obtained from all subjects.

Plasma CRP was measured with the Beckman Coulter High Sensitivity CRP assay on the Synchron LX-20 analyzer. All samples were run in duplicate. Inter- and intra-assay variability were reliably <10%. The values of CRP were log-transformed to improve the distribution.

Twins were kept as inpatients and wore an ambulatory ECG (Holter) monitor (GE Marquette SEER digital system) for 24 hours. Both twins in a pair were studied at the same time and their recording times, schedule and activity level during the recording were matched. All activity was restricted to quiet walking in the clinical facility or around the campus. Participants were instructed to refrain from smoking and drinking alcohol or coffee during the recording. HRV data were analyzed following published methodology.^{11,12} The power spectrum was computed from the fast Fourier transform (FFT) of the time series modified by a Parzen window to reduce spectral leakage and corrected for window attenuation and boxcar sampling.^{11,13} Four discrete frequency bands were integrated: ultra low frequency (ULF) <0.0033 Hz; very low frequency (VLF) 0.0033 to 0.04 Hz; low frequency (LF) 0.04 to 0.15 Hz; high frequency (HF) 0.15 to 0.40 Hz. HRV is believed to reflect the changing balance between sympathetic and parasympathetic activity. Which index best represents “autonomic balance” remains under investigation.¹⁴ Further, which indices are most predictive of outcome has varied across studies.¹⁴ For these reasons, data is presented on all frequency bands. Twins with >20% interpolation or <18 recorded hours were excluded from the analysis. All HRV data were log-transformed for analysis.

Body mass index (BMI) was calculated as weight/height². Physical activity was assessed with a modified version of the Baecke Questionnaire of Habitual Physical Activity,¹⁵ and the global physical activity score was used in the analysis. Cigarette smoking was classified into current versus never or past smoker.

Four SNPs in the *CRP* gene showed minor allele frequency (MAF) > 5% in the HapMap Caucasian panel (CEPH), including rs1205, rs1130864, rs1800947 and rs1417938. There was perfect linkage disequilibrium (LD) between rs1130864 and rs1417938 (pair-wise $r^2=1$). Thus, 3 SNPs including rs1205, rs1130864 and rs1800947 were selected and genotyped using the Beckman GenomeLab SNPstream Genotyping System (Beckman Coulter, Inc., Fullerton, CA). Genotyping accuracy for all SNPs was 99% as assessed by inclusion of duplicates (pairs of MZ twins) in the arrays, and negative controls (water blanks) were included on each plate.

In initial descriptive analyses we compared means (or prevalence) of CRP, HRV indices and other study variables between monozygotic (MZ) and dizygotic (DZ) twins. Associations between CRP levels and HRV indices were tested by using generalized estimating equations (GEE) which take into account the relatedness between co-twins. Two models were tested: 1) adjusting for only age; 2) adjusting for potential confounders including age, BMI, physical activity, smoking status and current use of medications (beta-blockers, statin and anti-depressants). These analyses were performed using STATA 8 (StataCorp, College Station, TX).

To estimate the relative contributions of genetic and environmental influences on the CRP plasma levels and HRV indices, structural equation models (SEM) were constructed using the software package Mx.¹⁶ The twin design allows separation of the observed phenotypic variance into underlying additive genetic variance (A), common environmental variance shared by a twin pair (C), and environmental variance specific to individuals (E). A greater similarity of phenotype(s) in MZ twins as compared with DZ twins, as indicated by a higher correlation in MZ than DZ twins, suggests a genetic effect.

To examine whether genetic and/or environmental factors contribute to the correlation between CRP and HRV, we constructed bivariate SEM fitting the association between these two traits. The phenotypic variation for each trait was decomposed into A, C and E components. The correlation between the two traits was similarly partitioned into components resulting from A, C and E. Models were fit by the method of maximum likelihood. A series of nested submodels (AE, CE and E) were each tested for their goodness of fit against a saturated model (ACE).

We tested the associations of each SNP in the *CRP* gene with CRP plasma levels and HRV indices. We then investigated to what extent the covariance between CRP and HRV indices could be explained by variants in the *CRP* gene. The software Haploview4.0 was used for computing pair-wise LD between the studied SNPs.¹⁷ Association analyses were performed using GEE. For individual SNP analysis, we tested the additive model of SNPs on CRP levels and HRV indices respectively. Similarly, two models were tested: 1) adjusting for only age; 2) adjusting for a priori specified covariates.

