Effect of the 252A>G of the lymphotoxin-α gene polymorphism on inflammatory markers in response to cigarette smoking in Korean healthy men

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July 2006

감사의 글

벌써 2년이란 시간이 흘렀다는 것이 믿겨지지 않을 만큼 바쁘게 달려온 것 같습니다. 2년 동안 연세에서 한 배움과 경험은 앞으로 제가 앞으로 살아가는데 있어서 소중한 밑거름이 될 것이라 믿어 의심치 않으며 먼저 저를 지금 여기까지 인도해 주신 하느님께 감사드립니다.

한없이 부족한 저에게 배움의 기회를 주시고 학문에 대한 진정한 열정을 가르쳐 주신 이종호 교수님께 감사 드립니다. 바쁘신 와중에도 항상 인자한 웃음으로 신경 써 주시는 장양수 교수님께 감사 드립니다. 언제나 매력적인 목소리로 강의해 주시고 학생의 입장에서 배려해 주시는 날개없는 천사 정지형 교수님, 최고의 강의, 언제나 화통하신 조홍근 교수님께도 머리 숙여 감사의 말씀을 드립니다.

영양학에 대한 학문의 자극을 주신 제가 이 길을 들어서기까지 가장 큰 영향을 주신 어머니 같은 변기원교수님, 카리스마 넘치시고 정열적이신 손숙미교수님 진심으로 감사합니다

진심으로 걱정해주시고, 언제나 정성껏 대해주시는 감사한 오연언니, 함께 있으면서 최고의 부러움을 자아낸 체육부장 지숙언니, 일이나 실험에 있어서 완벽을 추구하는 꼼꼼하신 지영언니, 이번 논문 나오기까지 가장 많이 도와줄, 느릿한 말투가 그리울 것 같은 따뜻한 눈빛의 수정언니, 넘치는 유머로 연구실에 웃음이 끊이지 않게 하면서도 세심히 신경써 주는 예정언니, 사랑스러운 웃음과 tea time이 언제나 유쾌한 혜진언니, 같이 보낸 시간이 적어서 아쉬울 예비 엄마 윤지숙 언니, 정말 성격이 좋아서 그 누구와도 잘 어울리는 절세미녀 현양언니 감사합니다.

짧게 나마 연구실 생활 같이 했지만 먼저 졸업해서 열심히 일하는 나의 자랑스런 선배들 … 부족한 후배 잘 챙겨주고 가뜩이나 귀 얇은 나에게 격려를 아끼지 않는 나에겐 행운인..똑똑 선배 여진언니, comet, adīponectīn 실험부터 MIE study까지 나의 연구실 생활에서 절대 빼놓을 수 없는 고마운 특별한 인연 슬희언니, 늘 따뜻하게 웃어주던 s혜진언니, 언제나 유쾌한 수경언니, 언제나 꼼꼼하게 일할 땐 일하고 밖에선 잘 챙겨주는 승은언니, 훤칠한 키에 언제나 애정어린 눈빛으로 바라봐 주는 센스쟁이 유란언니, 언제나 특별히 대해줄 예쁜 은정언니, 꼼꼼하게 후배들 가르쳐주고 신경써 주는 현지언니 감사합니다.

그리고 소중한 후배들, cholesterol 동고동락인연으로 맺어진 긍정적이고 마음 따뜻한 민지, 언제나 겸손하면서도 생각이 똑바른 노과의 sīnger 정현,

