Influence of the IL-6 -572C>G polymorphism on inflammatory markers according to cigarette smoking in Korean healthy men

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This certifies that the dissertation
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The Graduate School
Yonsei University
July 2007

감사의 글

처음 의사의 길을 택하던 심정으로 노화과학대학원의 문을 두드린 지가 엊그제 같은데 어느덧 그 결실이 이루어지려 합니다. 상상도 할 수 없는 은혜와 섭리로 이 길에 들어서도록 인도해주신 하느님께 무한한 감사를 드리며 더불어 보잘것없는 제가 지금까지 올 수 있도록 도와주신 많은 분들께 감사의 마음을 전하고자 합니다.

학문에 있어 넓은 시야를 가지도록 이끌어주시고 연구를 함에 있어 항상 세심하게 배려해주신 이종호 지도교수님께 진심으로 감사드립니다. 또한 깊이 있는 연구와 학문의 틀을 이룰 수 있도록 조언해주신 장양수 교수님께 감사드립니다. 학자의 고고함 속에서 항상 인자하게 가르침을 주신 이양자 교수님, 따뜻한 가르침과 격려로 지도해주신 하종원 교수님께도 감사드립니다. 영양학에 관심 가질 수 있도록 이끌어 주시고 박사과정의 계기를 마련해주신 이영진 교수님께 진심으로 감사드립니다. 앞으로도 교수님들의 가르침을 잊지 않고 더욱 정진하겠습니다.

가정의학 전문의로 키워주시고 열정적인 삶을 가르쳐주신 윤방부 교수님, 밤늦도록 교수실 불을 밝히시고 학자의 길은 꾸준함임을 보여주신 이혜리 교수님, 의대교수로서 자리 잡을 수 있도록 많은 가르침을 주신 강희철 교수님, 이덕철 교수님, 유병연 교수님, 배철영 선생님께도 감사를 드립니다.

명쾌한 강의에 감탄케 하면서도 의대동창으로서 친근하게 조언을 아끼지 않았던 조홍근 교수님, 멀어져있던 기초학문의 새로운 지식을 가르쳐주시고 그 중요성을 다시 깨닫게 해주신 정지형 교수님, 항상 성실함 그 자체의 모습으로 논문완성에 큰 도움을 주신 김오연 선생님, 연구진행과정에 안팎으로 신경써주신 채지숙 선생님, 크고 작은 힘든 일에 마다않고 도움 주셨던 김지영 선생님, 고수정 선생님, 현예정 선생님, 김혜진 선생님, 대학원 행정업무를 꼼꼼하게 챙겨준 정임씨, 연구대상자들을 모으기 위해 날마다 분당까지 먼 길을 와주었던 연세대 식품영양학과 대학원 식구들께 감사드립니다.

대학원 과정으로 본의 아니게 병원 일에 소홀해져도 이해해주시고 배려해주신 조덕연 원장님과 이경식 전 원장님, 힘든 병원 일 중에도 많이 격려해주신 오도연 교수님, 홍성표 교수님, 조용욱 교수님, 그동안 대학원 일로 귀찮은 부탁을 드려도 흔쾌히 도와주신 김문종 과장님께 감사드립니다. 묵묵히 빈 자리를 채워준 박경채 교수, 학회일로 함께 고생한 강영곤 선생, 힘들어 질 때 쳐다만 봐도 활력을 주던 박기헌 선생, 교실의

새로운 활력소 김귀순 선생, 김창오 선생, 힘들어도 티 안 내고 도와준 우리 분당차병원 가정의학과 외래 간호사들에게 감사의 마음을 전합니다.

항상 밝고 시원한 느낌의 여인 신영, 예쁘면서도 재미있게 웃음을 주던 현양, 똑똑한 모범생 은정, 털털해 보이지만 날카롭게 문제를 바라볼 줄 아는 봉준, 현명한 선생님이 되어있을 민희, 치매와 노인문제에 대해 많은 것을 알게 해준 홍창형 선생, 말없이 열정으로 가득 찬 천상 과학자 차승헌 선생, 후배이지만 창조적이어서 배울 점이 많았던 경철, 부드러우면서도 자기만의 색을 보여주던 석현, 조용하지만 속이 꽉 찬 정현, 정 많고 배려심 많은 숨은 일꾼 수현, 모든 일에 열성적이고 적극적인 현진, 우직한 듯하면서도 스마트한 강원, 많은 관심분야에 깊은 지식을 가진 기호, 막 전공의를 끝내고 대학원에 들어와 기대감에 차있던 태기, 나이 차이가 많이 나 본인들은 그렇게 생각 안 할지라도 대학원에 들어와 함께 강의를 들었기에 친구라고 부를 수 있어서 좋았던, 많이 그리워질 사람들 ... 노화과학 대학원 동문식구들에게도 감사의 마음을 전합니다.

또한 힘든 시기에 인생을 새로이 바라볼 수 있는 기회를 주고계신 이창재 선생님께 감사드리고 단조로운 생활의 한줌의 소금과도 같이 힘이 되어준 초목회 친구들 홍석, 준모, 수일, 동한, 호성, 광현에게도 고마움을 전합니다.

매사 미리 꼼꼼히 챙겨주시는 아버님, 지금도 공부할 때가 가장 행복하다고 하시는 어머님 – 어릴 땐 부담스럽기도 했지만 그게 큰 사랑의 표현이었음을 이제 압니다. 몸소 보여주신 그 모습이 지금의 저를 있게 했습니다. 감사합니다. 그리고 사랑합니다. 당신이 가셨던 길이라면 더 잘 도와줄 텐데 아쉬워 하시면서도 사위가 가는 길을 믿음으로 지켜봐주시는 장인어른, 맛있는 음식 할 때면 꼭 못 온다고 안타까와 하시며 늘 사랑으로 챙겨주시는 장모님, 정말 감사합니다. 늘 형을 존경해주며 힘이 되어준 착한 동생 형균, 형이라면 더 좋았을 든든한 느낌의 큰 처남과 식구들, 한 가족도 빠짐없이 자상하게 마음써주는 작은 처남과 식구들에게도 깊은 감사를 드립니다.

육체적으로나 정신적으로 힘들 때 항상 친구처럼 배려해주고 언제나 발 벗고 나서서 후원을 아끼지 않는 사랑하는 아내 예숙, 기발한 생각과 유머로 가족에게 웃음을 선사하는 정이 뚝뚝 떨어지는 아들 현욱, 아직까진 귀여움을 필살기로 아빠의 피로를 녹여버리는 예쁜 우리집 공주 현정에게 사랑과 감사를 전하며 이 논문을 바칩니다.

2007년 7월

신 경 균

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ABSTRACT

Influence of the IL-6 -572C>G polymorphism
on inflammatory markers according to cigarette smoking
in Korean healthy men

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Yonsei University

Objective : We investigated whether smoking would interact with the interleukin-6 (IL-6) polymorphisms (-174G>C and -572C>G, -597G>A and -1363G>T) in determining circulating levels of inflammatory markers and its consequence to oxidative stress.