Bivariate SEM for CRP and HRV was extended to examine the role of the *CRP* gene variants. Briefly, measured genotypes were incorporated in the SEM and genetic effects on CRP levels and HRV indices were tested. The contributions of genetic variants to the variances and covariance of CRP and HRV were also estimated. Details of this approach have been described previously.¹⁸ By involving measured genotypes, bivariate SEM has the advantage of allowing genetic effects on the means, variances and relations between two related phenotypes. The models were fitted with Mx software.¹⁶ Most of twins from the THS are Caucasians (94%). Further adjustment for race did not change the results. To avoid the potential influences of race/ethnicity, we repeated all the analyses after exclusion of Africa-Americans. The results were virtually identical and are not reported.

RESULTS

Subject characteristics are listed in Table 1. The mean age was 54 years old (range 47–60). None of the variables showed a significant difference between MZ and DZ twins. CRP plasma levels were significantly associated with decreased HRV indices including ULF, VLF and LF (all $P < 0.001$) but not with HF ($P = 0.52$) (Table 2). After adjusting for covariates, the associations between CRP and HRV indices were attenuated but remained statistically significant for ULF ($P < 0.001$) and VLF ($P = 0.002$).

For all HRV indices and CRP plasma levels, the correlations in MZ twins were consistently higher than those in DZ twins, indicating genetic influence (Table 3). This was confirmed by univariate SEM analysis. Moderate to high heritability was estimated for all HRV indices (56–64%). For CRP plasma levels, the heritability was estimated as 76% (95% CI: 64–84%). After adjusting for covariates, the heritability estimates were slightly changed but the overall results remained similar.

The best fitting bivariate models for the relationship between CRP levels and HRV indices were the AE models. The parameter estimates suggested that there were significant genetic correlations between CRP and ULF ($r_G = -0.30$, 95 CI% -0.34 to -0.16), as well as CRP and VLF ($r_G = -0.18$, 95 CI% -0.24 to -0.01). Most of the correlation between CRP and ULF ($r = -0.31$) was due to shared genetic factors. For CRP and VLF, about 64% of the correlation was influenced by the shared genetic factors. After adjustment for covariates, the genetic correlation between CRP and ULF remained statistically significant ($r_G = -0.26$, $P = 0.004$).

The locations and MAFs of the 3 SNPs are shown in Table 4. There was no strong LD among them (pair-wise r^2 : 0.03–0.25). Two SNPs were significantly associated with CRP levels (rs1205, $P = 0.003$ and rs1130864, $P = 0.024$). After adjusting for covariates, rs1205 remained significantly associated with CRP ($P = 0.004$). In addition, the SNP rs1205 was also significantly associated with ULF ($P = 0.005$), both before and after adjustment for covariates. Compared with the subjects homozygous for the rs1205 major C allele (CC genotype), those homozygous for the minor T allele (TT genotype) had, on average (using geometric means calculated from means of log-transformed values), a 58% lower CRP levels and a 36% higher ULF (Figure 2).

Figure 3 shows the best fitting bivariate model involving the genetic effects of *CRP* rs1205 on both CRP and ULF. The explained proportions of the phenotypic variance by rs1205 were 2% and 1% respectively. After taking into account the effect of this SNP, the residual heritabilities of CRP and ULF were estimated as 73% and 59%. As reported above, the overall genetic correlation between CRP and ULF was -0.30 . The residual genetic correlation was now estimated as -0.27 ($P < 0.001$) (Figure 3), indicating that about 11% of the shared genetic variance between these two phenotypes could be explained by the *CRP* rs1205.

DISCUSSION

In a sample of predominantly healthy middle-aged males free of CAD, we found a robust correlation between reduced HRV (ULF) and increased plasma levels of CRP. Bivariate genetic analyses indicated that a shared genetic vulnerability explains most of this association, even after adjustment for other CAD risk factors. In addition, one SNP located in the *CRP* gene (rs1205) was significantly associated with both CRP plasma levels and HRV (ULF), and explained about 11% of the genetic correlation between these two phenotypes.

