行政院國家科學委員會專題研究計畫 成果報告

C-反應蛋白基因啟動子區域核苷酸變異和心房顫動的關係-遺傳相關及功能性研究
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2314-B-002-134-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：國立臺灣大學醫學院內科

計 畫 主 持 人：黃瑞仁

計畫參與人員：學士級-專任助理：江怡萱

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 10 月 30 日

C-reactive Protein Gene G1059C Polymorphism and Atrial Fibrillation- Genetic and Functional Studies

Chia-Ti Tsai, MD, PhD,[1*] Juey-Jen Hwang, MD, PhD,[1, 2, 3*] Sheng-Nan Chang, MD,[2] Ling-Ping Lai, MD, PhD,[1, 2, 3] Li-Ying Huang, MS,[1] Chuen-Den Tseng, MD, PhD,[1] Jiunn-Lee Lin, MD, PhD,[1] Fu-Tien Chiang, MD, PhD,[1,4]

[*]Chia-Ti Tsai and Juey-Jen Hwang contributed equally to the manuscript

From the [1]Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [2]Cardiovascular Center, National Taiwan University Hospital Yun-Lin Branch, Douliou City, Taiwan, [3]Institute of Pharmacology, National Taiwan University, Taipei, Taiwan and [4]Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

Running title: CRP gene and AF
Total word count: 5313
No conflict of interest

This work was supported by two grants from the National Science Council, Taiwan, ROC (95-2314-B-002-087-MY3 and NSC-95-2314-B-002-134).

Corresponding author:

Fu-Tien Chiang, MD, PhD
Department of Laboratory Medicine
National Taiwan University Hospital
No. 1, Section 1, Jen-Ai Road, Taipei 100, TAIWAN
Tel: 886-2-23123456 ext. 5002
Fax: 886-2-82317099
E-mail: fang31@ms39.hinet.net

Abstract

Background Inflammation plays an important role in AF pathogenesis. Whether genetic variant in the C-reactive protein (CRP) gene predisposes to AF, and molecular effects of CRP on atrial myocytes and fibroblasts are unknown.

Objective To evaluate association between CRP gene G1059C polymorphism and non-familial atrial fibrillation (AF), and effect of CRP on atrial myocytes and fibroblasts.

Methods We performed genetic association study to investigate association between CRP gene G1059C polymorphism and non-familial AF. We used whole cell patch-clamp and reverse-transcription polymerase chain reaction to investigate the effect of CRP on the transmembranic ionic currents and expression of procollagens in atrial myocytes and fibroblasts, respectively.

Results Two-hundred and forty-three patients with non-familial AF and 348 controls were recruited. AF patients had a higher plasma CRP level (3.87±3.59 vs 2.91±3.30 mg/L, P=0.0008). The 1059C variant was associated with a lower CRP level, and a lower risk of AF (OR 0.54 [0.30-0.97] and 0.55 [0.32-0.94] for dominant and additive models, respectively, after multivariate adjustment). CRP significantly increased the inward L-type calcium channel, without significant change on T-type calcium current and other outward potassium currents in HL-1 atrial myocytes. CRP did not affect the expressions of type I alpha 1 (COL1A1), type III alpha 1 (COL3A1) and type 1 alpha

2 (COL1A2) procollagens in atrial fibroblasts.

Conclusions CRP gene G1059C polymorphism determines the basal level of CRP and the risk of common AF. The mechanism may be through an augmented inward calcium current by CRP in atrial myocytes, but not through atrial fibrosis.

Key words: C-reactive protein; Atrial fibrillation; Genetics; Atrial myocytes; Atrial fibroblasts

Abbreviation List

AF = Atrial fibrillation

COL1A1 = type I alpha 1 procollagen,

COL3A1 = type III alpha 1 procollagen

COL1A2 = type 1 alpha 2 procollagen

CRP = C-reactive protein

ICaL = L-type calcium current

ICaT = T-type calcium current

IKr = rapidly-activating delayed rectifier potassium current

IK1 = Inward rectifier potassium current

Ito = transient outward current

LAD = left atrial dimension

LVEF = left ventricular ejection fraction

LVESD = left ventricular end-systolic dimension

LVEDD = left ventricular end-diastolic dimensions

Reverse transcription–polymerase chain reaction = RT-PCR

VHD = valvular heart disease

Introduction

There is increasing evidence suggesting the role of inflammation and oxidative stress in the pathogenesis of AF **(1-6)**. It has also been speculated that these processes contribute to the atrial remodeling **(1)(6)**. Inflammatory markers, mainly C-reactive protein (CRP), an acute-phase reactant indicating active inflammation, have been used to predict the risk of AF **(4)** and AF recurrences after cardioversion **(7-9)**. It has also been shown that CRP was elevated in AF patients, and the CRP level correlated with the AF burden **(10)**. Furthermore, several pharmacological approaches with non-channel blocking agents with anti-inflammatory properties show favorable effects on AF, which include inhibitors of the renin-angiotensin system, statins, dietary antioxidants, corticosteroids, and others **(4)(6)(11-13)**. It has been shown that corticosteroid treatment significantly reduced the CRP levels and there was a strong correlation between CRP level and the risk of AF recurrence **(14)**.