Methods : Healthy male subjects (n=644) were recruited. The inclusion criteria were 38 $\leq$ age<72 y, no history or diagnosis of cardiovascular disease, diabetes mellitus, cancer or renal disease and no pathological electrocardiogram patterns. None of the subjects were taking medication. Subjects were classified into two groups: nonsmoking and

smoking. Smoking habits were assessed by standardized questionnaires. Based on smoking history, we categorized life-long nonsmokers or those who had quit smoking for at least 6 months as 'nonsmokers' (n=376), and subjects who were currently smoking >3 cigarettes per day as 'regular smokers' (n=268). Body weight, height, waist circumference, hip circumference, blood pressure were measured, and body mass index (BMI) was calculated. Interleukin-6 SNPs -174G>C, -572C>G, -597G>A and -1363G>T genotyping was performed. Fasting serum concentrations of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, fasting glucose, insulin were measured and insulin resistance was calculated with the homeostasis model assessment (HOMA). Serum IL-6, high-sensitive CRP, fibrinogen, oxidized LDL (ox-LDL) concentrations were measured. Urine was collected for the measurement of 8-epi-prostaglandin F2α (8-epi-PGF2α).

Results : The G/G genotype(n=26) of the -572C>G in nonsmokers (n=376) was associated with higher IL-6 (P=0.028), fibrinogen (P=0.007) and ox-LDL (P=0.006) than those with C/C (n=209) or C/G (n=141). Results were similar for nonsmokers and smokers (n=268), but in smokers, the -572G/G genotype was associated with a greater difference in levels of IL-6 (P=0.031), fibrinogen (P=0.001), ox-LDL (P=0.037) and PGF2α (P=0.050). IL-6 had positive relations with CRP, fibrinogen, ox-LDL and PGF2α. There was no evidence of an effect of -572C>G genotype on CRP levels in nonsmokers, however, this polymorphism was associated with a highly significant effect on CRP in smokers (P<0.001) (genotype-smoking interaction P=0.04, adjusted for age, BMI and IL-6). The C allele frequency at the -174 promoter region of IL-6 was very rare (<0.01) and -597G>A and -1363G>T were monomorphic in this study.

Conclusions : Our results suggest that IL-6 -572C>G has a greater response over time to

the inflammatory effects of smoking and this may result in smokers having higher oxidative stress in subjects with G/G compared to C/C or C/G.

Key words: IL-6 -572C>G polymorphism, smoking, inflammation, oxidative stress

PREFACE

Atherosclerosis is not only a degenerative disease but shares many characteristics of a chronic inflammatory process (36). There is increasing evidence that inflammation plays an important role in the development of atherosclerosis and coronary heart disease (CHD) (12). The inflammation is characterized by a local reaction, which may be followed by activation of a systemic acute-phase reaction. In this context, the acute-phase reactant, Interleukin-6 (IL-6) plays a major role by upregulating the synthesis of acute-phase proteins, including fibrinogen and CRP from hepatocyte (8).

Interleukin-6 (IL-6), a pleiotropic, proinflammatory cytokine, seems to play an important role in the pathogenesis of cardiovascular disease (1). IL-6 is produced by a variety of cells including macrophages after stimulation such as infection, trauma, or undergoing inflammatory activation in the vessel wall (2,3). However, increased IL-6 production may occur in the absence of infections and its role in CHD may be more central than that of CRP or fibrinogen, both of which are elevated inpatients with CHD (4). Genetic polymorphisms that determine the rate of acute-phase protein production would be important genetic risk factors for atherosclerosis (8).

Recent experimental work has identified the presence of 4 polymorphism in the IL-6 gene promotor on chromosome 7: -597G>A, -572C>G and -174G>C and a fourth polymorphism located at position -373 with vary numbers of As and Ts (10).

The -174G>C polymorphism has been reported as functionally important, since it influences the transcription rate of the gene and the plasma concentration of IL-6, the

results have however been conflicting (12).

The -572G allele (frequency 0.05, 0.04~0.06) was not associated with a significant effect on blood pressure, fibrinogen or relative of CHD in U.K. men (19). For the -572C>G polymorphism, no association was observed with levels of any trait or risk in Caucasian (8). This might be due to the low frequency of G/G homozygotes in Caucasian. Furthermore, allele frequencies at IL-6 -572C>G promoter polymorphism are quite different from Japanese and Korean (15-18) to Caucasian population (19).

Therefore, we determined the genotype effects of IL-6 promoter polymorphisms on circulating levels of IL-6 in healthy Korean men who have different allele frequencies of IL-6 promoter polymorphisms from Caucasian. We also investigated whether smoking would interact with IL-6 promoter polymorphisms in determining circulating levels of inflammatory markers and its consequence to oxidative stress such as lipoprotein oxidation and lipid peroxidation.

Ⅱ. BACKGROUND

2.1. Interleukin-6 (IL-6) and Interleukin-6 (IL-6) gene

Interleukin-6 is polypeptide with molecular masses of about 20kDa is classical secretory proteins synthesized with N-terminal signal peptides. The four-a-helix-bundle cytokines are one of the rare examples of proteins where pulling at the N- and C-termini would result in the formation of a knot.

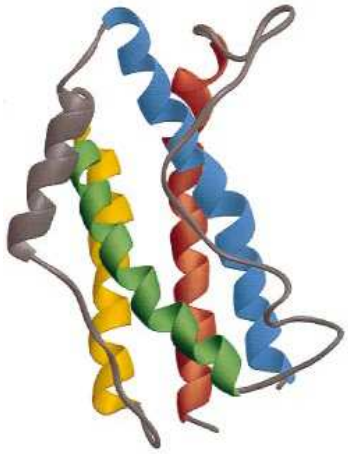

Figure 1. Structure of Interleukin-6 (IL-6) (Heinrich PC et al. Biochem. J. 334, 297-314, 1998)

IL-6 is secreted by different cell types including leukocytes and endothelial cells and has recently been shown to be released from muscle tissue and adipose cell. Its production is stimulated by TNF-α, IL-1, bacterial endotoxin, and

catecholamines, and is suppressed by glucocorticoids and estrogen.

It is now clear that IL-6 plays a central role in diverse host defense mechanisms such as the immune response, hematopoiesis, and acute-phase reactions (Fig. 2). While IL-6 appears to have little to do with the day-to-day "housekeeping" functions of the body, along with other cytokines it represents an important frontline component of the body's armory against infection or tissue damage.