There is growing evidence that both autonomic imbalance and inflammation play important roles in the development of CAD.^{19,20} Thus, it is perhaps not surprising that reduced HRV is accompanied by an increase of inflammation in patients with stable and unstable coronary disease.¹ Even among presumably healthy individuals, impaired HRV is associated with elevated inflammatory markers such as CRP.² However, most studies used a relatively short-time ECG reading for HRV evaluation (2–30 min), and only one study evaluated ULF.²¹ ULF, VLF, and LF power are influenced by both sympathetic and parasympathetic systems, whereas HF is largely thought to reflect short-term parasympathetic modulation.²² Our findings suggest the CRP may be more associated with longer-term autonomic fluctuations. ULF may also be affected by physical activity.²² Although ULF is the most predictive of mortality in post-myocardial infarction patients,¹¹ the exact physiological interpretation for ULF is still unclear.^{14,22,23} In our study, HRV was measured over 24-hours. Activity during the recording was matched between twins, and we also adjusted for habitual physical activity in the analysis.

The mechanisms responsible for the relationship between plasma CRP and cardiac autonomic alteration remain unclear. There are several possible explanations for this association. First, an imbalance in the autonomic nervous system (increased sympathetic activity or reduced parasympathetic activity) could theoretically modulate inflammatory response as both the bone marrow and the lymphoid system are innervated by autonomic nerves and are under the influence of these systems.³ Inflammation may, in turn, influence autonomic balance. For example, cytokines are known to act on the central nervous system by affecting the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS).^{4,5} Thus, autonomic imbalance and inflammation may potentiate each other.

In addition to the bidirectional relation between these 2 processes, another possible explanation for this relationship is that they may share a common underlying mechanism, such as a common genetic vulnerability. The role of genetic factors on HRV and inflammatory markers is well established from family and twin studies.^{6–8} Heritability is estimated to account for 35–48% of phenotypic variance for HRV indices.⁶ Studies of systemic levels of inflammatory markers in families and twins suggest that a considerable part of the variation in these biomarkers is explained by genetic factors, with heritability estimations of 40–60% for CRP.^{7,8} Our heritability estimates are consistent with these previous findings. Given the substantial genetic influence on both HRV and CRP, it is conceivable that common genes contribute to both these phenotypes. Our findings suggested a significant genetic correlation between HRV (ULF) and CRP. Common genes substantially contributed to the covariation of these two processes in our study: indeed, most of the correlation between ULF and CRP was due to shared genetic factors.

Common genes that may be involved in the regulation of both autonomic function and inflammation include those coding for various molecules in the HPA axis and the SNS. For example, hypothalamic corticotrophin-releasing hormone (CRH) influences sympathetic activation with increased norepinephrine production and decreased HRV.²⁴ CRH is also capable of exerting immunoregulatory effects through the CRH receptor.²⁵ In addition, genetic variants in the catecholaminergic/beta-adrenergic pathway were also reported to influence CRP plasma levels.²⁶ Clarification of the roles of these common genetic variants will improve our understanding of the mechanisms underlying autonomic-immune interactions.

Several prior studies have examined the relations between *CRP* polymorphisms and CRP levels and reported individual associations of rs1130864 and rs1205 with CRP levels.^{27,28} More recently, 9 of 13 *CRP* SNPs were found to be associated with blood CRP levels in the Framingham Heart Study.²⁹ Of these 9, only a triallelic SNP (rs3091244) or a 2-SNP haplotype (rs2808630 and rs1205) remained associated with blood CRP levels after accounting for correlation among SNPs. In our study, we also observed a significant association between rs1205 and CRP levels after adjustment for potential CAD risk factors. However, this SNP only explained 2% of the phenotypic variation of CRP levels, which is consistent with previous findings.²⁹ The function of rs1205 is not clear. This SNP is partially correlated with the triallelic SNP rs3091244, and the latter has been associated with CRP promoter activity and might be functional.³⁰ Thus, the association between rs1205 and CRP levels may reflect the LD of rs1205 with rs3091244.

We found that this same SNP (rs1205) was also significantly associated with ULF, both before and after adjustment for covariates and CRP levels. Further bivariate genetic modeling in MZ and DZ twins found that this SNP explained about 11% of the shared genetic covariation between ULF and CRP levels. These results suggest that these two phenotypes may be involved in the expression of a common pathophysiological mechanism involving autonomic-immune dysregulation. Our data, however, also indicate that the remaining portion (90%) of the shared genetic variance between ULF and CRP is still unexplained. Future studies should evaluate other genetic pathways that are involved in the link between autonomic dysfunction and inflammation.

