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Effects of the Interaction between Interleukin-6 -634C/G Polymorphism and Smoking on Serum C-Reactive Protein Concentrations
(インターロイキン6-634C/G多型と喫煙との相互作用が、血清中C反応性タンパク質濃度に及ぼす作用)

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Effects of the interaction between interleukin-6 -634C/G polymorphism and smoking on serum C-reactive protein concentrations

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Running head: IL-6 promoter gene polymorphism, smoking, and CRP

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ABSTRACT

Smoking and interleukin-6 (IL-6) are major factors in inflammation. The aim of this study was to investigate whether or not the IL6 -634C/G polymorphism (rs1800796) and its interaction with smoking influence serum C-reactive protein (CRP) concentrations. The subjects were 347 Japanese male employees of a transit company. CRP and conventional cardiovascular risk factors were evaluated. IL6 -634C/G polymorphisms were genotyped by allelic discrimination using fluorogenic probes and the 5´nuclease assay. The mean values of CRP were significantly higher in current smokers than in nonsmokers after adjustment for age, BMI, systolic blood pressure, total cholesterol, log triglycerides, HDL cholesterol, fasting glucose, and drinking habit (p=0.011). Comparison of three genotypes revealed significant interaction between smoking and the IL6 -634C/G genotype manifested by CRP concentrations (p=0.007) after the adjustments cited above. After stratification by smoking status, CRP differed significantly among IL6 -634C/G genotypes groups in nonsmokers (p=0.010, p for trend=0.007), whereas no significant difference was found in current smokers. Comparison between -634CC and C/G + G/G groups revealed also

a significant interaction between smoking and the IL6 -634C/G genotype ($p = 0.007$). These findings suggest that the impact of the -634G allele on CRP elevation is greater in nonsmokers than in current smokers. Since gene-environment interactions have been insufficiently examined, further studies are required to clarify their effect on inflammation, including CRP elevation.

Key Words: C-reactive protein, smoking, interleukin-6, gene polymorphism, interaction

INTRODUCTION

Atherosclerosis is now generally accepted as an inflammatory disorder in the arterial wall (1), and the C-reactive protein (CRP) level is a strong predictor of cardiovascular events (2-5). The influences of lifestyle and genetic factors on CRP, and their interactions, are therefore important.

The synthesis of CRP in the liver is mainly under the control of interleukin-6 (IL-6) (6). Smoking is known to increase IL-6 (7,8). Many trials, including ECAT (9) and the MONICA study (3) have documented increased CRP concentrations in smokers.

There have been several reports on the relationships between IL6 gene polymorphisms and CRP. One study has reported that the presence of the C allele of the IL6 -174G/C polymorphism was significantly associated with higher CRP concentrations in 98 hypertensive probands and their families with or without hypertension (n=588) (10). Another study has failed to show a significant association of IL6 -174C/G polymorphism with CRP concentration in 160 coronary artery disease patients (11). On the other hand, a significant association of the -174G allele with

increased levels of CRP in 290 type 2 diabetes patients (12) has been reported. These inconclusive results may suggest that the -174C/G polymorphism could influence underlying diseases and conditions. Furthermore, in young and healthy subjects carrying the -174C allele, those who smoked had higher leukocytes, lymphocytes, and monocytes than those who did not smoke (13). Thus, gene-environment interactions in the pathogenesis of inflammation should also be elucidated.

The C allele of the IL6 -174G/C polymorphism is common among Caucasians but extremely rare among East Asians (14-17). However, the G allele of the IL6 -634C/G polymorphism is common among East Asians, and this genotype is significantly related to recurrent pregnancy loss (14) and to the loss of bone mineral density (18). The -634G allele was significantly more common in patients with macroalbuminuria than in those with normoalbuminuria among type 2 diabetic patients, and a study reported that the -634G allele was associated with elevated production and secretion of IL-6 by peripheral blood mononuclear cells in vitro (19).

Thus, the IL6 -634C/G polymorphism may affect inflammation and, both in itself and

through its interaction with smoking, may affect CRP concentrations. The aim of this study was to investigate whether or not the IL6 -634C/G polymorphism and its interaction with smoking influence serum CRP concentrations in healthy Japanese male workers.