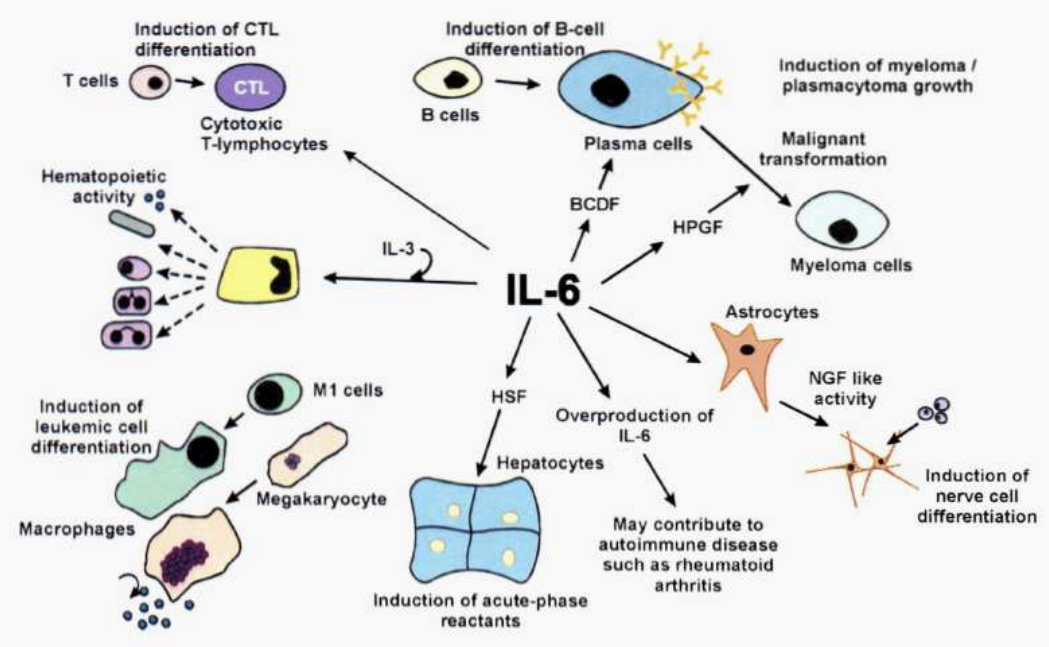

Figure 2. Biologic activities of interleukin-6. (Simpson RJ et al. Protein Science , 6929-955, 1997.)

In accordance with its functional pleiotropy, IL-6 has been implicated in the pathology of many diseases including multiple myeloma, rheumatoid arthritis, Castleman's disease, AIDS, mesangial proliferative glomerulonephritis, psoriasis, Kaposi's sarcoma, sepsis, and osteoporosis.

IL-6 exerts diverse proliferative, differentiative, and maturation events depending on the nature of the target cell. Such functional pleiotropy is a common feature of most cytokines and growth factors that have a role in the immunohemopoietic system. Since a single cell often responds to numerous cytokines and growth factors that act in synergy, overlapping biological activities (functional redundancy) or modulation between such cytokines may occur either at their receptors or along their intracellular signal transduction pathways.

IL-6 gene is located in chromosome 7p21and its span is 5kb. It is composed of promotor region, 5 exons and 4 introns.

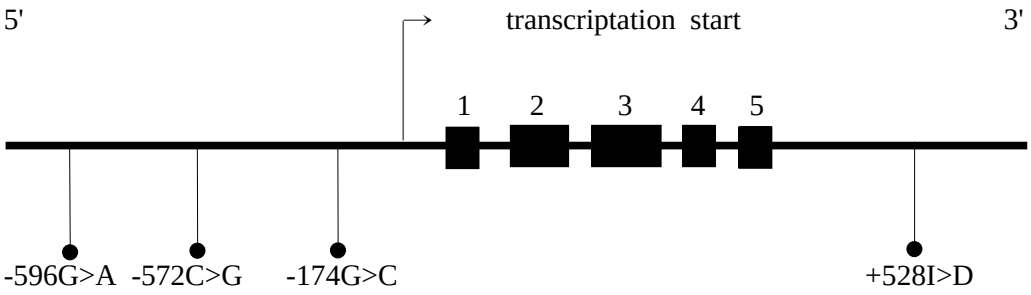

Figure 3. IL-6 genomic structure and polymorphism location.

Within the protein coding region of the gene, the positions of exon/ intron boundaries, exon lengths, and location of cysteine residues within exons are conserved across species. However, differences occur at the 5' boundary of exon 1 and the 3' boundary of exon **5,** which lie outside the coding region. This conservation of genomic structure is also observed in the gene for granulocyte colony-stimulating factor (G-CSF), which is related to IL-6 by sequence homology.

2.2. Inflammation and CVD

Inflammation is now strongly implicated in the process of atherosclerosis and clinical cardiovascular disease (CVD) (8), with inflammation being involved in all stages of atherosclerotic development , including oxidative damage, cell proliferation and plaque development and destabilization, as well as coagulation, thrombosis, and fibrinolysis. If inflammation is both a cause and a consequence of CHD, the plasma levels of acute-phase proteins, such as IL-6, fibrinogen, and CRP, will be markers of the magnitude of inflammatory response and the severity of CVD, and genetic polymorphisms that determine the rate of acute-phase protein production would be important genetic risk factors. WOSCOPS have previously reported that mean plasma levels of fibrinogen and CRP were higher at baseline in men who subsequently had a CHD event than in those who remained event free, and Basso et al. examined the impact on CHD traits and CHD risk for 2 promotor polymorphisms in the IL-6 gene that have been shown in vitro to affect transcriptional strength and are thus functional (8).

Inflammatory cells are present both during atherogenesis and in ruptured atherosclerotic plaque causing myocardial infarction (MI). In patients with CHD, increased levels of systemic markers of inflammation, such as the acute phase reactants CRP and fibrinogen, are found and associated with a poor prognostic outcome (12). Many cell types produce IL-6, major biological effects which are proliferation and differentiation of B and T lymphocytes and stimulation of the hepatic acute phase response including CRP and fibrinogen. IL-6 activates the endothelium, causes leukocyte recruitment to the vessel wall, and stimulates vascular smooth muscle cell proliferation, effects that may lead to plaque growth and/or instability (12).

The source of IL-6 is generally assumed to be macrophages activated by infection, or undergoing inflammatory activation in the vessel wall, with additional contributions from fibroblasts and endothelial cells. However, increased production of IL-6 may occur in the absence of infections, and its role in CHD may be more central than that of CRP or fibrinogen, both of which are elevated in patients with CHD (19).

Increased circulating levels of inflammatory mediators have been documented in subjects with COPD. Elevated levels of IL-6 and IL-8 have also been shown. Van Eeden SF et al. have shown increased levels of circulating IL-6 and IL-1 in smokers without COPD. They also showed that subjects exposed to increased levels of ambient particleshave elevated circulating cytokines levels such as IL-1, IL-6, and granulocyte macrophage colony-stimulating factor (GMCSF) (42).

2.3. Relationship between plasma levels of IL-6 and CVD

Inflammatory process play a pivotal role in the pathogenesis of atherosclerosis. Within the inflammatory pathway, cytokines fulfill a multitude of functions. IL-6 is a pleiotropic inflammatory cytokine. It plays an important part in the acute phase response and inflammatory cascade, such as upregulation of acute phase proteins such as CRP. CRP levels have been found to be associated with risk of CHD, Several studies have also shown as association between IL-6 plasma levels and cardiovascular pathology. Ridker et al. found elevated IL-6 levels to be associated with increased risk of MI. This finding was replicated in another large population by Bennet et al., who found an increased risk of MI for those in the upper quartile levels of IL-6 versus those in the lowest quartile levels, and by Cesari et al, when comparing subjects with highest and lowest IL-6 tertiles in an American population (11).

2.4. Relationship between genotypes of IL-6 and CVD

For the IL-6 -572C>G polymorphism, no association was observed with levels of any trait or risk in Caucasian (8). This might be due to the low frequency of G/G homozygotes in Caucasian. The low frequency of -572G allele means that the sample has adequate power to detect only a 1.85-fold higher risk in carriers, and although it cannot be ruled out that this polymorphism may have an effect on risk in some situations, the WOSCOPS data (19) confirm that this polymorphism has negligible or extremely modest effects on determining plasma levels of inflammatory markers, such as CRP and fibrinogen, and risk in middle-aged men.