There have been several reports addressing the genetic control of familial AF **(15-17)**. However, the genetic study for non-familial AF is scarce in the literature **(18-20)**. Based upon the aforementioned studies on the relationship between inflammatory state, CRP and AF, we hypothesized that the CRP gene might be one of the susceptible genes of non-familial AF. There are several single nucleotide polymorphisms in the CRP gene, such as G1059C and C1444T in the exon 2 and

intronic T to A transversion polymorphisms **(21)(22)**. Prior studies have shown that G1059C was associated with baseline CRP level **(21)**. There are also reports investigating the association between CRP G1059C polymorphism with cardiovascular diseases, such as myocardial infarction, stroke, and post-angioplasty restenosis **(21)(23)**.

In the present study, we conducted a case-control study to investigate the association between the CRP gene G1059C polymorphism and non-familial AF. The effect of CRP on atrial myocytes and cardiac fibroblasts were also investigated to elucidate the possible molecular mechanism.

Methods

Study population

The study included 243 consecutive patients at the Cardiovascular Clinic and Adult cardiology ward of the National Taiwan University Hospital with a documented history of AF, either by surface 12-lead ECG or Holter monitoring. Patients with hyperthyroidism were excluded. Patients with familial AF were also excluded. The patient was defined as having familial AF if any of his or her directly-related family members also has AF. The control patients, comparable for age and gender, consisted

of 343 consecutive patients and were recruited from the same clinic and ward. The control patients had no documented history of AF and no symptoms suggesting the attack of AF, such as palpitation, chest discomfort, shortness or breath or dizziness.

Hypertension was defined as the presence of elevated systolic (>140 mm Hg) and/or diastolic (>90 mm Hg) blood pressure and/or the current use of antihypertensive drugs. Non- insulin-dependent diabetes mellitus was defined as history of hypoglycemic treatment and/or a fasting blood glucose of >126 mg/dl.

All of the patients underwent the echocardiographic examination. All standard measurements were obtained from parasternal long- and short-axis views and the apical 4-chamber view according to American Society of Echocardiography guidelines **(24)**. M-mode measurements of left atrial anteroposterior dimension (LAD), left ventricular end-systolic and end-diastolic dimensions (LVESD and LVEDD) and left ventricular ejection fraction (LVEF) were used for the analyses. Left ventricular mass was calculated with echocardiographic parameters and the Devereux formula **(25)**. Doppler and color Doppler studies were performed for detection of valvular heart disease (VHD). Significant VHD was defined as at least moderate aortic or mitral stenosis/regurgitation. The study was approved by the local institutional review board and informed consent was obtained from the study subjects.

Genotyping of CRP gene G1059C polymorphism and measurement of plasma CRP concentration

Blood was withdrawn, centrifuged soon, then DNA extraction was performed with standard phenol–chloroform method and plasma was stored in 80°C freezer before analysis. Genotyping of CRP gene G1059C polymorphism was performed by polymerase chain reaction (PCR) and further digestion by *Mae*III restriction enzyme, as previously described **(26)**. Briefly, PCR was performed by using the following primers: Forward-5′GATCTGTGTGATCTGAGAAACCTCT3′ and Reverse- 5′GAGGTACCAGAGACAGAGACGTG3′. Target DNA was amplified using 94°C for 5 minutes, then 30 cycles of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 30 seconds, followed by extension step with 72°C for 10 minutes. Two percent agarose gel was used for visualization. Quality control for the experiments was evaluated by re-genotyping of 20 random samples blinded to the technician. The plasma CRP concentration was measured by a high sensitivity Immunonephelometry (Nephelometry, Behring Nephelometer II, Dade Behring Marburg GmbH, Germany) with the lowest detection limit of 0.16 mg/L. All laboratory analyses were performed blinded with respect to the diagnosis and patients' characteristics. Subjects with CRP level more than 20 mg/L were excluded from the study due to the possibility of the presence of acute stress reaction.

Transmembraneous ionic current measurement

Transmembrane currents were measured by using a patch-clamp amplifier (8900; Dagan Corporation, Minneapolis, Minn) by applying a whole-cell recording technique as previously described **(27)**. The extracellular solution contained NaCl 137 mM, KCl 5.4 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.8 mM, KH_2PO_4 1.2 mM, dextrose 22 mM, and HEPES buffer 10 mM (pH 7.4). The internal pipette solution contained K-aspartate 120 mM, KCl 20.0 mM, Mg adenosine 5'-triphosphate 5.0 mM, K_2 ethylene glycol tetraacetic acid 11.0, HEPES buffer 10.0 mM, $MgCl_2$ 2.0 mM, $CaCl_2$ 1 mM, Na creatine phosphate 5, mM Na guanosine 5'-triphosphate. 0.2 mM, and adenosine 3',5'-cyclic monophosphate 0.2 (pH 7.2).

The L-type (ICaL) and T-type (ICaT) calcium currents were recorded as previously described **(27).** The contamination of I_{Na} and I_K was abolished by replacing the sodium ion with NMDG (137 mM) and adding Cs^+ (12 mM) in the bath solution. The pipette internal solution also contained TEA (30 mM) and Cs+ (130 mM). Ba^{2+} (20 mM) was employed as the charge carrier in the bath solution. Total calcium currents, including ICaL and ICaT currents, were obtained by using a series of depolarization steps to +60 mV from the holding potential –80 mV. ICaL was obtained by a family of depolarization steps to +70 mV from the holding potential –50

mV. ICaT was obtained after subtracting the ICaL total calcium current. HL-1 myocytes exhibit either dominant L (calcium currents obtained from holding potential -80 mV were identical to those obtained from holding potential -50 mV) or dominant T type (no current obtained from holding potential -50 mV) calcium currents, which were sensitive to nifedipine 3 μmol/L and nickel 1 μmol/L, respectively **(27)**.