더 많이 가까워 지지 못한 것이 아쉬울 똑 부러지는 미진, LDL - particle size로 인연이 된 눈웃음이 예쁜 유미, MIQ하면서 고생 많이 한 생각하면 미안한 박수언니 감사합니다. 함께한 시간이 짧아서 아쉬움이 크고 많이 챙겨주지 못한 것이 한없이 미안한 우리 1학기들. 꼼꼼하고 정 많은 애교덩어리 소의, 밝은 모습이 너무 사랑스러울 미란, 훤칠한 키에 시원시원한 여진, 언제나 밝게 인사하는 주영, 사투리가 매력적인 소녀 주연, 밝고 명랑한 귀염둥이 소연, 매사에 적극적이고 열심인 효희, 웃는 모습이 너무 예쁜 시내 그리고 0학기 첫인상과 다르다는데 아직 본 모습을 드러내지 않는 승현이, 그리고 5층에 묻을 열면.. 유일하게 웃으면서 인사해 주는 고마운 정임언니. 그리고 옆 실험실. 언제나 예쁘게 인사해 주는 고마운 유경이, 곧 신부가 되는 너무 부러운 현아. 모두 감사한 인연입니다. 언제나 행복하길 바랍니다.

빼놓을 수 없는 우리 노과 식구들 ~ 언제나 반갑게 웃어주시는 인자하신 노과의 기둥 신경균샘, 멀리 있고 비록 떨어져 생활했지만 후배 꼼꼼하게 챙겨줄 신영언니, 언제나 환한 웃음 잃지 않으시는 차승헌 샘, 노과연의 유일한 미남 홍창형샘, 나이 차이는 엄청나지만 친구처럼 잘 대해주신 감사한 김경철샘, 굉장히 가정적이시고 언제나 친절하신 조석현샘, 모르는 것이 없으시고 학문적 열정이 대단하신 이기호샘, 너무너무 털털하시고 학교에서 조차 뵙기 힘든 배태기샘, 입학 때부터 많이 챙겨주고 이야기를 참 잘 들어주는 봉준오빠, 후배답지 않게 의젓한 강원이, 정말 독특한 계획을 갖고 있어서 졸업논문이 기대되는 태원이, 늘 과묵한 승원씨, 정말 재밌고 귀여운 동생 수혁이 모두 감사합니다.

그리고 정말 소중한 내 동기들.. 가장 많이 부딪히기도 했지만 그만큼 이제 눈 빛만봐도 뭘 말하는지 알 수 있을 것 같은 매력만점 인기쟁이 현진, 이뿐 얼굴에 완전 동안, 그리고 상냥함까지 갖춘 완벽한 매력녀 계영언니, 얼음같이 차가운 줄 알았는데 정말 따뜻하고 강한 Auto Smile 진경이..이 소중한 동기들이 있었기에 무사히 연구실 생활을 하고 졸업까지 할 수 있었던 것 같습니다.

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끈질긴 인연, 잘생긴 오원석 선생님 정말 감사합니다. 철없던 어린시절부터 나의 가치관 형성에 큰 영향을 준 지향이, 나의 대녀, 마음의 안식처 귀옥이, 언제나 즐겁게 사는 이쁜이 보경. 성실하게 살아가는 즐거운 친구 탄, 그리고 옴팡 친구들. 한참 물오른 귀여운 척의 대명사 도리스, 권양 귀

러, 곧 신부가 되는 언제나 고맙고 사랑하는 친구 에로틱 무지개 정숙, 일이 너무 힘들지만 언제 나 웃음을 잃지 않는 고마운 천박한 언어의 마술사 승희, family 즐거운 맥주 친구. 마음씨 좋은 초리 철희, 그리고 즐거운 성당친구 진희언니, 의외로 속 깊은 고니보미 건범. 우리 중 제일 먼저 유부남이 되어 깨가 쏟아지는 우열이, B형 친구 기봉이 기현, 언제나 한결같이 무뚝뚝한 형근이, 모두 감사합니다

나의 특별한 인연 사미 친구들.
같이 입학해서 한 학교 아래 다른 건물에서 석사 과정을 마친 정말 든든한 친구 짠질이 주연, 언제나 행복을 빌어주는 러블리 리니지 정민, 꿈을 위해 나아가는 재미난 친구 민경, 듬직한 감자라는 표현이 안 어울릴 정도로 갸냘프게 변신한 감자 해경이 소중한 인연..계속 이어지길 바랍니다. 정말로 저에겐 과분한 인연들 입니다. 감사합니다.