The Northwick Park Heart Study (NPHS), a prospective study of healthy men (19), reported that compared with risk in the -174G>C G/G group, risk in the -174G>C G/C group was 1.55 (1.06 to 2.22), but in the -174G>C C/C group, the risk was not significantly elevated (1.07 [0.65 to 1.77]). In NPHS, there was evidence that the -174C allele risk effect was greater in current smokers.

In a recent study (12), the -174G>C polymorphism was not associated with cardiovascular death or a new MI, whereas the -572C>G polymorphism showed a borderline significant increase in risk in univariate analysis.

Recent experimental work has identified the presence of 4 polymorphism in the IL-6 gene promotor on chromosome 7: -597G>A, -572C>G and -174G>C and a fourth polymorphism located at position -373 with vary numbers of As and Ts (10).

The -174G>C polymorphism has been reported as functionally important, since it influences the transcription rate of the gene and the plasma concentration of IL-6,

however, the results have been conflicting (12).

Regarding association between IL-6 genotype and plasma IL-6 concentrations, the -174C allele was associated with raised IL-6 levels in patients with abdominal aneurysms (12). In patients homozygous for the -174C allele and heterozygous for the -572C allele, plasma concentrations of IL-6, stimulated by coronary artery bypass grafting (CABG), were significantly higher 6 hour after surgery. The latter study indicates that IL-6 promotor polymorphism influence the IL-6 response to inflammatory stimuli (14).

The -572G allele (frequency 0.05, 0.04~0.06) was not associated with a significant effect on blood pressure, fibrinogen or relative of CHD in U.K. men (19).

High levels of IL-6 could promote plaque growth and rupture by increasing expression of several key genes and could lead to the progression of atherosclerosis and plaque rupture (19).

Smoking is known to damage endothelial cells, to induce macrophage recruitment in the lungs and to be associated with increased plasma levels of inflammatory markers such as CRP and fibrinogen (19). IL-6 genotype showed a strong interaction with smoking in respect to CHD risk in NPHS-II. If the IL-6 -174C allele shows a greater response over time to the inflammatory effects of smoking, it may result in smokers having higher tissue levels of IL-6 compared to -174G>C G/G subjects. The very likely underlying mechanism for this is due to the direct inflammatory consequences of smoking on the pulmonary and vascular endothelium. If a high basal degree of inflammation is associated with the -174C allele then this would be exaggerated in smokers.

2.5. IL-6 -174G>C promotor polymorphism and the risk of CHD

Fishman et al (9) detected a functional polymorphism in the promotor region of the human IL-6 gene (174bp upstream from start site). IL-6 -174G>C promotor polymorphism appears to influence transcription of the IL-6 gene and also plasma levels of IL-6, and IL-6 is, therefore, a candidate gene for additional study into its role in CVD (9). However, results from previous studies on the -174G>C polymorphism and CHD were inconsistent (8). Five studies, most of which were case-control studies, conducted in western, mainly white, populations, found the C allele to be associated with an increased risk of CHD or CVD (36). Four other studies, however, did not find a significant association.

Relationship from previous studies on this IL-6 -174G>C promotor polymorphism and CHD was inconsistent. All of the studies on IL-6 genotype and CHD were performed in western populations with predominantly male subjects with an average of more than 50 years old. It is, therefore, unlikely that differences in findings are because of ethnicity, gender, or age difference between the studies.

2.6. Relationship between genotypes of IL-6 and plasma levels of IL-6

Recent findings suggest that the transcriptional activity of the IL-6 gene and the plasma levels of IL-6 protein are associated with a single G/C base exchange polymorphism sited at -174 promotor region. Homozygotes for allele G and G/C heterozygotes have been shown to have higher plasma IL-6 levels, higher IL-6 gene transcriptional activity and higher IL-6 inducible responses than subjects homozygous for C allele (9).

The C allele frequency at the -174 promotor region of IL-6 is very low (<0.01%) in the Korean population (38), although this SNP is commonly detected with about a 40% prevalence in Caucasian. In contrast, IL-6 -636C>G promotor polymorphism, which Tanaka et al. clarified to have a significant association with blood pressure and carotid IMT in Japanese women, has been recognized with only about a 5% prevalence in Caucasian. In the African-American population, IL-6 -636C>G promotor polymorphism is common (9.5%) and IL-6 -174G>C promotor polymorphism is rare (4%). These findings are very different from those in Caucasian; however, circulating IL-6 levels in African-American are similar to those in Caucasian (35). Thus, IL-6 -174G>C promotor polymorphism is important in the regulation of IL-6 production in Caucasian, but other gene polymorphisms, such as IL-6 -636C>G promotor polymorphism, may play important roles in IL-6 production in East Asian and African-American populations (35).

The relationship between IL-6 genotypes and plasma levels of IL-6 appear to be complex. The higher IL-6 concentrations in CVD patients support the exaggeration of the

C allele raising effect in an inflammatory situation. The -572G allele, and also the -174C allele, have been associated with increased IL-6 levels in an inflammatory situation.

There is also no consensus on the effect of the genotype on plasma levels of IL-6 and CRP. Sie et al (11) found that the -174G>C C-allele was significantly associated with higher CRP levels. In most studies showing an effect of the -174G>C C-allele on plasma levels of both IL-6 and CRP, the C-allele was associated with higher levels of both IL-6 and CRP. This is pathophysiologically plausible, because CRP is produced in the liver, and IL-6 is a hepatocyte stimulant, so elevated IL-6 levels will result in higher CRP levels.

Sie et al did not find a clear association between genotype and the risk of CHD. This may be related to the fact that the functionality of the polymorphism, at least with respect to the extent of the influence on IL-6 plasma levels, has not been definitively established. Although an effect on transcription and IL-6 levels was described, the view presented by Terry et al. (10),whereby the effect is cell specific dependent on complex interactions between several polymorphisms, rather than on an individual polymorphism, might be more applicable. This implies that the solitary -174G>C genotype might influence plasma levels but not in a substantial way.

There is evidence for the IL-6 -174G>C promotor polymorphism to be in linkage disequilibrium with other functional bur less frequently investigated polymorphisms, such as -597G>A, -572C>G, and with possibly functional polymorphisms in the -373AT run (43). (The -174G>C and -597G>A polymorphisms were in strong linkage disequilibrium (R^2=0.95). The IL-6 -174G>C promotor polymorphism was not observed in Japanese population.)

Several polymorphisms, for examples, those for TNF α and β, TGF β1, IL-1ra, CD14, E selectin, have been found to be functional(44), that is, to have direct effects on gene transcription and protein function. However, although A allele may clearly be associated with protein function or concentrations, and protein concentrations with disease, it still may not be possible to relate a given genetic variant to disease, as an individual polymorphism contributes only a fraction to the entire heritable variance in protein concentrations (45). Additionally, the small contribution of a single novel polymorphism to the overall risk of a multifactorial disorders such as IHD may be obscured by the presence of one or more dominant classical risk factors (46).

The IL-6 -572C>G substitution, which is considerably rather than IL-6 -174G>C in Caucasian, has been associated with IL-6 expression levels after coronary artery bypass grafting (CABG) but was not associated with IL-6 levels in patients with ST-elevation MI and hypercholesterolemic men (8,12,14).

Functional studies using the reporter gene showed that -174G>C, -572G>C, and the A and T repeat variation, AnTn tract, which lies between -174G>C and -572G>C, intricately cooperate in regulating IL-6 gene expression(10). Although several association studies on -174G>C in Caucasian have been reported, this polymorphism is recognized only with low allele frequency in South Chinese, Korean, and Japanese population. One SNP -636C>G, was identical to the previously described -572C>G (10,35). -636C>G in the promotor of IL-6 had positive associations with blood pressure and carotid atherosclerosis in a large scale Japanese general population (35).