The rapidly-activating delayed rectifier potassium current, or IKr, which is sensitive to specific blockers such as E4031 or dofetilide, is a prominent component of the outward rectifier potassium currents of HL-1 cells **(28)(29)**. We also measured the effect of CRP on IKr. IKr was obtained by applying a series of depolarization steps (500 ms) from -80 mV and was measured as the tail currents at the repolarization step to -40 mV, which was sensitive to E4031 (1 μmol/L). We also measured the effect of CRP on other outward currents. The transient outward current (Ito) was measured as the 4-aminopyridine-sensitive peak current (10 mM). The inward rectifier potassium current (IK1) was obtained by using a series of hyperpolarization steps (200 ms) to -140 mV after a pre-pulse to -20 mV (200 ms) from a holding potential –80 mV.

HL-1 myocytes culture

The HL-1 atrial cell line was derived from adult mouse atria, which were obtained from Louisiana State University in New Orleans, LA, USA. The culture and maintenance of HL-1 atrial myocytes were as previously described **(27)(28)**. HL-1 myocytes were serum starved for 24 hours on the 3rd day after subculture and then used for experiments (CRP stimulation).

Isolation and Culture of Neonatal Rat Atrial Fibroblasts

Both atria from week-old neonatal Wistar rats were cut into chunks and were subjected to trypsin (0.125%) digestion in a balanced salt solution. The disaggregated cells were collected by centrifugation at 300 g for 10 min. The cell pellet was re-suspended in serum-containing medium (Ham's F-12:DMEM;1:1 with 20% fetal bovine serum and 1% penicillin-streptomycin), plated onto a Petri dish and kept for 2.5h in a 5% CO_2 atmosphere at 37°C to allow fibroblasts to attach to the bottom of the dish. The enriched cells were subsequently incubated with DMEM supplemented with 10% fetal calf serum and 1% penicillin-streptomycin for an additional 3 days. After 3 days, the cells were transferred to a serum-free medium (Ham's F-12:DMEM;1:1 for myocytes and DMEM for nonmyocytes) overnight and then used for the subsequent experiments (CRP stimulation).

Extraction of RNA and Reverse Transcription–Polymerase Chain Reaction

Accumulation of extracellular matrix is an important structural change in AF **(30-32)**. Therefore, we used the expression of type I alpha 1 (COL1A1), type III alpha 1 (COL3A1) and type 1 alpha 2 (COL1A2) procollagens to evaluate the change of collagen synthesis by atrial fibroblasts after CRP stimulation.

The extraction and quantification of mRNA by means of reverse transcription–polymerase chain reaction (RT-PCR) were performed as reported **(27)(33)**. In brief, 5 μg total RNA was isolated and reverse transcribed. Single-stranded cDNA was amplified with PCR. PCR with each specific primer pair yielded only one DNA band of predicted size. The PCR products were confirmed by means of direct sequencing. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as the internal control for equal loading. The reaction products were analyzed with agarose gel electrophoresis. Optic densitometry was performed after the gel was stained with ethidium bromide to measures its DNA amount. Expression of mRNA was represented by its ratio to the mRNA for GAPDH. The primer sequences were as follows:

Rat COL1A1 (250 bp):

Forward: 5’TCTCCACTCTTCTAGTTCCT3’

Reverse: 5’TTGGGTCATTTCCACATGC3’

Rat COL3A1 (557 bp):

Forward: 5’AGCTGGTCAGCCTGGAGATA3’

Reverse: 5’GACCTCGTGCTCCAGTTAGC3’

Rat COL1A2 (352 bp):

Forward: 5’CACCTGGTCCTGTTGGAAGT3’

Reverse: 5’ATACCTGGCAGACCACGTTC3’

Rat GAPDH (408 bp):

Forward: 5’TTGCCATCAACGACCCCTTC3’

Reverse: TTGTCATGGATGACCTTGGC

Statistic method

For comparisons of the baseline characteristics, the between-group data were compared with Student’s unpaired *t* test for continuous data and with the $\chi 2$ or Fisher’s exact test for categorical data (degree of freedom [df] =1). Hardy-Weinberg equilibrium of the genotype distribution of polymorphisms was tested using a $\chi 2$ test (df=2).

We compared the allele and genotype frequencies between the cases and controls with the $\chi 2$ test or Fisher’s exact test (df=1 for the two by two allele frequencies $\chi 2$ test, and 2 for the three by two genotype frequencies $\chi 2$ test). The

genotype-phenotype correlation was examined with additive, dominant, and recessive models with logistic regression. The dominant genetic model compares individuals with one or two C alleles (GC+CC) with the baseline group with no C allele (GG genotype). The recessive genetic model compares the CC genotype with the combined GG+GC genotypes, which form the baseline group. The additive genetic model assumes that there is a linear gradient in risk between the GG, GC, and CC genotypes (GG genotype as the baseline). Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. Because LAD, LVEF and the percentage of patients with significant VHD were not balanced in the cases and controls **(Table 1)**, we also performed the analyses with adjustment for LAD, LVEF and the percentage of patients with significant VHD. The STATA software was used for analyses and $P<0.05$ was considered statistically significant.

Results

Basline characteristics

The baseline characteristics of the entire study population according to the presence (cases) or absence (controls) of AF are shown in **Table 1**. The AF patients had a lower mean LVEF, higher mean LAD, and a greater incidence of significant VHD. The AF patients also had a higher mean plasma CRP level than the controls.