METHODS

Subjects

The subjects were transit company employees (1255 men and 94 women aged 35 to 60 years) who had an annual health checkup between April 2003 and March 2004. We used a self-administered questionnaire including items on clinical history, family history, smoking, and alcohol consumption. The questionnaire was distributed to the subjects prior to the checkup and was collected at the checkup. Answers to the questionnaire and written informed consent to view health checkup data were obtained from 413 men and 5 women (response rate: men 32.9%, women 5.3%). A total of 71 subjects were excluded for the following reasons: women (n=5; because of sample size), history of hypertension (n=34), history of dyslipidemia (n=17), history of diabetes (n=18), history of coronary disease or stroke (n=9), or blood samples not analyzed (n=8). Finally, we analyzed 347 male employees who had no history of cancer and no no past or present systemic inflammation such as active chronic arthritis.

This study was conducted with written informed consent from all subjects and approved by

the institutional ethics board for epidemiological studies of the Hokkaido University Graduate School of Medicine.

Data collection

Subjects were classified as either current smokers or nonsmokers, with the latter group including both never- and ex-smokers. Drinkers were defined as those who consumed alcohol once a week or more.

Anthropometric measures (height, body weight, and waist and hip circumferences) were recorded by a standardized protocol. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Resting blood pressure was measured in the sitting position using an automated blood pressure monitor (BP-103iII, Omron Colin, Tokyo, Japan) following at least 5 minutes of rest.

Blood samples were drawn from the antecubital vein of the seated subject with minimal tourniquet use after a 12-hour fast. Total cholesterol (TC), triglyceride (TG), uric acid (UA), and glucose levels were measured by enzymatic methods. The high-density lipoprotein cholesterol

(HDL-C) level was measured by a direct method. CRP was measured by nephelometry, with a latex particle-enhanced immunoassay (N Latex CRP II; Dade Behring, Tokyo, Japan). The assay could detect 0.004 mg/dL of CRP. Undetectable CRP values were recorded as 0.002 mg/dL.

Genomic DNA was extracted from peripheral blood lymphocytes using the EZ1 DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. IL-6 -634C/G gene polymorphisms were determined by the TaqMan polymerase chain reaction (PCR) method using a Minor Groove Binder (MGB) probe as described previously (20). To detect a polymorphism of IL-6 -634C/G (rs1800796), two MGB probes were prepared: a C allele–specific probe, 5'-FAM CAA CAG CCC CTC ACA G-MGB-3', and a G allele–specific probe, 5'-CAA CAG CCG CTC ACA G-MGB-3'. Each of the reporters was quenched by MGB, which was typically located at the 3' end. The primers for PCR of the promoter region including the -634C/G polymorphism of IL-6 were as follows: forward, 5'- GGA TGG CCA GGC AGT TCT A -3'; reverse, 5'- CCA GTC ATC TGA GTT CTT CTG TGT T-3'. The reaction mixture contained approximately 40 ng of template DNA, 5.0 μl of TaqMan Universal PCR master mixture, and 0.3

μl of 40× assay mixture in a volume of 10 μl. Real-time PCR was performed on a 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) using a procedure consisting of incubation at 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. FAM and VIC fluorescence levels of the PCR products were measured at 60°C for 1 min, resulting in the clear identification of the three genotypes of the IL-6 promoter region on a two-dimensional graph.

Statistical Analysis

The subjects were categorized according to smoking status (current smoker or nonsmoker) and IL-6 -634C/G genotype (C/C, C/G, or G/G). The data were presented as means±SD or as median values (and interquartile ranges) for variables with skewed distributions or percentages, and the data were compared among groups using Student's unpaired t-test, analysis of variance (ANOVA), the Wilcoxon rank-sum test, the Kruskal-Wallis test, or the chi-square test.

Because of its skewed distribution, the mean log-transferred CRP was compared between

current smokers and nonsmokers using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL-C, fasting glucose, and drinking habit. Next, a GLM was employed to evaluate the significant contributions of smoking status and genotype interaction to CRP levels, after adjustment for the possible confounders named above. The back-transformed means and standard errors (SE) of log-transferred CRP in each group are presented in the results and the figures.

A p value of <0.05 was considered statistically significant. All analyses were conducted using the SPSS software package version 14.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics of the groups according to smoking status are shown in Table 1. TG and CRP were significantly higher in current smokers than in nonsmokers. Age, systolic and diastolic blood pressure, HDL-C, and fasting glucose were significantly lower in current smokers than in nonsmokers.

The characteristics of the groups according to IL6 -634C/G genotypes are shown in Table 2. None of the variables, including CRP, differed significantly among the genotypes. The distribution of the genotypes was in Hardy-Weinberg equilibrium.

Figure 1 shows the back-transformed adjusted means and SE values of log CRP according to smoking status. The back-transformed adjusted mean values of log CRP in nonsmokers and current smokers were 0.039 and 0.052 mg/dL, respectively (p=0.011).