마지막으로 나의 사랑하는 가족들. 어릴 적부터 길러주시고, 가르쳐 주시고 예뻐 해 주신 정말 사랑하는 두번째 엄마, 아줌마. 오래오래 사셔야 해요. 친딸처럼 아껴주는 사랑하는 우리 이모, 이모부 감사합니다. 언제나 부족한 동생인데 챙겨주고 아껴주는 선희언니 정말 고맙습니다. 언제나 듬직한 버팀목이었던 나의 오빠, 눈물 많고 마음 여린 새언니, 하나밖에 없는 처제라며 진심으로 걱정해주고 행복을 바래주는 멋쟁이 형부, 겉으로는 내색 안 하지만 진심으로 아껴주는 나의 사랑하는 언니 정말 감사합니다. 사랑스런 나의 조카들 어릴 적 내 모습과 많이 닮은 정연이, 뚱땡이 이모를 너무 좋아해주는 고마운 시원이, 하는 짓 마다 너무 예쁜 연우. 마지막으로 성실하게 살아가는 법을 가르쳐 주시고, 늦둥이 딸 낳아서 지금까지 키워주시고 아낌없이 사랑해 주신 아빠, 엄마께 진심으로 감사드리며, 이 작은 결실을 바칩니다.

2006년 7월

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ABSTRACT

Effect of the 252A>G polymorphism of the lymphotoxin-α gene on inflammatory markers of response to cigarette smoking in Korean healthy men

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Background: The systemic inflammatory response is heightened in smokers. This study examined whether the established cardiovascular risk factor, smoking status, might interact with the lymphotoxin-α (LTA) 252A>G gene polymorphism in determining plasma levels of TNF-α and eventually IL-6, adiponectin and CRP downstream in the inflammatory cascade.

Methods & results: After adjustment for age, 208 smokers with an average consumption of 18±1 cigarettes/d had higher levels of TNF-α, IL-6, CRP and urinary excretion of 8-epi $PGF_{2\alpha}$ than nonsmokers (n=272). Nonsmokers with G/G (n=58) had

higher concentrations of TNF-α and 8-epi $PGF_{2\alpha}$ than those with A/A (n=90) or A/G (n=124). TNF-α concentrations were higher in smokers than nonsmokers of the same genotype. Smokers with G/G (n=36) showed higher concentrations of TNF-α than those with A/A (n=65). Smokers with G/G had higher levels of circulating IL-6 and urinary 8-epi $PGF_{2\alpha}$ than those with A/G (n=107) or A/A. Furthermore, smokers with A/G or G/G showed lower adiponectin concentrations than those with A/A. The adjusted model TNF-α levels showed main effects of genotype (F=4.897, P=0.028) and for smoking (F=7.240, P=0.001), as well as the smoking status-genotype interaction (F=3.882, P=0.001). TNF-α concentrations had a positive relation with serum concentrations of IL-6 (r=0.385, P<0.001) and a negative relation with adiponectin (r=-0.123, P<0.05).

Conclusion: Our results suggest that LTA 252A>G polymorphism may modulate the inflammatory effects and oxidative stress of smoking in healthy men. The detrimental effect of smoking on inflammatory markers and oxidative stress is most clearly seen in men of genotype 252G/G, suggesting a genotype-specific interaction with smoking

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Key Word: Lymphotoxin-α gene, 252 A>G polymorphism, smoking, TNF-α, 8-epi $PGF_{2\alpha}$, IL-6, CRP, adiponectin

1. Introduction

Smoking, one of the environmental oxidative stimuli, might be the strongest cardiovascular disease (CVD) risk factors for atherosclerosis (1). Several studies have revealed circulating levels for tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) in smokers are elevated as compared to non-smokers (2-4). This suggests that part of the coronary risk associated with smoking may relate to increased inflammatory activity. Interestingly, only some people similarly exposed to cigarette smoke develop CVD (5), indicating that genetic factors are important determinants of interpersonal susceptibility to biological pathways linking smoking with CVD risk (6).