G1059C polymorphism and AF

CRP gene G1059C polymorphism significantly affected the basal CRP level. Subjects with GC and CC genotypes had significantly lower basal plasma CRP concentration than those with GG genotype (GG: 3.02 ± 3.33; GC: 2.50 ± 3.21;CC: 1.85 ± 1.76 mg/L, all p <0.01 compared to the those with GG genotype).

No deviation from the expected population genotype proportions predicted by Hardy–Weinberg equilibrium was detected **(Table 2)**. A significant difference in genotype distribution and allele frequencies between the AF patients and controls were found for G1059C polymorphisms **(Table 2)**. The allele frequency of 1059C variant was significantly lower in the AF patients. The genotype frequencies of GC and CC were also significantly lower in the AF patients.

In the univariate analysis, C allele of the CRP gene G1059C polymorphism was significantly associated with a protecting effect on AF under dominant and additive models **(Table 3)**. Because the mean LAD, LVEF and the percentage of patients with significant VHD were significantly different in the AF patients and controls, we also performed the multivariate analysis to adjust for the possible confounding effects from LAD, LVEF and the presence of significant VHD. In the multivariate analysis, the association of C allele with a protecting effect on AF remained significant after

adjustment for LAD, LVEF and the presence of significant VHD **(Table 3)**. In other words, G allele of the CRP gene G1059C polymorphism was associated with a higher basal CRP level, and a higher risk of AF.

Effect of CRP on the transmembranic ionic currents of HL-1 atrial myocytes

The Effect of CRP on the transmembranic ionic currents of HL-1 atrial myocytes was evaluated by using a tight-sealed whole-cell recording technique. The CRP was applied and the currents were recorded after a steady-state of current amplitude (around 3 min after membrane rupture). The cells could sustain a tight-sealed condition without leak for around 20 min. Cells with leak were not used for study.

CRP significantly increased ICaL current density **(Figure 1A and 1B)**, without a significant change in ICaT (data not shown). HL-1 myocytes also had typical IKr currents **(27)**. CRP (1 mg/L) did not affect IKr current density (data now shown)**.** We also observed no significant changes in IK1 and Ito after CRP stimulation (data not shown).

Effect of CRP on cardiac fibroblasts

Accumulation of extracellular matrix is an important structural change in AF **(30-32)**. We used the expressions of COL1A1, COL3A1 and COL1A2 to evaluate the

amount of collagen synthesis by atrial fibroblasts. We found that CRP did not increase or decrease COL1A1, COL1A3 and COL1A2 expressions in atrial fibroblasts with either different concentrations or different stimulation times **(Figure 2)**.

Discussion

Main findings

To the best of our knowledge, this is the first report to demonstrate the association between the CRP gene variant with non-familial AF. The 1059G allele was associated with a higher basal CRP level, and predisposition to AF. The mechanism of the association may be through an augmented inward calcium current induced by CRP in atrial myocytes, which may play an important role in the genesis of calcium overload and initiation of AF.

Mechanisms of the Association

Our results are similar to those of other studies that CRP gene G1059C polymorphism affects the basal CRP level, with the C allele associated with a lower plasma CRP level **(21)**. So far the mechanism of this association has never been established. The CRP G1059C polymorphism locates in exon 2 of the CRP gene, which is silent at the amino acid level (CTG-> CTC, Leu-> Leu). The most possible

mechanism to explain the association between G1059C polymorphism and plasma CRP level is that it may be in tight linkage disequilibrium with polymorphisms in the promoter region of the CRP gene **(34)**.

In the present study, we also demonstrated that CRP increases the ICaL current in atrial myocytes, which has never been reported in the literature. The effect could be observed with a physiological range of CRP concentration (0.5-1 mg/L). The CRP-induced increase in inward calcium current may increase calcium influx during rapid depolarization and thus exacerbate the condition of calcium overload in AF. However, the change of ICaL current induced by CRP (up-regulation) is the reverse of those observed in animal models of AF **(35)** or human AF **(36)**(down-regulation).

The possible explanation is that increased ICaL may induce calcium overload and thus initiation of AF. Using a computer simulation model, it has been demonstrated that increased ICaL plays a critical role in induction of dynamic spatial dispersions of repolarization, which caused conduction block, reentry, and thus initiation of AF **(37)**. In humans, it has also been found that increased ICaL is a strong predictor of post-operative AF **(38).** Therefore, it is possible that CRP increases the risk of AF through augmenting ICaL. This hypothesis is in accordance with the finding that baseline CRP predicted risk for developing future AF in the general population **(39)**. The down-regulation of ICaL in animal models of AF **(35)** or human

AF **(36)** may be an adaptive process **(38)** to prevent further damage from calcium overload after a longer term of AF-induced calcium overload, and is important for the mechanism of shortening of atrial refractory period and maintenance of AF.

In addition to the electrophysiological mechanisms, atrial fibrosis also plays a very important role in the promotion of AF **(30-32)**. However, in the present study, we found that CRP did not affect the expression of procollagens in atrial fibroblasts. Therefore, it is relatively unlikely that CRP promotes AF through atrial fibrosis.