2.7. Relationship between genotypes of IL-6 and insulin

The pathogenic impact of IL-6 in insulin-resistant states is underscored by the effect of the functional IL-6 -174G>C promotor polymorphism. The -174G>C variant has been shown to influence the transcriptional IL-6, and human -174G allele carriers exhibit higher plasma IL-6 levels compared with homozygous C allele carriers, an affect which is modulated by age and gender.

The -174 C allele was associated with lower serum insulin levels among male controls but did not significantly influence MI risk or IL-6 levels in Swedish (40). The association between the -174G allele and increased insulin levels among the male controls could be a chance finding. However, the significant associations persisted after adjustment for potential confounders and the finding is in agreement with results form a study by Fernandez-Real et al. (41). That paper also reports an association between the -174G allele and reduced insulin sensitivity, providing further support that genetic determinants of IL-6 levels may play a role in insulin resistance. The -572C>G polymorphism has been reported to be associated with progression of diabetic nephropathy in Japanese type 2 diabetic patients (15). The lack of associations among the cases could be due to the effect of IL-6 genotypes on insulin levels being diluted by other insulin stimulating factors that are more common among the cases than the controls (40). Cohort studies on healthy subjects are therefore required to replicate the findings and to explore the molecular mechanisms behind these associations.

Ⅲ. Influence of the IL-6 -572C>G polymorphism on inflammatory markers according to cigarette smoking in Korean healthy men

3.1. INTRODUCTION

Interleukin-6 (IL-6), a pleiotropic, proinflammatory cytokine, seems to play an important role in the pathogenesis of cardiovascular disease (1). IL-6 is produced by a variety of cells including macrophages after stimulation such as infection, trauma, or undergoing inflammatory activation in the vessel wall (2,3). However, increased IL-6 production may occur in the absence of infections (4). Smoking status, the established cardiovascular risk factors, is known to be associated with increased levels of systemic markers of inflammation, such as acute-phase proteins, IL-6, C-reactive protein (CRP) and fibrinogen (5,6). Interestingly, only some people exposed to cigarette smoke develop cardiovascular disease (CVD), indicating that genetic factors are important determinants of susceptibility (7) Thus, genetic polymorphisms that determine the rate of acute-phase protein production would be important genetic risk factors for atherosclerosis (8).

In vitro study showed that polymorphisms at positions -174 and -572 (-634) in the IL-6 gene affect transcriptional strength (9, 10) which are functionally important. However, previous population genetic studies are inconclusive regarding associations between these polymorphisms and circulating IL-6 levels (8 11-15), but the reasons for the discrepancies among studies are unclear. Furthermore, allele frequencies at -572 are quite different from Japanese and another Korean (C>G, G: 0.249) (15-18) to Caucasian population (G>C, C:0.04~0.06) (19).

Therefore, we determined the genotype effects of IL-6 promoter polymorphisms on circulating levels of IL-6 in healthy Korean men who have different allele frequencies of IL-6 promoter polymorphisms from Caucasian. We also investigated whether smoking would interact with IL-6 promoter polymorphisms in determining circulating levels of

inflammatory markers and its consequence to oxidative stress such as lipoprotein oxidation and lipid peroxidation.

3.2. Subjects and methods

3.2.1. Study Subjects

Healthy male subjects (n=644) were recruited concomitantly from participants in prospective human genetic study, supported by a Genome Research Development Project on Health and Medicine (project#: A000385), Ministry of Health & Welfare. The inclusion criteria were $38 \leq$ age<72 y, no history or diagnosis of CVD, diabetes mellitus, cancer or renal disease and no pathological electrocardiogram patterns. None of the subjects were taking medication. Written informed consent was obtained from all subjects and the protocol was approved by an ethical committee, the Institute of Review Board of Yonsei University. Subjects were classified into two groups: nonsmoking and smoking. Smoking habits were assessed by standardized questionnaires. Based on smoking history, we categorized life-long nonsmokers or those who had quit smoking for at least 6 months as 'nonsmokers' (n=376), and subjects who were currently smoking >3 cigarettes per day as 'regular smokers' (n=268).

3.2.2. Anthropometric parameters, blood pressure measurements and blood collection

Body weight and height were measured unclothed and without shoes in the morning. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m^2). Waist and hip circumferences were measured with paper tape horizontally at the umbilicus in the standing position after normal expiration. Blood pressure was read from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 min of rest. The average of three measurements was recorded for each subject.

Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-hour fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1-3 hours) and were stored at -70°C until analysis.

3.2.3. Genotyping of interleukin-6 at positions -174(G>C) and -572(C>G), -597(G>A) and -1363(G>T)

Genomic DNA was prepared from peripheral blood samples using a Puregene® DNA purification kit (Gentra, Minneapolis, MN), following the manufacturer's protocol. Interleukin-6 SNPs -174G>C, -572C>G, -597G>A and -1363G>T genotyping was performed by SNP-IT™ assays using SNP stream 25K®System (Orchid Biosciences, Princeton, NJ).

3.2.4. Serum lipid profiles and glucose and insulin concentrations

Fasting serum concentrations of total cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd. Tokyo, Japan). After using dextran sulfate magnesium to precipitate serum chylomicron, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), the remaining high-density lipoprotein (HDL) cholesterol from the supernatant was measured by an enzymatic method. LDL cholesterol was indirectly estimated in subjects with serum triglyceride concentrations <4.52 mol/l (400 mg/ml) by using the Friedewald formula. In subjects with serum triglyceride concentration ≥4.52 mol/l, LDL cholesterol was measured directly. Fasting glucose was measured by a glucose oxidase method using the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Insulin was measured by radioimmuno-assays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA). Insulin resistance was calculated with the homeostasis model assessment (HOMA) using the following equation: insulin resistance (IR)={fasting insulin(μIU/ml)×fasting glucose(mmol/l)}/22.5 (20).

3.2.5. Circulating concentrations of IL-6, CRP, fibrinogen and oxidized LDL

Serum IL-6 was measured using an enzyme immunoassay (R&D Systems) and the resultant color reaction was read by Victor2 at 450 nm with wavelength correction set to 540 nm. Serum high-sensitive CRP concentrations were measured with an Express+ autoanalyzer (Chiron Diagnostics Co., Walpole, MA) using a high-sensitivity CRP-Latex (II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan) that allowed detection of CRP concentrations as low as 0.001 mg/dl and as high as 32 mg/dl. Plasma fibrinogen concentration was measured as follows. After adding plasma with some thrombin, ball which is in cuvette is binding clotted material. The motionless time is measured using a Stago compact (DIAGNOSTICA STAGO, France) and fibrinogen mass is calculated from a calibration curve. Plasma oxidized LDL (ox-LDL) was measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden). The resultant color reaction was read at 450 nm with a Victor2 (Perkin Elmer Life Sciences, Turku, Finland).