A physiological role for the -308G>A polymorphism of the TNF-α gene has been indicated in several studies (7-9). However, no association could be found between the -308G>A polymorphism and either circulating levels of TNF-α (10, 11) or myocardial infarction and CVD (8). Since the genes for TNF-α and lymphotoxin-α (LTA) are located close to each other within the human leukocyte antigen class III gene cluster on chromosome 6p (12), another candidate polymorphism that might mediate the cardiovascular risk is the LTA gene. In fact, the LTA 252G allele was found to have higher TNF-α secretary capacity than 252A allele (13), as well as higher circulating levels of TNF-α (14). TNF-α upregulates production of IL-6, a cytokine that causes hepatic production of acute phase proteins (15). Recently, Ozaki et al. (16) provided evidence that the 252A>G polymorphism of the LTA gene affects

susceptibility to MI in Japanese. Therefore, we investigated whether smoking would have possible interactive effects with LTA polymorphisms in determining circulating levels of inflammatory markers such as TNF-α, IL-6, adiponectin, and CRP and its consequence to oxidative stress.

2. Background

2.1 The characteristics of LTA

The proinflammatory cytokine lymphotoxin-a (LTA, LT-α, or TNF-β) is also a key mediator in the initiation of a local vascular inflammatory response. Its action is characterized by the stimulation of adhesion molecule production, thrombogenesis, smooth muscle proliferation, platelet activation, and release of vasoactive agents (17,18,19,20). Lymphotoxin –α is a cytokine produced by T cells that is homologous to and bind to the same receptors as TNF(21-493p) . Tumor necrosis factor (TNF) and lymphotoxin-*a* were isolated more than 10 years ago, on the basis of their ability to kill tumor cells in vitro and to cause hemorrhagic necrosis of transplantable tumors in mice(22). LT- α is structurally similar to TNF-α and also has an important role in the inflammatory response by inducing monocyte migration, as well as by promotion of lymphocyte activation and proliferation (23, 24) but TNF-β is synthesized primarily bt T cell and acts as an autocrine growth factor for Epistein-Barrvis-infectes cells, whereas TNF-α is produced mainly by monocytes, activated macrophages and adipocytes in the obese status(25,26).

LT-α (TNF-β) and TNF-α are proinflammatory cytokines coded for by the *LTA* and *TNFA* genes, respectively. Both cytokines induce apoptosis in cells upon binding to TNF receptor type 1, whereas they induce inflammatory responses by activating NFκB nuclear protein upon binding to TNF receptor type 2 (28).