Limitations

First, we only studied a single polymorphism in the CRP gene. However, this polymorphism has been reported to affect the basal plasma CRP level in many studies **(21) (40)(41)**, and thus has functional significance. Second, we only investigated the effect of CRP in the cellular level, and did not provide a mechanic link between CRP and AF by using an animal model. There are several gene over-expression models of AF **(42)(43)**. However, the animal model with cardiac-specific CRP gene over-expression has been lacking so far. Finally, we also did not provide the mechanism by which CRP augments ICaL. The CRP receptor and the detailed signaling pathway of CRP in cardiomyocytes has been unknown so far, and have never been reported in the literature.

In conclusion, the evaluation of CRP gene G1059C polymorphism has demonstrated a possible role for CRP gene as a predisposing factor to AF. Future studies to substantiate our results in other ethnic population are warranted. Furthermore, from a pharmacogenetic point of view, whether this polymorphism could also predict the efficiency of non-channel blocking drugs with anti-inflammatory properties, such as statin, to treat AF also warrants further studies.

References

1. Korantzopoulos P, Kolettis T, Siogas K, Goudevenos J. Atrial fibrillation and electrical remodeling: the potential role of inflammation and oxidative stress. *Med Sci Monit* 2003;9:RA225–229.
2. Liu T, Li G. Is atrial fibrillation an inflammatory disease? *Med Hypotheses* 2005;64:1237– 1238.
3. Engelmann MDM, Svendsen JH. Inflammation in the genesis and perpetuation of atrial fibrillation. *Eur Heart J* 2005;26:2083–2092.
4. Boos CJ, Anderson RA, Lip GY. Is atrial fibrillation an inflammatory disorder? *Eur Heart J* 2006;27:136–149.
5. Madrid AH. C-reactive protein and atrial fibrillation. An old marker looking for a new target. *Rev Esp Cardiol* 2006;59:94–98.
6. Korantzopoulos P, Kolettis TM, Galaris D, Goudevenos JA. The role of oxidative stress in the pathogenesis and perpetuation of atrial fibrillation. *Int J Cardiol* 2007;115:135– 143.
7. Wazni O, Martin DO, Marrouche NF, Shaaraoui M, Chung MK, Almahameed S, Schweikert RA, Saliba WI, Natale A. C reactive protein concentration and recurrence of atrial fibrillation after electrical cardioversion. *Heart* 2005;91:1303–1305.

8. Malouf JF, Kanagala R, Al Atawi FO, Rosales AG, Davison DE, Murali NS, Tsang TS, Chandrasekaran K, Ammash NM, Friedman PA, Somers VK. High sensitivity C-reactive protein: a novel predictor for recurrence of atrial fibrillation after successful cardioversion. *J Am Coll Cardiol* 2005;46:1284 –1287.
9. Watanabe E, Arakawa T, Uchiyama T, Kodama I, Hishida H. High-sensitivity C-reactive protein is predictive of successful cardioversion for atrial fibrillation and maintenance of sinus rhythm after conversion. *Int J Cardiol* 2006;108:346 –353.
10. Chung MK, Martin DO, Sprecher D, Wazni O, Kanderian A, Carnes CA, Bauer JA, Tchou PJ, Niebauer MJ, Natale A, Van Wagoner DR. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. *Circulation* 2001;104:2886-2891.
11. Korantzopoulos P, Kolettis TM, Kountouris E, Dimitroula V, Karanikis P, Pappa E, Siogas K, Goudevenos JA. Oral vitamin C administration reduces early recurrence rates after electrical cardioversion of persistent atrial fibrillation and attenuates associated inflammation. *Int J Cardiol* 2005;102:321– 326.
12. Liu T, Li GP, Huang TG. Anti-inflammatory therapies in atrial fibrillation. *Int J Cardiol* 2005;104:359–360.
13. Dorian P. The future of atrial fibrillation therapy. *J Cardiovasc Electrophysiol*

2006;17:S11– S16.

14. Dernellis J, Panaretou M. Relationship between C-reactive protein concentrations during glucocorticoid therapy and recurrent atrial fibrillation. *Eur Heart J* 2004;25:1100 –1107.

15. Brugada R, Tapscott T, Czernuszewicz GZ, Marian AJ, Iglesias A, Mont L, Brugada J, Girona J, Domingo A, Bachinski LL, Roberts R. Identification of a genetic locus for familial atrial fibrillation. *N Engl J Med* 1997;336:905–911.

16. Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, Jin HW, Sun H, Su XY, Zhuang QN, Yang YQ, Li YB, Liu Y, Xu HJ, Li XF, Ma N, Mou CP, Chen Z, Barhanin J, Huang W. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* 2003;299:251–254.

17. Yang Y, Xia M, Jin Q, Bendahhou S, Shi J, Chen Y, Liang B, Lin J, Liu Y, Liu B, Zhou Q, Zhang D, Wang R, Ma N, Su X, Niu K, Pei Y, Xu W, Chen Z, Wan H, Cui J, Barhanin J, Chen Y. Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. *Am J Hum Genet* 2004;75:899–905.

18. Lai LP, Su MJ, Yeh HM, Lin JL, Chiang FT, Hwang JJ, Hsu KL, Tseng CD, Lien WP, Tseng YZ, Huang SK. Association of the human MinK gene 38G allele with atrial fibrillation: Evidence of possible genetic control on the pathogenesis of atrial fibrillation. *Am Heart J* 2002;144:485–490.

19. Tsai CT, Lai LP, Lin JL, Chiang FT, Hwang JJ, Ritchie MD, Moore JH, Hsu KL, Tseng CD, Liau CS, Tseng YZ. Renin-angiotensin system gene polymorphisms and atrial fibrillation. *Circulation* 2004;109:1640-1646.