Comparison of the three genotypes revealed a significant interaction between smoking and the IL6 -634C/G genotypes, as manifested by CRP concentrations (p =0.007, Figure 2 (a)). After stratification by smoking status, CRP was found to differ significantly among the IL-6 -634C/G

genotype groups among nonsmokers after adjustment for age, BMI, systolic blood pressure, TC, log TG, HDL-C, fasting glucose, and drinking habit (p=0.010, p for trend=0.007), whereas no significant differences among genotype groups were found in current smokers (p=0.49, p for trend=0.56).

Then, comparison between -634CC and C/G + G/G groups revealed a significant interaction between smoking and IL6 -634C/G genotypes, as manifested by CRP concentrations (p=0.007, Figure 2 (b)). After stratification by smoking status, CRP was found to differ significantly between two genotype groups among nonsmokers after adjustment for age, BMI, systolic blood pressure, TC, log TG, HDL-C, fasting glucose, and drinking habit (p=0.017), whereas no significant differences between two genotype groups were found among current smokers (p=0.235).

DISCUSSION

In this study, smoking status was related to CRP concentration in whole subject analysis, but IL6 -634C/G polymorphism was not. However, significant interaction was found between smoking and the IL6 -634C allele, thus affecting CRP concentrations. The IL6 -634C/G polymorphism was significantly associated with CRP concentration in nonsmokers but not in current smokers.

The synthesis of CRP in the liver is mainly under the control of IL-6 (6). Therefore, a genotype or haplotype that influences IL-6 production could affect CRP concentrations.

Ota et al. (18) speculated that a decrease in bone mineral density found in carriers of the -634G allele, but not in subjects with the -634C allele, was due to effects of transcriptional activation that is caused by the presence of the G allele, since IL-6 is known to stimulate osteoclast development. Those with the -634G variant had a lower risk of abortion (14). We attribute this to increased IL-6 production in the -634G variant, because low serum concentrations of IL-6 during early pregnancy in women with recurrent pregnancy loss were found to be

associated with unsuccessful pregnancy outcome (21). The -634G allele was significantly more common in patients with macroalbuminuria than in patients with normoalbuminuria among type 2 diabetic patients, and the study reported that the -634G allele was associated with elevated production and secretion of IL-6 by peripheral blood mononuclear cells in vitro (19).

Meanwhile, the -634C allele (denote -572G/C in Refs. 22-24) is less frequent in Caucasians (22-24). In Caucasians, the -634C allele was associated with increased serum insulin release during an oral glucose tolerance test (23). The -634C allele induced higher gene expression levels than the G allele after stimulation with interleukin-1β and dexamethasone, and the C allele was related to higher serum CRP levels in Caucasian postmenopausal women (22). However, an in vitro study found that the -634C/G allele was not associated with IL-6 production by leukocytes after lipopolysaccharide stimulation (25).

Several studies among Caucasians have shown that the combination of polymorphisms in the promoter region of IL6 affects its gene expression (25-27). But, as mentioned above, the -174C allele is extremely rare and the -634C allele is common in Japanese,whereas in Caucasians the

-174C allele is relatively frequent and the -634C allele is less frequent. Tanaka et al. speculated that racial variation of the -634C/G polymorphism affects IL-6 production, since there are such racial differences in allelic distribution (28).

In young and healthy subjects who carry the IL6 -174C allele, those who smoked had higher levels of leukocytes, lymphocytes, and monocytes than those who did not smoke (13). If the -634C/G polymorphism had had the same effect as the-174G/C polymorphism on inflammation, the CRP concentrations in current smokers with the -634G allele would have been higher than in nonsmokers with the -634C allele. However, the authors of the -174G/C polymorphism study did not explain the mechanism underlying the IL6 -174C allele's enhancement of inflammation in smokers (13). Therefore, we can only speculate as to the possible reason for the difference in the -634G allele's effect between smoker and nonsmokers. A possible reason for the lack of a -634C/G genotype effect on CRP concentrations in current smokers is that continuous smoking, which stimulates inflammation, could reduce the -634G allele's effect on CRP concentration; the -634G allele may increase CRP concentration when smoking does not stimulate inflammation.

Also, glutathione S-transferase (GST) M1 and P1, phase II detoxification enzymes, were related to increased risk of smoking-related diseases (29,30) and to the metabolism of many carcinogenic compounds in tobacco smoke (31). It has been reported that the GSTM1 and GSTP1 gene polymorphisms are related to inflammation (32). Thus, smokers' detoxification enzyme gene polymorphisms may affect the -634G allele's influence on inflammation in smokers.