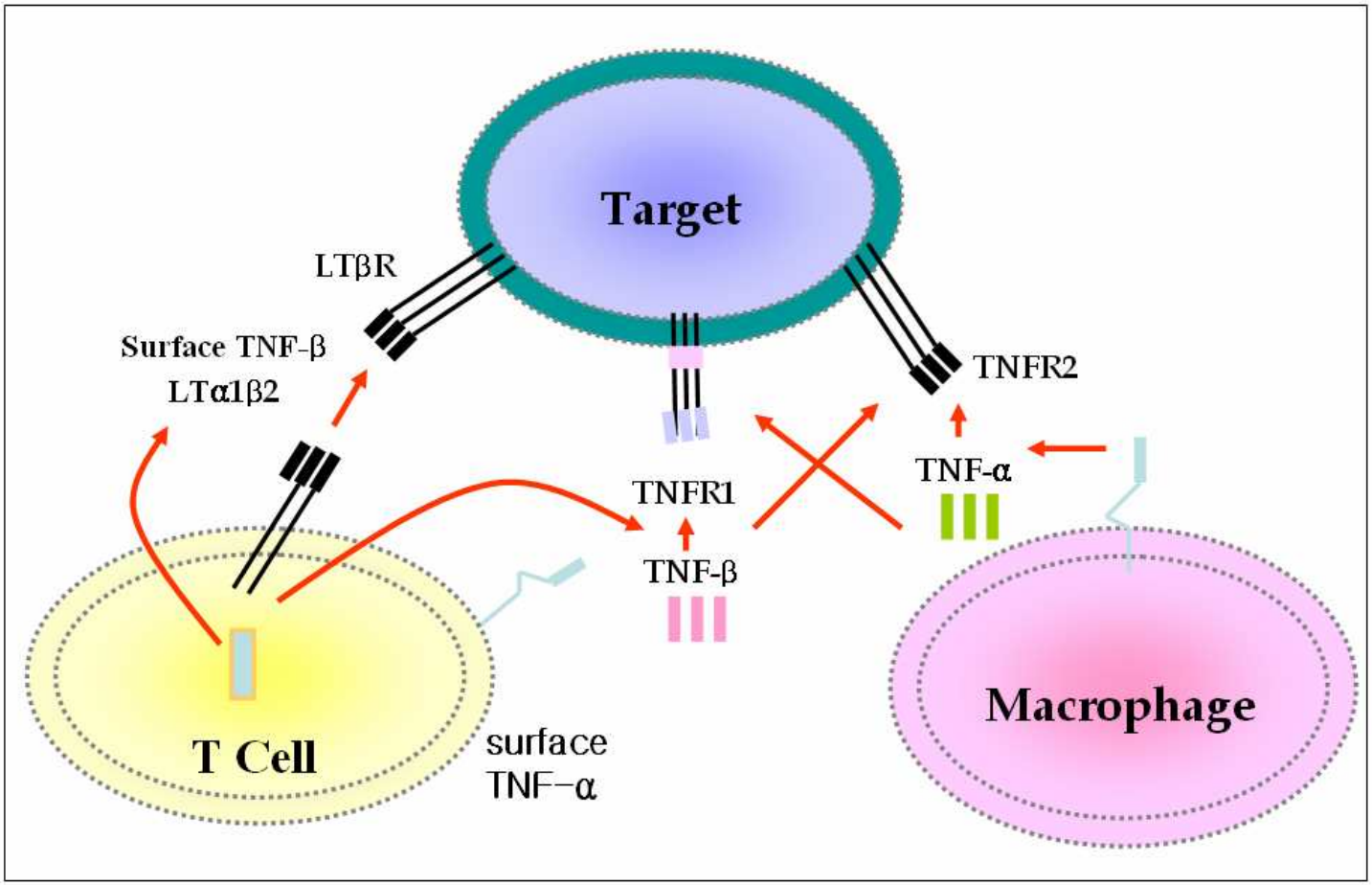
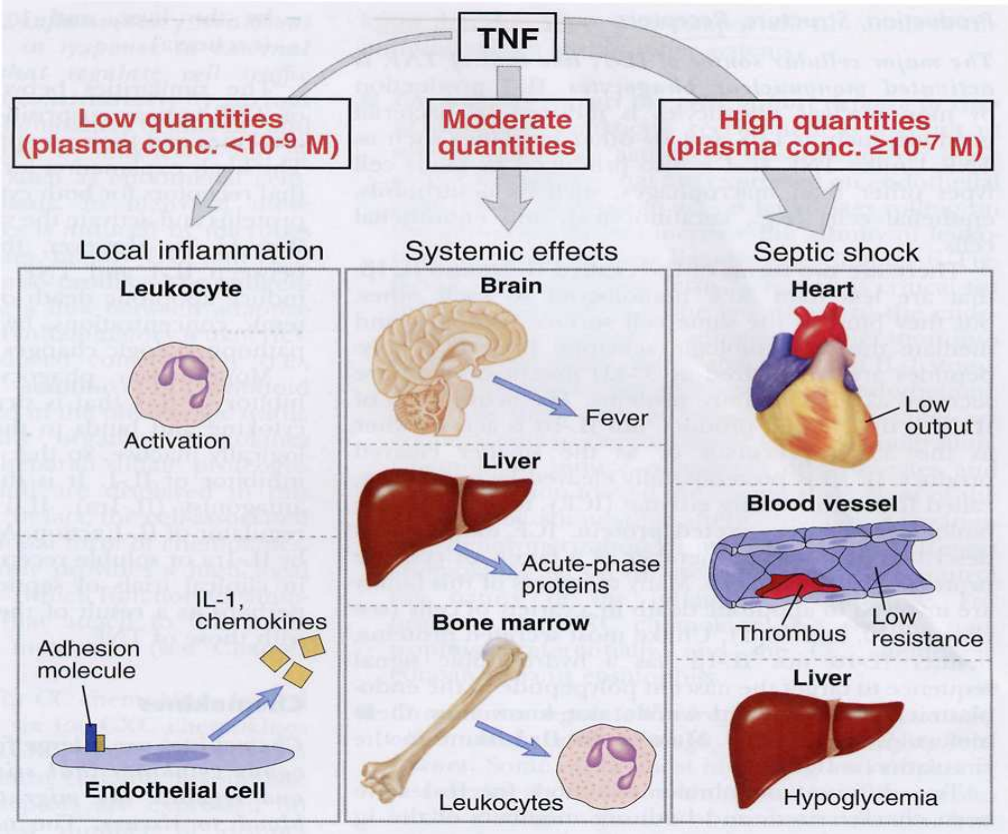