20. Juang JM, Chern YR, Tsai CT, Chiang FT, Lin JL, Hwang JJ, Hsu KL, Tseng CD, Tseng YZ, Lai LP. The association of human connexin 40 genetic polymorphisms with atrial fibrillation. *Int J Cardiol* 2007;116:107-112.

21. Zee RY, Ridker PM. Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. *Atherosclerosis* 2002;162:217–219.

22. 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.

23. Zee RY, Hegener HH, Fernandez-Cruz A, Lindpaintner K. C-reactive protein gene polymorphisms and the incidence of postangioplasty restenosis. *Atherosclerosis* 2004;176:393–396.

24. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantification in M-mode echocardiography: results of a survey of

echocardiographic measurements. *Circulation* 1978;58:1072–1083.

25. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; 57: 450 – 458.

26. Dai DF, Chiang FT, Lin JL, Huang LY, Chen CL, Chang CJ, Lai LP, Hsu KL, Tseng CD, Tseng YZ, Hwang JJ. Human C-reactive protein (CRP) gene 1059G>C polymorphism is associated with plasma CRP concentration in patients receiving coronary angiography. *J Formos Med Assoc* 2007;106:347-354.

27. Tsai CT, Wang DL, Chen WP, Hwang JJ, Hsieh CS, Hsu KL, Tseng CD, Lai LP, Tseng YZ, Chiang FT, Lin JL. Angiotensin II increases expression of alpha1C subunit of L-type calcium channel through a reactive oxygen species and cAMP response element-binding protein-dependent pathway in HL-1 myocytes. *Circ Res* 2007;100:1476-1485.

28. Claycomb WC, Lanson NA, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, Izzo NJ. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proc Natl Acad Sci USA* 1998;95:2979-2984.

29. Yang T, Wathen MS, Felipe A, Tamkun MM, Snyders DJ, Roden DM. K+ currents and K+ channel mRNA in cultured atrial cardiac myocytes (AT-1 cells). *Circ Res*

1994;75:870-878.

30. Li D, Shinagawa K, Pang L, Leung TK, Cardin S, Wang Z, Nattel S. Effects of angiotensin-converting enzyme inhibition on the development of the atrial fibrillation substrate in dogs with ventricular tachypacing-induced congestive heart failure. *Circulation* 2001;104:2608–2614.

31. Kumagai K, Nakashima H, Urata H, Gondo N, Arakawa K, Saku K. Effects of angiotensin II type 1 receptor antagonist on electrical and structural remodeling in atrial fibrillation. *J Am Coll Cardiol* 2003;41:2197-2204.

32. Chiu YT, Wu TJ, Wei HJ, Cheng CC, Lin NN, Chen YT, Ting CT. Increased extracellular collagen matrix in myocardial sleeves of pulmonary veins: an additional mechanism facilitating repetitive rapid activities in chronic pacing-induced sustained atrial fibrillation. *J Cardiovasc Electrophysiol* 2005;16:753-759.

33. Lai LP, Su MJ, Lin JL, Lin FY, Tsai CH, Chen YS, Huang SK, Tseng YZ, Lien WP. Down-regulation of L-type calcium channel and sarcoplasmic reticular Ca(2+)-ATPase mRNA in human atrial fibrillation without significant change in the mRNA of ryanodine receptor, calsequestrin and phospholamban: an insight into the mechanism of atrial electrical remodeling. *J Am Coll Cardiol* 1999;33:1231-1237.

34. Inoue I, Nakajima T, Williams CS, Quackenbush J, Puryear R, Powers M, Cheng T, Ludwig EH, Sharma AM, Hata A, Jeunemaitre X, Lalouel JM. A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. *J Clin Invest* 1997; 99: 1786 – 1797.

35. Yue L, Feng J, Gaspo R. Ionic Remodeling Underlying Action Potential Changes in a Canine Model of Atrial Fibrillation. *Circ Res* 1997;81:512-525.

36. Bosch RF, Zeng X, Grammer JB, Popovic K, Mewis C, Kuhlkamp V. Ionic mechanisms of electrical remodeling in human atrial fibrillation. *Cardiovasc Res* 1999; 44: 121 – 131.

37. Gong Y, Xie F, Stein KM, Garfinkel A, Culianu CA, Lerman BB, Christini DJ. Mechanism underlying initiation of paroxysmal atrial flutter/atrial fibrillation by ectopic foci: a simulation study. *Circulation* 2007;115:2094-2102.

38. Van Wagoner DR, Pond AL, Lamorgese M, Rossie SS, McCarthy PM, Nerbonne JM. Atrial L-type Ca2+ currents and human atrial fibrillation. *Circ Res* 1999;85:473-476.

39. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, Tracy RP, Van Wagoner DR, Psaty BM, Lauer MS, Chung MK. Inflammation as a risk factor for atrial fibrillation. *Circulation*

2003;108:3006-3010.

40. Thalmaier D, Dambacher J, Seiderer J, Konrad A, Schachinger V, Pfennig S, Otte JM, Crispin A, Goke B, Ochsenkuhn T, Lohse P, Brand S. The +1059G/C polymorphism in the C-reactive protein (CRP) gene is associated with involvement of the terminal ileum and decreased serum CRP levels in patients with Crohn's disease. *Aliment Pharmacol Ther* 2006;24:1105-1115.

41. Eklund C, Lehtimaki T, Hurme M. Epistatic effect of C-reactive protein (CRP) single nucleotide polymorphism (SNP) +1059 and interleukin-1B SNP +3954 on CRP concentration in healthy male blood donors. *Int J Immunogenet* 2005;32:229-232.