After comparing constructs of the 5' flanking region in a luciferase reporter vector transiently transfected in HeLA cells, Fishman et al. (26) reported that the -174C construct showed lower expression than the -174G construct. After stimulation with lipopolysaccharide or IL-1, expression from the -174C construct did not significantly change after 24 h, whereas expression from the 174G construct increased significantly. Terry et al. (27) showed that more than one of the IL6 promoter polymorphic sites was functional, but when the -174G/C polymorphism was considered alone, variants containing a C allele showed lower expression than the G/G genotype. Thus, these studies provide evidence of an enhancement in IL-6 transcription of IL-6 associated with the G-allele, whereas the -634G/C genotype had no transcriptional evidence.

However, as previously described, the results of studies on the association of the IL6 -174G/C genotype with CRP concentrations were inconclusive (10-12). Furthermore, the association between the genotype and plasma IL-6 concentrations is also controversial. Fishman et al. showed that the mean plasma IL-6 concentration was lower in individuals with the -174C/C genotype than in those with the -174G/C or -174G/G genotypes (26). On the other hand, Jones et al. reported that abdominal aortic aneurysm patients with the -174G/G genotype had lower plasma concentrations of IL-6 than those with the -174G/C or -174C/C genotypes (33). Meanwhile, Brull et al. found no association between the genotype and baseline plasma IL-6 levels in 127 patients undergoing CABG, although, 6 hours after CABG, peak IL-6 levels were significantly higher in those with genotype -174C/C compared with -174G allele carriers (34).

These discrepancies may occur because baseline diseases and conditions influence the genotype's effects on IL-6 transcription. Thus, since gene-environment interactions in chronic diseases are very important (35), we should investigate these interactions.

Furthermore, it has been reported that the haplotype of the IL6 gene is associated with hypertension (28). Therefore, more haplotype studies of the IL6 gene are needed to elucidate the effects on inflammation.

In general, CRP concentrations increase as cigarette consumption increases (36). Among smokers in present study, the back-transformed adjusted mean values of log CRP in those who smoked $\geq$ 20 cigarettes per day was significantly higher than that among smokers of $\leq$ 19 cigarettes per day. But, the -634C/G genotype did not significantly affect CRP concentration among smokers of $\geq$ 20 cigarettes per day or smokers of $\leq$ 19 cigarettes per day (data not shown). Further studies are needed to clarify whether or not the interaction between the amount of tobacco consumption and IL6 genotypes affects CRP concentration.

We believe that this type of findings on the relationships between cytokine polymorphisms and inflammation will lead to tailor-made treatment. For example, a -634C carrier with mild dyslipidema may have to take statins as early as possible because statins attenuate systemic inflammatory activity (37).

The present study has several limitations. First, the IL-6 concentrations were not measured. DeMichele et al. insisted that many studies failed to find an association between the IL6 -174C/G polymorphism and spot serum IL-6, since there is tremendous intraindividual variation in IL-6 levels, even in nonpathological circumstances (38). Therefore, we evaluated the CRP level because of its popularity and measurement stability. Second, we hypothesize that the -634G allele, by increasing IL6 gene transcription, raises serum CRP concentrations. However, linkage disequilibrium between this polymorphism and others that affect IL-6, other cytokines, or CRP gene transcription directly could influence serum CRP concentrations. Further studies are needed to clarify the mechanism underlying this association. Third, the smaller sample size of subjects with the -634G/G allele might be responsible for the nonsignificant relationship of -634C/G polymorphisms to CRP level among subjects overall or smokers. Fourth, the restriction of the current study to Japanese males aged 35 to 60 years means that additional work will be required to confirm these findings and to extend this work to older and younger men, women, and non-Japanese populations.

In summary, we observed significant interaction between smoking and the IL6 -634C/G polymorphism, which is common among Japanese, and this interaction influenced CRP concentrations. The IL6 -634G allele was significantly associated with CRP concentrations in nonsmokers but not in current smokers. Our results suggest that the impact of the -634G allele on CRP elevation is greater in nonsmokers than in current smokers. Since the gene-environment interaction has been insufficiently examined, further studies are required to clarify the gene-environment interactions affecting inflammation, including elevated CRP.

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References

1. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340: 115-126.

2. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342: 836-843.

3. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999; 99: 237-242.

4. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, Gallimore JR, Pepys MB. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Bmj 2000; 321: 199-204.

5. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002; 347: 1557-1565.

6. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990; 265: 621-636.