3.2.6. Urine collection and 8-epi-prostaglandin F2α

Urine was collected after a 12-h fast in polyethylene bottles containing 1% butylated hydroxytoluene before blood collection. The tubes were immediately covered with aluminum foil and stored at -70℃ until analysis. 8-epi-prostaglandin F2α (8-epi-PGF2α) was measured with the use of an enzyme immunoassay (BIOXYTECH urinary 8-epi-PGF2α Assay kit; OXIS International Inc, Portland, OR). The resulting color reaction was read with the use of a Victor 2 (Perkin Elmer Life Sciences, Turka, Finland) at 650 nm. Urinary creatinine was determined by using the alkaline picrate (Jeffe) reaction.

3.2.7. Statistical methods

Statistical analyses were performed with SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, U.S.A.). Hardy Weinberg Equilibrium (HWE) was examined using the Executive SNP Analyzer 1.0 (http://www.istech.info/SilicoSNP/index.html). An independent t-test was performed to compare the difference in general characteristics between non-smokers and smokers. Oneway analysis of variance (ANOVA) followed by Bonferroni test were used to compare the differences in biomarkers among genotype groups. Associations between genotypes and concentrations of inflammatory markers were determined in smokers and nonsmokers. The null hypothesis was that smokers and nonsmokers with different IL-6 genotypes do not differ in circulating concentrations of inflammatory markers. Using the general linear models to assess whether the IL-6 genotypes, smoking status and the interaction between IL-6 genotypes with cigarette smoking made a statistically significant contribution to inflammatory markers, the proportion of variance explained of inflammatory markers (dependent variables) and independent variables of smoking status (smokers vs. nonsmokers), genetic variant, and an interaction term between smoking status and gene variant were elucidated with an adjustment. Pearson correlation test were used to examine the relation of IL-6 concentrations and other inflammatory markers. Each variable was examined for normal distribution patterns. Significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented using untransformed and unadjusted values. Results are expressed as mean±S.E. A 2-tailed value of $P<0.05$ was considered statistically significant.

3.3. RESULTS

3.3.1. Main characteristics of the study subjects

Table 1 presents the main characteristics of the study population, which included 268 smokers with an average consumption of 18±1 cigarettes/d. There were no significant differences between smokers and nonsmokers (n=376) in age, BMI, waist-hip ratio (WHR), blood pressure, serum glucose, insulin, HOMA-IR, lipid profiles, and alcohol intake. However, smokers had a trend toward higher concentrations of IL-6 (P=0.143), fibrinogen (P=0.069) and PGF2α (P=0.060) compared to nonsmokers. Ox-LDL concentrations were higher in smokers than nonsmokers (P=0.033).

Table 1. Main characteristics of non-smokers and smokers

	Nonsmokers (n=376)	Smokers (n=268)
Age (years)	52.8 ± 0.45	54.0 ± 0.50
Body mass index(kg/m2)	24.5 ± 0.14	24.6 ± 0.15
Waist-hip ratio	0.90 ± 0.00	0.90 ± 0.00
Blood Pressure (BP)		
Systolic BP (mmHg)	126.0 ± 0.94	128.9 ± 1.06
Diastolic BP (mmHg)	81.0 ± 0.65	82.3 ± 0.66
Alcohol intake (g/day)	26.5 ± 2.30	23.3 ± 2.56
Tobacco (cigarettes/day)	—	18.0 ± 0.80
Glucose (mg/dL)	92.3 ± 0.68	91.6 ± 0.81
Insulin (μU/mL)†	8.28 ± 0.20	8.30 ± 0.24
HOMA-IR[1]	1.91 ± 0.05	1.92 ± 0.06
Triglyceride (mg/dL)†	140.7 ± 3.69	144.7 ± 4.63
LDL-cholesterol (mg/dL)	121.6 ± 1.78	124.5 ± 2.17
HDL-cholesterol (mg/dL)	47.0 ± 0.60	48.8 ± 0.83
Total cholesterol (mg/dL)	197.5 ± 1.85	203.4 ± 2.22
IL-6 (pg/mL)†	3.18 ± 0.25	3.78 ± 0.33
CRP (mg/dL) †	1.06 ± 0.18	1.31 ± 0.27
Fibrinogen (mg/dL)†	311.0 ± 3.64	323.4 ± 5.08†
Ox-LDL (mg/dL)	58.1 ± 1.33	63.0 ± 1.83*
PGF2α (pg/mg creatinine)†	900.6 ± 32.4	965.1 ± 45.6†

Mean±S.E. † log-transformed. † $p<0.1$, *$p<0.05$ compared to nonsmokers.

[1]Insulin Resistance = {fasting insulin(μU/mL)×fasting glucose(mmol/L)}/22.5

3.3.2. Genotypes and allele frequencies of the IL-6-174G>C and -572C>G, -597G>A and -363G>T polymorphisms

The Genotype distributions for IL-6-174G>C and -572C>G polymorphisms were in Hardy-Weinberg equilibrium in the population as a whole or in nonsmokers and smokers separated. In the -174G>C, 638 (99%) were G/G homozygotes, 6 (1%) were G/C heterozygotes, and none (0%) were C/C homozygotes. Since the C allele frequency at the -174 promoter region of IL-6 was less than 0.01, data for the IL-6 -174G>C polymorphism will not be presented. In the -572 C>G, there were 367 participants (57.0%) with the C/C genotype, 233 (36.2%) with the C/G genotype and 44 (6.8%) with the G/G genotype. The allele frequency of the C allele of IL-6 -572 was 0.751 and that of G allele was 0.249 in the whole study population, which is quite different from those reported in European Caucasians (C>G, C: 0.04 to 0.06) (3,15-18). Nonsmokers and smokers had similar genotype distribution at the -572C>G polymorphism. In nonsmokers, the genotype distribution was 55.6% for C/C, 37.5% for C/G, and 6.9% for G/G. In smokers, the genotype distribution was 59.0% for C/C, 34.3% for C/G, and 6.7% for G/G. On the other hand, IL6 -597G>A (G:A=1:0) and -1363G>T (G:T=1:0) were monomorphic in this study.

3.3.3. Clinical characteristics according to IL-6-572 C>G polymorphism

In smokers, there were no significant genotype-related differences among IL-6 -572C>G genotypes with respect to age, BMI, WHR, alcohol intake, blood pressure, serum glucose, insulin, HOMA-IR, lipid profiles, cigarette and alcohol consumption (data not shown). Similarly, in nonsmokers, differences between genotype groups were not found in theses variables.

3.3.4. Relationship between IL-6 -572C>G polymorphism and circulating levels of IL-6, CRP, fibrinogen or oxidative stress

Fig. 4 showed the genotype effect of the -572C>G polymorphism on serum concentrations of IL-6 and CRP in smokers compared to non-smokers. The raising effect associated with -572G/G genotype on circulating levels of IL-6 was greater in smokers [3.38pg/mL higher than C/C (P=0.042) and 3.65pg/mL higher than C/G (p=0.029), P=0.031] than in nonsmokers [2.04pg/mL higher than C/G (P=0.025), P=0.028], although the genotype-smoking interaction was not statistically significant. There was no evidence of an effect of -572C>G genotype on CRP levels in nonsmokers, however, this polymorphism was associated with a highly significant effect on CRP in smokers (P<0.001) (genotype-smoking interaction P=0.04, adjusted for age, BMI and IL-6). Smokers with the G/G genotype had significantly higher concentrations of CRP (about 130%) than those with C/G or G/G genotype. To extend the analysis of the effect of the -572C>G polymorphism on fibrinogen, ox-LDL and PGF2α, data are presented in Fig. 5 showing the genotype effect on these variables in smokers compared to non-smokers. In nonsmokers, the -572G/G genotype was associated with significantly higher circulating concentrations of fibrinogen (P=0.007) and ox-LDL (P=0.006) than C/G or G/G genotypes and a tendency toward higher urinary excretion of PGF2α (P=0.161). Results were similar for nonsmokers and smokers, but in smokers, the -572G/G genotype was associated with a greater difference in levels of fibrinogen (P=0.001), ox-LDL (P=0.037) and PGF2α (P=0.050). There were no significant genotype-smoking interaction on levels of fibrinogen and oxidative stress.