A limitation of our study is its cross-sectional nature, thus limited in the ability to discern any causal relationships between autonomic dysfunction and inflammation. However, based on our results, the covariation of these two processes is due in large part to a common genetic precursor rather than a cause-effect relationship. Also, because our twins were all middle-aged male military veterans, caution should be used in generalizing our results to women or older individuals. In addition, we only investigated the SNPs within the *CRP* gene and did not genotype polymorphisms in the promoter region, which limited the comparison to previous studies. Finally, a broad evaluation of the relationship between HRV and other inflammatory markers is required in future study.

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References

1. Lanza GA, Sgueglia GA, Cianflone D, Rebuzzi AG, Angeloni G, Sestito A, Infusino F, Crea F, Maseri A. Relation of heart rate variability to serum levels of C-reactive protein in patients with unstable angina pectoris. *Am J Cardiol* 2006;97:1702–1706. [PubMed: 16765117]
2. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Hansen JF. C-reactive protein, heart rate variability and prognosis in community subjects with no apparent heart disease. *J Intern Med* 2006;260:377–387. [PubMed: 16961675]
3. Maestroni GJ. Sympathetic nervous system influence on the innate immune response. *Ann N Y Acad Sci* 2006;1069:195–207. [PubMed: 16855146]
4. Tracey KJ. The inflammatory reflex. *Nature* 2002;420:853–859. [PubMed: 12490958]
5. Juttler E, Tarabin V, Schwaninger M. Interleukin-6 (IL-6): a possible neuromodulator induced by neuronal activity. *Neuroscientist* 2002;8:268–275. [PubMed: 12061506]
6. Kupper NH, Willemsen G, van den Berg M, de Boer D, Posthuma D, Boomsma DI, de Geus EJ. Heritability of ambulatory heart rate variability. *Circulation* 2004;110:2792–2796. [PubMed: 15492317]
7. MacGregor AJ, Gallimore JR, Spector TD, Pepys MB. Genetic effects on baseline values of C-reactive protein and serum amyloid a protein: a comparison of monozygotic and dizygotic twins. *Clin Chem* 2004;50:130–134. [PubMed: 14633907]
8. Su S, Snieder H, Miller AH, Ritchie J, Bremner JD, Goldberg J, Dai J, Jones L, Murrah NV, Zhao J, Vaccarino V. Genetic and environmental influences on systemic markers of inflammation in middle-aged male twins. *Atherosclerosis* 2008;200:213–220. [PubMed: 18243214]
9. Goldberg J, Curran B, Vitek ME, Henderson WG, Boyko EJ. The Vietnam Era Twin Registry. *Twin Res* 2002;5:476–481. [PubMed: 12537880]
10. Scherrer JF, Xian H, Bucholz KK, Eisen SA, Lyons MJ, Goldberg J, Tsuang M, True WR. A twin study of depression symptoms, hypertension, and heart disease in middle-aged men. *Psychosom Med* 2003;65:548–557. [PubMed: 12883104]
11. Bigger JT Jr, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992;85:164–171. [PubMed: 1728446]
12. Lampert R, Ickovics JR, Viscoli CJ, Horwitz RI, Lee FA. Effects of propranolol on recovery of heart rate variability following acute myocardial infarction and relation to outcome in the Beta-Blocker Heart Attack Trial. *Am J Cardiol* 2003;91:137–142. [PubMed: 12521623]
13. Berger RD, Akselrod S, Gordon D, Cohen RJ. An efficient algorithm for spectral analysis of heart rate variability. *IEEE Trans Biomed Eng* 1986;33:900–904. [PubMed: 3759126]
14. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996;93:1043–1065. [PubMed: 8598068]
15. Richardson MT, Ainsworth BE, Wu HC, Jacobs DR Jr, Leon AS. Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity. *Int J Epidemiol* 1995;24:685–693. [PubMed: 8550264]
16. Neale, MC.; Boker, SM.; Xie, G.; Maes, HH. *Mx: Statistical Modeling*. 6. Richmond: Virginia Commonwealth University Medical School; 2003.
17. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265. [PubMed: 15297300]