7. Mendall MA, Patel P, Asante M, Ballam L, Morris J, Strachan DP, Camm AJ, Northfield TC. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. Heart 1997; 78: 273-277.

8. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arterioscler Thromb Vasc Biol 2002; 22: 1668-1673.

9. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Lancet 1997; 349: 462-466.

10. Vickers MA, Green FR, Terry C, Mayosi BM, Julier C, Lathrop M, Ratcliffe PJ, Watkins HC, Keavney B. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc Res 2002; 53: 1029-1034.

11. Latkovskis G, Licis N, Kalnins U. C-reactive protein levels and common polymorphisms of the interleukin-1 gene cluster and interleukin-6 gene in patients with coronary heart disease. Eur J Immunogenet 2004; 31: 207-213.

12. Libra M, Signorelli SS, Bevelacqua Y, Navolanic PM, Bevelacqua V, Polesel J, Talamini R,

Stivala F, Mazzarino MC, Malaponte G. Analysis of G(-174)C IL-6 polymorphism and plasma concentrations of inflammatory markers in patients with type 2 diabetes and peripheral arterial disease. J Clin Pathol 2006; 59: 211-215.

13. Ortlepp JR, Metrikat J, Vesper K, Mevissen V, Schmitz F, Albrecht M, Maya-Pelzer P, Hanrath P, Weber C, Zerres K, Hoffmann R. The interleukin-6 promoter polymorphism is associated with elevated leukocyte, lymphocyte, and monocyte counts and reduced physical fitness in young healthy smokers. J Mol Med 2003; 81: 578-584.

14. Saijo Y, Sata F, Yamada H, Kondo T, Kato EH, Kishi R. Single nucleotide polymorphisms in the promoter region of the interleukin-6 gene and the risk of recurrent pregnancy loss in Japanese women. Fertil Steril 2004; 81: 374-378.

15. Hayakawa T, Takamura T, Hisada A, Abe T, Nomura G, Kobayashi K. IL-6 gene polymorphism -174G/C does not contribute substantially to hyperlipidaemia and Type II diabetes mellitus in Japanese men. Diabetologia 2002; 45: 453-454.

16. Zhai R, Liu G, Yang C, Huang C, Wu C, Christiani DC. The G to C polymorphism at -174 of the interleukin-6 gene is rare in a Southern Chinese population. Pharmacogenetics 2001; 11: 699-701.

17. Lim CS, Zheng S, Kim YS, Ahn C, Han JS, Kim S, Lee JS, Chae DW. The --174 G to C polymorphism of interleukin-6 gene is very rare in koreans. Cytokine 2002; 19: 52-54.

18. Ota N, Nakajima T, Nakazawa I, Suzuki T, Hosoi T, Orimo H, Inoue S, Shirai Y, Emi M. A nucleotide variant in the promoter region of the interleukin-6 gene associated with decreased bone mineral density. J Hum Genet 2001; 46: 267-272.

19. Kitamura A, Hasegawa G, Obayashi H, Kamiuchi K, Ishii M, Yano M, Tanaka T, Yamaguchi M, Shigeta H, Ogata M, Nakamura N, Yoshikawa T. Interleukin-6 polymorphism (-634C/G) in the promotor region and the progression of diabetic nephropathy in type 2 diabetes. Diabet Med 2002; 19: 1000-1005.

20. Saijo Y, Sata F, Yamada H, Suzuki K, Sasaki S, Kondo T, Gong YY, Kato EH, Shimada S, Morikawa M, Minakami H, Kishi R. Ah receptor, CYP1A1, CYP1A2 and CYP1B1 gene polymorphisms are not involved in the risk of recurrent pregnancy loss. Mol Hum Reprod 2004; 10: 729-733.

21. Makhseed M, Raghupathy R, Azizieh F, Farhat R, Hassan N, Bandar A. Circulating cytokines and CD30 in normal human pregnancy and recurrent spontaneous abortions. Hum Reprod 2000; 15: 2011-2017.

22. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab 2003; 88: 255-259.

23. Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, Kristiansen OP, Mandrup-Poulsen T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia 2005; 48: 251-260.

24. Muller-Steinhardt M, Fricke L, Muller B, Ebel B, Kirchner H, Hartel C. Cooperative influence of the interleukin-6 promoter polymorphisms -597, -572 and -174 on long-term kidney allograft survival. Am J Transplant 2004; 4: 402-406.

25. Rivera-Chavez FA, Peters-Hybki DL, Barber RC, O'Keefe GE. Interleukin-6 promoter haplotypes and interleukin-6 cytokine responses. Shock 2003; 20: 218-223.

26. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998; 102: 1369-1376.

27. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem 2000; 275: 18138-18144.

28. Tanaka C, Mannami T, Kamide K, Takiuchi S, Kokubo Y, Katsuya T, Kawano Y, Miyata T, Ogihara T, Tomoike H. Single nucleotide polymorphisms in the interleukin-6 gene associated with blood pressure and atherosclerosis in a Japanese general population.

Hypertens Res 2005; 28: 35-41.

29. Saarikoski ST, Voho A, Reinikainen M, Anttila S, Karjalainen A, Malaveille C, Vainio H, Husgafvel-Pursiainen K, Hirvonen A. Combined effect of polymorphic GST genes on individual susceptibility to lung cancer. Int J Cancer 1998; 77: 516-521.

30. Sasaki S, Kondo T, Sata F, Saijo Y, Katoh S, Nakajima S, Ishizuka M, Fujita S, Kishi R. Maternal smoking during pregnancy and genetic polymorphisms in the Ah receptor, CYP1A1 and GSTM1 affect infant birth size in Japanese subjects. Mol Hum Reprod 2006; 12: 77-83.

31. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995; 30: 445-600.

32. Kim JH, Park SG, Lee KH, Choi JH, Ha EH, Myung SK, Hong YC. GSTM1 and GSTP1 polymorphisms as potential factors for modifying the effect of smoking on inflammatory response. J Korean Med Sci 2006; 21: 1021-1027.

33. Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, Humphries SE, Powell JT. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 2001; 103: 2260-2265.

34. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, Lowe GD,

Humphries SE. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 2001; 21: 1458-1463.

35. Tiret L. Gene-environment interaction: a central concept in multifactorial diseases. Proc Nutr Soc 2002; 61: 457-463.

36. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 2003; 138: 891-897.

37. Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. Jama 2001; 286: 64-70.

38. DeMichele A, Martin AM, Mick R, Gor P, Wray L, Klein-Cabral M, Athanasiadis G, Colligan T, Stadtmauer E, Weber B. Interleukin-6 -174G-->C polymorphism is associated with improved outcome in high-risk breast cancer. Cancer Res 2003; 63: 8051-8056.

FIGURE LEGENDS

Figure 1

Adjusted mean (SE) concentrations of CRP according to smoking status

Using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL cholesterol, fasting glucose, and drinking habit, adjusted mean (SE) values of log CRP were back-transformed.

Figure 2

(a)

Adjusted mean (SE) concentrations of CRP in relation to smoking status and interleukin-6 -634C/G genotype (comparing all three genotypes)

Using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL cholesterol, fasting glucose, and drinking habit, adjusted mean (SE) values of log CRP were back-transformed.

*p value for difference (p for trend).

(b)

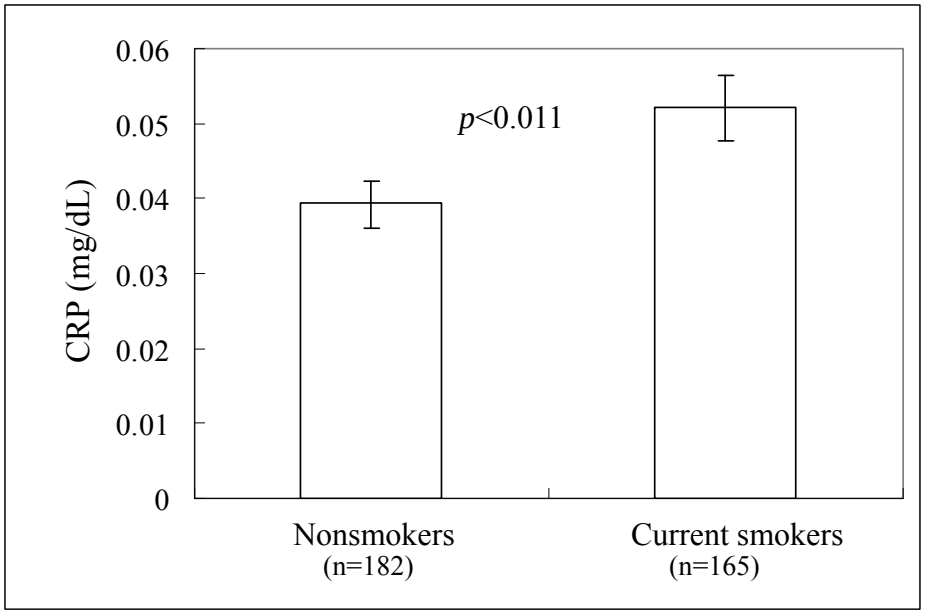
Adjusted mean (SE) concentrations of CRP in relation to smoking status and interleukin-6 -634C/G genotype (C/C vs. C/G＋G/G)

Using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL cholesterol, fasting glucose, and drinking habit, adjusted mean (SE) values of log CRP were back-transformed.