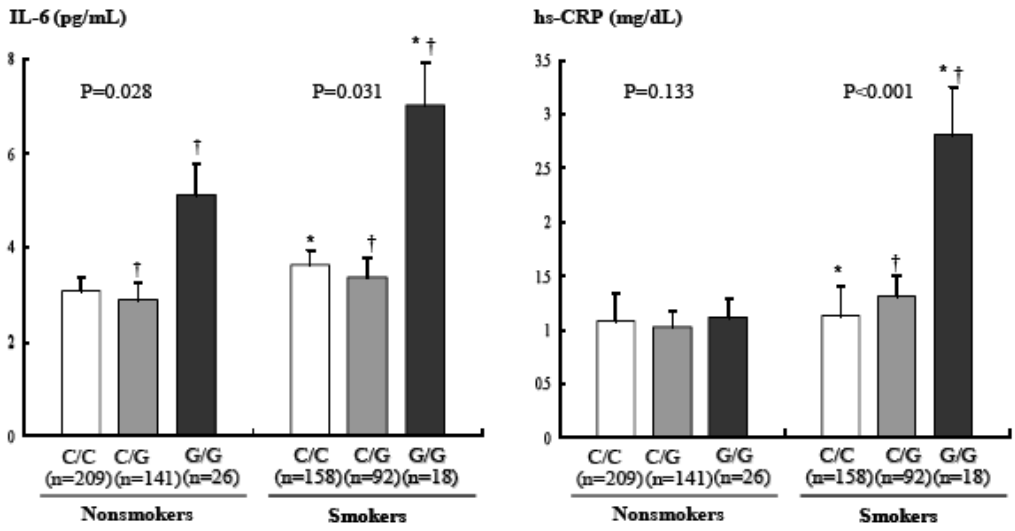

Figure 4. IL-6 and CRP concentrations in relation to smoking and the IL-6 -572C>G polymorphism in 644 healthy men

*p<0.05 between C/C and G/G, † p<0.05 between C/G and G/G based on one-way analysis of variance (ANOVA) followed by Bonferroni test.

All data were log-transformed.

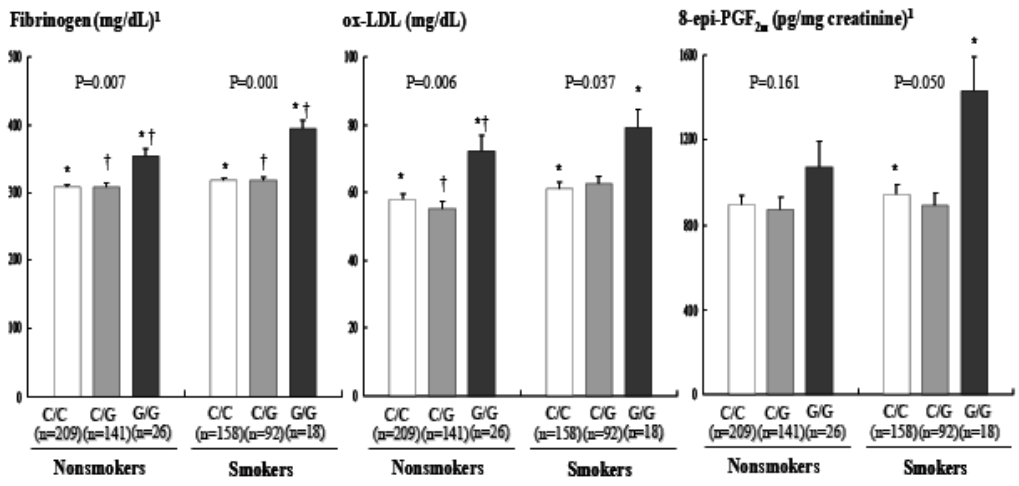

Figure 5. Fibrinogen and ox-LDL concentrations and urinary excretion of 8-epi-PGF2α in relation to smoking and the IL-6 -572C>G polymorphism in 644 healthy men

*p<0.05 between C/C and G/G, † p<0.05 between C/G and G/G based on one-way analysis of variance (ANOVA) followed by Bonferroni test.

[1]log-transformed.

3.3.5. Relation of IL-6 concentrations to CRP and fibrinogen and oxidative stress in all subjects

Pearson correlation test showed that IL-6 had positive correlations with CRP ($r=0.133$, $P<0.01$), fibrinogen ($r=0.273$, $P<0.001$), ox-LDL ($r=0.173$, $P<0.001$) and PGF2 α ($r=0.159$, $P<0.01$). In addition, plasma fibrinogen levels positively correlated with IL-6, CRP ($r=0.328$, $P<0.001$), ox-LDL ($r=0.195$, $P<0.001$) and PGF2α ($r=0.185$, $P<0.001$).

3.4. DISCUSSION

In this study, we observed associations between IL-6 -572C>G promoter polymorphism and circulating levels of IL-6. The -572 G/G genotype (6.8%) was associated with significantly higher circulating levels of IL-6 than the C/C or C/G genotype. Especially, an absolute difference in the mean estimates of IL-6 between the G/G and G/C or C/C genotypes in smokers was greater as compared to nonsmokers. This might indicate that the higher IL-6 concentration in smokers support the exaggeration of the G/G genotype raising effect in an inflammatory situations. Kitamura et al also reported that the -634(-572) G allele had a higher IL-6 secretion capacity than those without the G allele in patients with type 2 diabetes (15). In Caucasian or western population, on the other hand, -572 C allele was associated with the increased IL-6 levels (14), but the association was inconsistent among studies; no association was shown between IL-6 -572G>C genotype and circulating IL-6 levels in patients with MI and in hypercholesterolemic men (8 12, 14). These discrepancies might be due to the differences in subject characteristics among studies; race, population size, sex, disease status and so on. Our study subjects were all men who has non- diagnosed disease in $38 \leq$ age<72 yr.

IL-6 induces the hepatic synthesis of CRP, a known proinflammatory marker of atherothrombotic vascular disease, but IL-6 is not the only determinant of CRP levels (21). Bermudez et al. (22) suggest that IL-6-independent pathways exist to upregulate circulating concentrations of CRP. The -572C>G polymorphism in this study was associated with increased CRP in smokers but showed no association in nonsmokers. Since smokers did not show the genotype-related differences in BMI, WHR and

HOMA-IR levels, independent correlates of circulating concentrations of CRP (23), the differences in CRP production between IL-6 -572C>G genotypes in smokers might be due to the combined effects of smoking and IL-6 levels. Thus, -572C>G polymorphism may exert an influence on CRP concentrations in the presence of a constant stimulus, such as that provided by smoking. This environmental genotype interaction supports previous studies (24, 25), which found an interaction between smoking and polymorphisms of genes participating in the inflammatory response.