18. van den Oord EJ, Snieder H. Including measured genotypes in statistical models to study the interplay of multiple factors affecting complex traits. *Behav Genet* 2002;32:1–22. [PubMed: 11958539]
19. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;340:115–126. [PubMed: 9887164]
20. Lahiri MK, Kannankeril PJ, Goldberger JJ. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. *J Am Coll Cardiol* 2008;51:1725–1733. [PubMed: 18452777]
21. Lampert R, Bremner JD, Su S, Miller A, Lee F, Cheema F, Goldberg J, Vaccarino V. Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men. *Am Heart J* 2008;156:759, e751–757. [PubMed: 18926158]
22. Roach D, Wilson W, Ritchie D, Sheldon R. Dissection of long-range heart rate variability: controlled induction of prognostic measures by activity in the laboratory. *J Am Coll Cardiol* 2004;43:2271–2277. [PubMed: 15193692]
23. Tulppo M, Huikuri HV. Origin and significance of heart rate variability. *J Am Coll Cardiol* 2004;43:2278–2280. [PubMed: 15193693]
24. Arlt J, Jahn H, Kellner M, Strohle A, Yassouridis A, Wiedemann K. Modulation of sympathetic activity by corticotropin-releasing hormone and atrial natriuretic peptide. *Neuropeptides* 2003;37:362–368. [PubMed: 14698679]
25. Miller, AH.; Pearce, BD.; MPC. Immune system and central nervous system interactions. In: Sadock, BM.; Sadock, VA., editors. *Kaplan & Sadock's comprehensive textbook of psychiatry*. Philadelphia: Lippincott Williams & Wilkins; 2000.
26. Wessel J, Moratorio G, Rao F, Mahata M, Zhang L, Greene W, Rana BK, Kennedy BP, Khandrika S, Huang P, Lillie EO, Shih PA, Smith DW, Wen G, Hamilton BA, Ziegler MG, Witztum JL, Schork NJ, Schmid-Schonbein GW, O'Connor DT. C-reactive protein, an 'intermediate phenotype' for inflammation: human twin studies reveal heritability, association with blood pressure and the metabolic syndrome, and the influence of common polymorphism at catecholaminergic/beta-adrenergic pathway loci. *J Hypertens* 2007;25:329–343. [PubMed: 17211240]
27. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, Sharma P, Lowe GD, World MJ, Humphries SE, Hingorani AD. Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. *Arterioscler Thromb Vasc Biol* 2003;23:2063–2069. [PubMed: 12842840]
28. Russell AI, Cunninghame Graham DS, Shepherd C, Robertson CA, Whittaker J, Meeks J, Powell RJ, Isenberg DA, Walport MJ, Vyse TJ. Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. *Hum Mol Genet* 2004;13:137–147. [PubMed: 14645206]
29. Kathiresan S, Larson MG, Vasan RS, Guo CY, Gona P, Keaney JF Jr, Wilson PW, Newton-Cheh C, Musone SL, Camargo AL, Drake JA, Levy D, O'Donnell CJ, Hirschhorn JN, Benjamin EJ. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. *Circulation* 2006;113:1415–1423. [PubMed: 16534007]
30. Szalai AJ, Wu J, Lange EM, McCrory MA, Langefeld CD, Williams A, Zakharkin SO, George V, Allison DB, Cooper GS, Xie F, Fan Z, Edberg JC, Kimberly RP. Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. *J Mol Med* 2005;83:440–447. [PubMed: 15778807]

Figure 1.
Flow chart showing the construction of the Twins Heart Study sample.

Figure 2.

The C-reactive protein (CRP) plasma levels **(a)** and the ultra low frequency (ULF) **(b)** according to the genotypes of rs1205. CRP levels were geometric means calculated from means of log-transformed values.

Figure 3.

Bivariate structural equation model for the variances of C-reactive protein (CRP) and ultra-low frequency (ULF) that is explained by the SNP rs1205 of the CRP gene. The variance for each trait is divided into three components: effect of rs1205, residual additive genetic factors (A_{CRP} and A_{ULF}), and unique environmental factors (E_{CRP} and E_{ULF}). Paths, which are the standardized regression coefficients, must be squared to equal the proportion of variance accounted for. The symbol “ $\sqrt{}$ ” indicates the square-root. The phenotypic correlation between CRP and ULF is, in this model, decomposed into that due to additive genes (upper curve) and to unique environmental factors (lower curve). A negative correlation indicates that genetic factors causing an increase in CRP are also associated with lower ULF.