42. Verheule S, Sato T, Everett T, Engle SK, Otten D, Rubart-von der Lohe M, Nakajima HO, Nakajima H, Field LJ, Olgin JE. Increased vulnerability to atrial fibrillation in transgenic mice with selective atrial fibrosis caused by overexpression of TGF-beta1. *Circ Res* 2004;94:1458-1465.

43. Xiao HD, Fuchs S, Campbell DJ, Lewis W, Dudley SC Jr, Kasi VS, Hoit BD, Keshelava G, Zhao H, Capecchi MR, Bernstein KE. Mice with cardiac-restricted angiotensin-converting enzyme (ACE) have atrial enlargement, cardiac arrhythmia, and sudden death. *Am J Pathol* 2004;165:1019-1032.

Acknowledgement

This work was supported by two grants from the National Science Council, Taiwan, ROC (95-2314-B-002-087-MY3 and NSC-95-2314-B-002-134).

Figure Legends

Figure 1. C-reactive protein (CRP) increases L-type calcium current (ICaL) in HL-1 atrial myocytes. **A,** Representative recordings of whole-cell ICaL currents are shown for the control cells and CRP-treated (1 mg/L, 10 min). ICaL was obtained by a family of depolarization steps to +70 mV from the holding potential –50 mV. Inset, Voltage protocol. **B,** Representative density-voltage relationships of ICaL in control cells and CRP-treated cells (all n=6).

Figure 2. No effect of C-reactive protein (CRP) on expressions of procollagens in atrial fibroblasts. **A,** Total RNA was isolated from atrial fibroblasts left untreated (controls) or treated for indicated times with CRP (1 mg/L). Reverse Transcription–Polymerase Chain Reaction (RT-PCR) with specific primers for type I alpha 1 (COL1A1), type III alpha 1 (COL3A1) and type 1 alpha 2 (COL1A2) procollagens were performed, and the PCR products were visualized by electrophoresis and ethidium bromide staining. **B,** Total RNA was isolated from atrial fibroblasts left untreated (controls) or treated with indicated concentrations (1 denotes 1 mg/L) of CRP for 24h. RT-PCR for COL1A1, COL1A3 and COL1A2 were performed and the PCR products were visualized by electrophoresis and ethidium bromide staining. GAPDH was used for internal control to show equal loading of the cDNAs. The results represent 3 independent experiments.

Figure 1.