*p value for difference.

Figure 1.

Adjusted mean (SE) concentrations of CRP according to smoking status

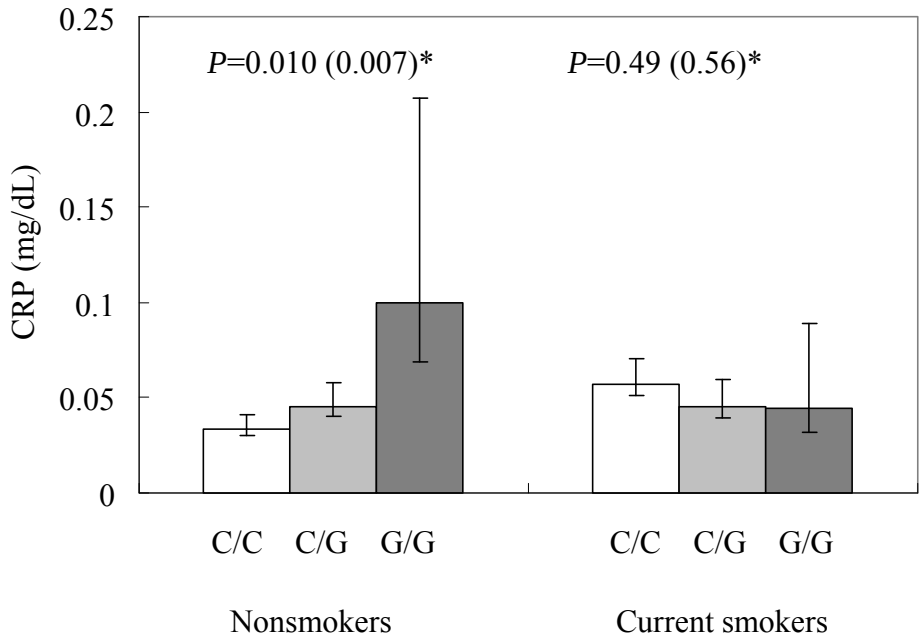


Using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL cholesterol, fasting glucose, and drinking habit, adjusted mean (SE) values of log CRP were back-transformed.

F**igure 2**

(a)

Adjusted mean (SE) concentrations of CRP in relation to smoking status and interleukin-6 -634C/G genotype (comparing all three genotypes)



Using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL cholesterol, fasting glucose, and drinking habit, adjusted mean (SE)

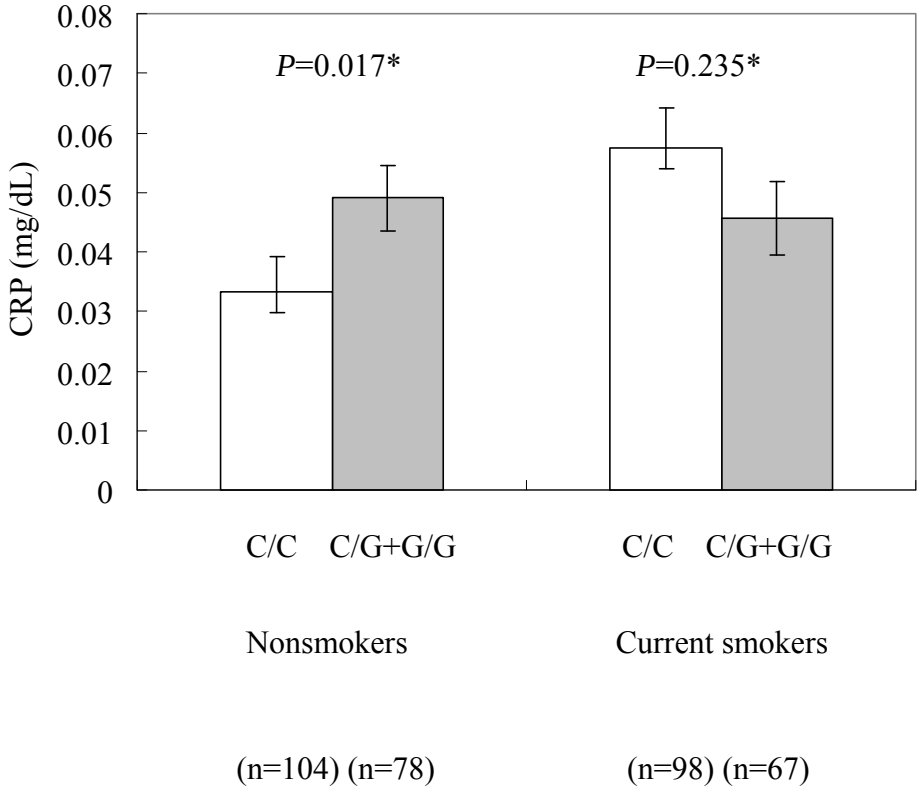
values of log CRP were back-transformed.