Table 1

Characteristics in the Twins Heart Study subjects

Variable	MZ Twins (N = 144)	DZ Twins (N = 92)
Age (years)	54.2 ± 2.9	54.7 ± 2.9
Current smoker	24 (17%)	13 (14%)
Body mass index (kg/m ²)	28.9 ± 4.5	29.5 ± 4.9
Physical activity (Baecke) score	7.6 ± 1.6	7.4 ± 1.6
Taking beta-blockers	8 (6%)	3 (3%)
Taking statins	31 (22%)	18 (20%)
Taking anti-depressants	19 (13%)	15 (16%)
C-reactive protein (mg/dL)	2.2 ± 2.8	2.4 ± 3.8
Index of heart rate variability		
Ln Ultra Low Frequency (ms ²)	9.2 ± 0.5	9.1 ± 0.6
Ln Very Low Frequency (ms ²)	7.7 ± 0.5	7.6 ± 0.6
Ln Low Frequency (ms ²)	6.8 ± 0.6	6.6 ± 0.8
Ln High Frequency (ms ²)	5.4 ± 0.9	5.3 ± 0.9

Mean ± SD was reported unless stated otherwise.

MZ, monozygotic; DZ, dizygotic; SD, standard deviation; Ln, logarithm transformation

Table 2

Associations between C-reactive protein plasma levels and heart rate variability indices

HRV Indices	Ln CRP			
	Model 1 ^a		Model 2 ^b	
	$\beta^{\dagger}$	<i>P</i> Values	$\beta^{\dagger}$	<i>P</i> Values
Ln ULF	−0.51	< 0.001	−0.47	< 0.001
Ln VLF	−0.60	< 0.001	−0.41	0.001
Ln LF	−0.35	0.001	−0.19	0.059
Ln HF	−0.05	0.60	−0.001	0.99

Ln, logarithm transformation; CRP, C-reactive protein; HRV, heart rate variability; ULF, ultra low frequency; VLF, very low frequency; LF, low frequency; HF, high frequency

^a Adjusting for age

^b Adjusting for age, body mass index, physical activity, current smoking and use of medications (beta-blockers, statin and anti-depressants)

[†] Regression coefficients (β) and *P* values were derived from generalized estimating equation (GEE) models.

Table 3
Heritability estimation of C-reactive protein levels and heart rate variability indices

Variable	Correlations		Heritability (95% CI)	
	MZ	DZ	Model 1 ^a	Model 2 ^b
Ln CRP	0.74	0.43	0.76 (0.63–0.84)	0.70 (0.55–0.80)
Ln ULF	0.60	0.07	0.64 (0.43–0.76)	0.58 (0.37–0.73)
Ln VLF	0.54	0.23	0.64 (0.44–0.76)	0.64 (0.46–0.76)
Ln LF	0.54	0.38	0.63 (0.46–0.76)	0.62 (0.42–0.75)
Ln HF	0.57	0.39	0.54 (0.35–0.68)	0.56 (0.36–0.70)

MZ, monozygotic; DZ, dizygotic; CI, confidence interval; Ln, logarithm transformation; CRP, C-reactive protein; ULF, ultra low frequency; VLF, very low frequency; LF, low frequency; HF, high frequency

^a Adjusting for age

^b Adjusting for age, body mass index, physical activity, current smoking and use of medications (beta-blockers, statin and anti-depressants)

Table 4Associations of individual *CRP* genetic variants with C-reactive protein plasma levels and heart rate variability indices

SNP	Variant Type	Major Allele/Minor Allele	Minor Allele Frequency	P Values Associated with CRP and HRV					
				Ln CRP	Ln ULF	Ln VLF	Ln LF	Ln HF	
rs1205	3' UTR	C/T	0.30	0.003 (0.004)	0.005 (0.003)	0.29 (0.37)	0.37 (0.54)	0.51 (0.62)	
rs1130864	3' UTR	C/T	0.26	0.022 (0.083)	0.54 (0.83)	0.58 (0.86)	0.42 (0.84)	0.46 (0.59)	
rs1800947	Synonymous, coding	G/C	0.06	0.093 (0.076)	0.054 (0.062)	0.089 (0.098)	0.072 (0.099)	0.57 (0.66)	

Ln, logarithm transformation; CRP, C-reactive protein; HRV, heart rate variability; ULF, ultra low frequency; VLF, very low frequency; LF, low frequency; HF, high frequency; UTR, un-translated region
P values < .05 are indicated in bold. P values in parentheses were adjusted for age, body mass index, physical activity, current smoking and use of medications (beta-blockers, statin and anti-depressants).