The positive relation of IL-6 to fibrinogen in this study supports the previous findings (26-28) that IL-6 plays a major role in upregulation of hepatic synthesis of fibrinogen and is correlated with plasma concentrations of fibrinogen (29, 30). The effects of the G/G genotype on fibrinogen levels were more marked in smokers compared with nonsmokers. However, there was no significant interaction of the IL-6 -572C>G genotypes with cigarette smoke on fibrinogen concentrations. Thus, the effect of IL-6 -572C>G polymorphism on fibrinogen in smokers may be mediated by the modulation of IL-6 on fibrinogen levels through the regulation of fibrinogen synthesis. Also, recently Mannila et al reported that fibrinogen haplotypes are associated with serum IL-6 concentrations in healthy men; the FGA 2224G>A htSNP was significantly associated with serum IL-6 concentration ($P < 0.05$) and the FGG-FGA haplotype (containing the major FGG 9340T and FGA 2224G alleles) was associated with increased risk of MI and with higher IL-6 concentrations (31).

In the inflammatory response to cigarette smoke mediated via proinflammatory gene expression, oxidative stress has been suggested to play a critical role (32). In fact, increased inflammation has been suggested to alter the degree or extent of lipoprotein oxidation (33). The group with the G/G genotype in this study also had higher

inflammation and LDL oxidation such as higher circulating concentrations of IL-6, fibrinogen and ox-LDL as compared with the group with C/C or C/G. Recently, Holvoet et al (33) have found in different ethnic groups that fibrinogen is a strong predictor of ox-LDL, which is known to be associated with subclinical and established CVD. In this study, we also observed the positive relation of fibrinogen and ox-LDL.

The 8-epi PGF2α, the gold standard for quantification of *in vivo* oxidative stress, was observed to be associated with several risk factors for atherosclerosis including diabetes, obesity and hypercholesterolemia (34). However, the subjects of the present study did not show the genotype-related differences in these variables. Therefore, higher levels of 8-epi PGF2α in smokers with the G/G genotype as compared to those with C/C or C/G might relate to the synergistic effects of cigarette smoking and the inflammatory roles of IL-6, CRP and fibrinogen. Our results suggest that interaction of genetic background with cigarette smoke is an important component in inflammatory responses and oxidative stress. However, the number of smokers in the G/G genotype group was small, and this result needs to be confirmed in large population study. Limitations of this study also include the cross-sectional nature of the analyses. We could evaluate the association, but not the prospective prediction of the IL-6 genotype effect on cardiovascular morbidity. The strength of this study is, however, its homogeneous population, consisting of subjects with the same gender, race, and nationality and health status.

In Caucasians, the -174G>C has been reported as functionally important (12), and this SNP is commonly detected with about a 40% prevalence (19, 35-37). However, allele frequency at this position was less than 0.01 in this study population, which is consistent with the previous finding in Korean population (38). At position -572, major allele and

minor allele were in reverse between our subjects (major allele: C, 75%) and Caucasians (major allele: G, ~95%) (19) and the minor allele frequency was also quite different between two populations, which was important in the circulating levels of IL-6 in this study population. In addition, according to Fife's report, allele frequenciesof 579G>A and -1363G>T were 0.402 and 0.088 respectively in 103 simplex families with systemic arthritis. However they turn out monomorphic in our study.

Furthermore, in subjects with homozygous for the -572 G allele, serum concentrations of CRP were significantly higher only in smokers. Therefore, we could indicate that individuals with the G/G genotype of the IL-6 -572C>G polymorphism, who represent about 7% of Korean population, have an elevated inflammatory response to cigarette smoking. From our results, we found that the inflammatory response is probably related to the genetic variation of the promoter region of the IL-6 gene. However, when we suggest a recommendation of stop-smoking, we have to give it to everyone regardless of genetic predisposition to inflammatory response.

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국 문 요 약

남성에서 흡연에 따른 Interleukin-6 -572C>G promotor 유전자 다형성이 염증성 인자에 미치는 영향

Interleukin-6 (IL-6)는 심혈관 질환의 발병기전에 있어서 중요한 역할을 하는 것으로 여겨지는 다면발현성의 염증전구성 물질로서 감염, 외상이나 혈관벽의 염증 활성과 같은 자극 후에 대식세포 등의 여러종류의 세포로부터 생성된다. 그러나 감염이 없이도 IL-6의 생성 증가는 발생할 수 있으며 심혈관질환의 위험인자로 알려진 흡연은 IL-6, C-reactive protein이나 fibrinogen 등의 급성기 염증성 단백질의 증가와 관련이 있다고 알려진 바 있다.

본 논문에서는 IL-6 -572 promotor 유전자 다형성과 염증성 인자와의 관련성을 살펴보고자 남성에서 흡연의 여부와 관련이 있는지를 횡단적 연구를 통해 살펴보았다.

연구는 심혈관 질환, 당뇨병, 고혈압 등의 특정 질환을 가지지 않고 약물을 복용하고 있지 않은 건강한 남성 644명을 대상으로 하였다. 비흡연군 (376명)에서 IL-6 -572C>G promotor 유전자 다형성의 G/G genotype을 가진 대상자 (26명)가 C/C (209명) 혹은 C/G genotype (141명)을 가진 대상자에 비하여 IL-6 (P=0.028), fibrinogen (P=0.007), oxidized LDL (P=0.006)의 혈중 농도가 유의하게 높았고, 흡연군에서도 결과는 GG genotype을 가진 대상자(18명)와 C/C (158명) 혹은 C/G genotype (92명)을 가진 대상자에 비하여 IL-6 (P=0.031), fibrinogen (P=0.001), oxidized LDL (P=0.037) 및 PGF2α (P=0.050)의 혈중 농도가 유의하게 높았으나 비흡연군에 비해 혈중농도의 차이가 더 많았다. 또 IL-6는 CRP, fibrinogen, oxidized LDL, PGF2α 와 양의 상관관계를 가지

고 있음을 보여주고 있었다.

IL-6 -572C>G promotor 유전자 다형성의 genotype에 따른 CRP와의 관련성이 있다는 증거가 비흡연자에서는 없었으나 흡연자에서는 유의한 관련성이 있었다 (P=0.001). 연령, 체질량지수, IL-6를 보정한 후 genotype과 흡연간의 상호작용은 유의한 관련성이 있었다 (P=0.04).

본 연구에서 IL-6의 -174 promotor의 C 대립유전자의 빈도는 매우 낮았으며 (P<0.01) -597G>A , -1363G>T 유전자 다형성 위치의 다형성 형태는 보이지 않았다.

이상의 결과로 본 연구에서는 IL-6 -572C>G promotor 유전자 다형성의 G/G genotype을 가진 경우 C/C 혹은 C/G genotype을 가진 경우에 비하여 염증 반응성 단백질의 혈중 농도가 유의하게 높았고, 흡연군에서는 그 변화가 더 강하다는 것을 알 수 있었다. 이 결과에서 IL-6 -572C>G promotor 유전자 다형성은 장기간 흡연으로 인한 염증성 효과에 더 많은 영향을 줄 수 있고 G/G genotype을 가진 경우 C/C 혹은 C/G genotype을 가진 경우에 비하여 더 높은 산화 스트레스를 가질 수 있음을 제시할 수 있었다.

핵심되는 말 : IL-6 -572C>G 유전자 다형성, 흡연, 염증, 산화 스트레스