*p value for difference (p for trend).

F**igure 2**

(b)

Adjusted mean (SE) concentrations of CRP in relation to smoking status and interleukin-6 -634C/G genotype (C/C vs. C/G＋G/G)



Using general linear model (GLM) univariate analyses adjusted for age, BMI, systolic blood pressure, TC, log TG, HDL cholesterol, fasting glucose, and drinking habit, adjusted mean (SE) values of log CRP were back-transformed.

*p value for difference.

Table 1

Patient characteristics according to smoking status

	Nonsmoker (n=182)	Current smoker (n=165)	p value*
Age (y)	49.7 ± 5.1	48.0 ± 4.6	0.004
BMI (kg/m^2)	23.9 ± 2.4	23.6 ± 2.9	0.24
SBP (mmHg)	126.4 ± 15.8	119.1 ± 15.8	<0.0001
DBP (mmHg)	82.9 ± 10.9	76.8 ± 10.6	<0.0001
Total cholesterol (mg/dL)	204.8 ± 29.2	201.0 ± 33.2	0.27
Triglycerides (mg/dL)	108 (73-153)	123 (88-188)	0.011
HDL cholesterol (mg/dL)	55.8 ± 14.8	51.1 ± 12.1	0.002
Fasting glucose (mg/dL)	100.3 ± 13.5	97.0 ± 8.5	<0.001

Uric acid (mg/dL)	5.7 ± 1.1	5.8 ± 1.2	0.38
Drinker (%)	81.9	77.6	0.32
CRP (mg/dL)	0.036	0.051	0.013
	(0.020-0.071)	(0.026-0.092)	

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage

*Student's unpaired t-test, the Wilcoxon rank-sum test, or the chi-square test.

Table 2

Patient characteristics according to interleukin-6 —634C/G genotype

	Interleukin-6 —634C/G genotype			P value*
	C/C (n=202)	C/G (n=128)	G/G (n=17)	
Age (y)	48.7 ± 5.4	49.1 ± 5.2	50.2 ± 4.6	0.44
BMI (kg/m^2)	23.8 ± 2.8	23.6 ± 2.3	23.4 ± 2.9	0.61
SBP (mmHg)	122.8 ± 15.9	122.3 ± 16.6	128.5 ± 16.6	0.33
DBP (mmHg)	80.0 ± 11.5	80.0 ± 11.0	80.8 ± 9.3	0.96
Total cholesterol (mg/dL)	203.4 ± 32.3	201.3 ± 28.3	210.8 ± 38.4	0.48
Triglycerides (mg/dL)	119.0 (84-175)	112.0 (83-156)	94.0 (55-157)	0.26
HDL cholesterol (mg/dL)	53.7 ± 13.8	53.1 ± 13.9	55.6 ± 13.4	0.76

Fasting glucose (mg/dL)	98.7 ± 12.2	98.6 ± 10.6	100.0 ± 9.5	0.89
Uric acid (mg/dL)	5.8 ± 1.3	5.7 ± 1.0	5.9 ± 1.3	0.66
Current smoker (%)	48.5	45.3	52.9	0.77
Drinker (%)	80.7	76.6	94.1	0.21
CRP (mg/dL)	0.037	0.044	0.050	0.56
	(0.021-0.083)	(0.024-0.076)	(0.026-0.125)	

Variables are presented as mean ± SD, median (interquartile range) for skewed variables, or percentage

*Analysis of variance (ANOVA), the Kruskal-Wallis test, or the chi-square test.

Abbreviations

IL-6: interleukin-6

CRP: C-reactive protein

BMI: body mass index

TC: total cholesterol

TG: triglyceride

UA: uric acid

HDL-C: high-density lipoprotein cholesterol

PCR: polymerase chain reaction

MGB: Minor Groove Binder

ANOVA: analysis of variance

GLM: general linear model

SE: standard error