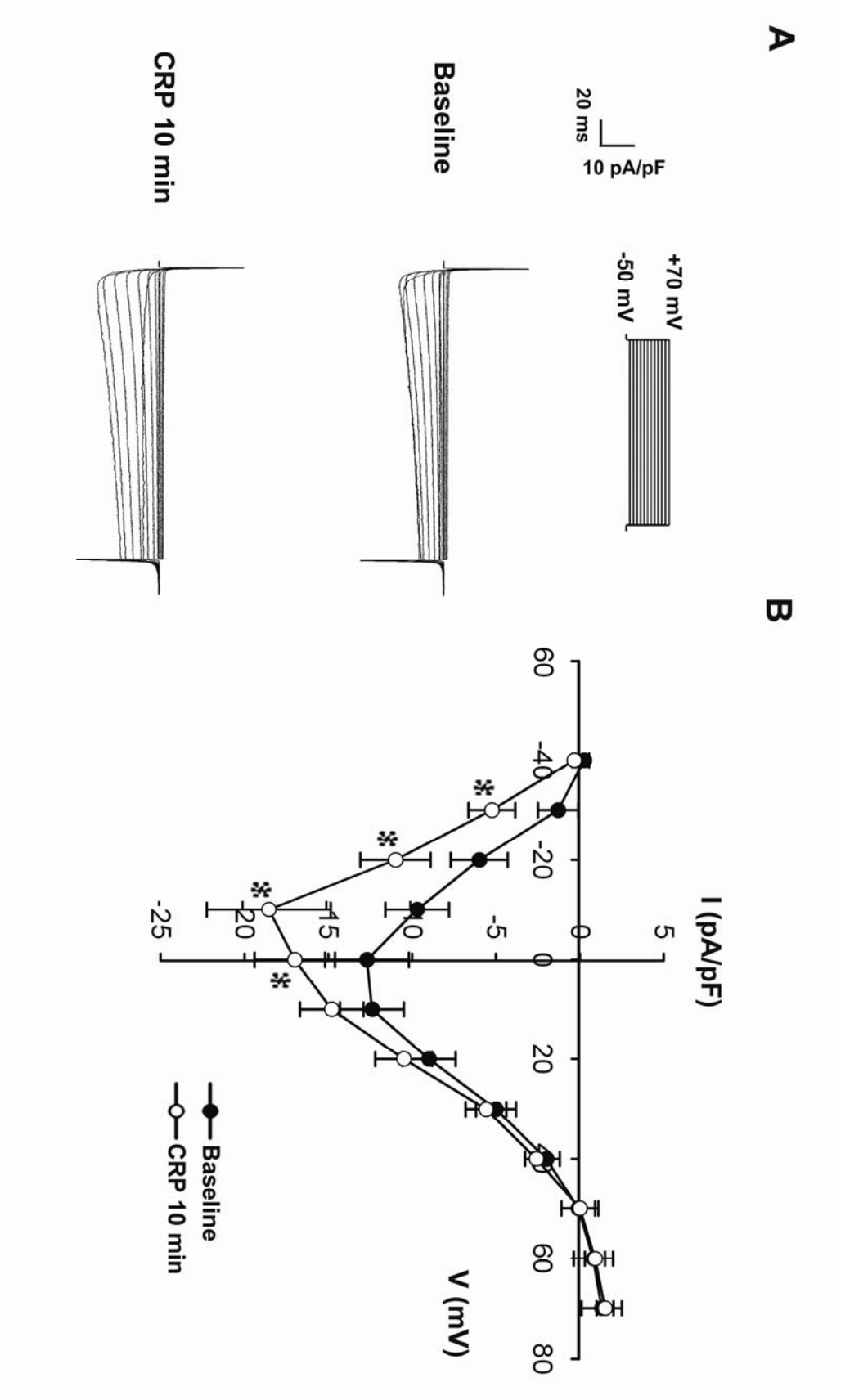
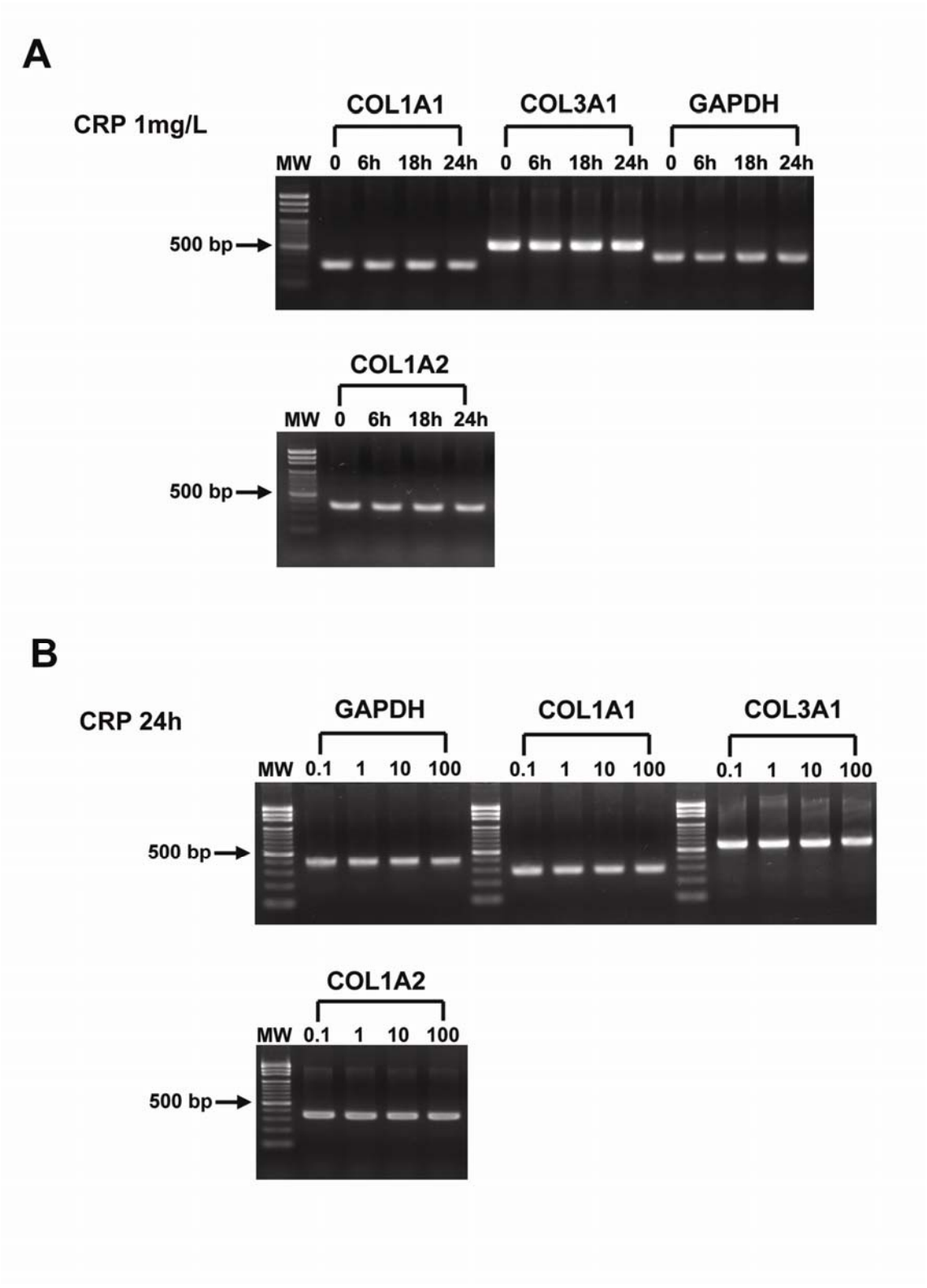

A
20 ms
10 pA/pF
+70 mV
-50 mV
Baseline
CRP 10 min
B
I (pA/pF)
V (mV)
Baseline
CRP 10 min

Figure 2.


A
CRP 1mg/L
COL1A1
COL3A1
GAPDH
MW 0 6h 18h 24h 0 6h 18h 24h 0 6h 18h 24h
500 bp
COL1A2
MW 0 6h 18h 24h
500 bp
B
CRP 24h
GAPDH
COL1A1
COL3A1
MW 0.1 1 10 100 0.1 1 10 100 0.1 1 10 100
500 bp
COL1A2
MW 0.1 1 10 100
500 bp