Fig 1. TNF-α, TNF-β form and receptor binding **Adapted from 27**

To compare biologic effects of TNF and LT in vivo, Both cytokine gene was in tumor cell. The both molecules have similar antitimor activity, but LT is less toxic than TNF (29). The tumoricidal and tumorstatic effects of TNF-a and TNF-β raised much interest and led to the rapid cloning of the genes in mouse and man, as well as to the production of rTNF-a and rTNF-β (30).

A reduction of atherosclerosis lesion in LTA knockout mice, but not in TNF-_ knockout mice, suggesting that LTA may be more important in the proatherogenic response (31).



Frg 2. Biologic action of TNF adapted from 21

2.2. Gene polymorphism of LT-α (252G>A)

Lymphotoxin alpha (LTA, formerly named TNF-β) is a member of the TNF family

that plays a critical role in inflammation and is located within the human leukocyte antigen class III gene cluster on human chromosome 6p21. Many studies have focused on TNF- α as a key cytokine involved in heart failure and atherosclerosis (32,33). A polymorphism that affects LT-a expression was found in the first intron of the gene at nucleotide position -252. Because this genetic variation results in the disappearance of a *Nco* I restriction site by replacing A by G, both allelic forms are referred to, respectively, as LTA (5.5 kB) and LTA (10.5 kB). This LTA -252 polymorphism, conserved in both human and mice, is located within a phorbol ester-responsive DNA element (TRE) with high affinity for the AP-1, jun, and c-fos heterodimer transcription factor family. The presence of LTA (5.5kB) allele was shown to result in significantly higher LTA production by phytohemag glutinin-stimulated peripheral blood mononuclear cells due to increased LTA gene transcription. (35).

The mutant allele results in a significantly increased production of LT-α in vitro-stimulated mononuclear cells, related to increased gene transcription (36). This variation results because polymorphisms in the promoter regions of the TNF-α and lymphotoxin-α genes influence the amount of TNF-α produced after an inflammatory stimulus (37). Investigated the discribution of TNF-β gene variants in relation to insulin. C-peptide and lipid levels as is risk factrs for coronary artery disease(CAD).

Increased serum insulin has been shown to bean independent risk factor for CAD(38). TNF-β alleles might either directly accelerate inflammatory processes in atherosclerosis intima or affect the intima indirectly by an increased insulin secretion in pancreatic β-cells(39).

The LTA gene were associated with the onset of myocardial infarction or coronary heart disease. The LTA polymorphism is associated with recurrent cardiovascular events confirms the important role of this cytokine, as is shown in these previous reports and suggests the need for adjunctive treatment for patients with the polymorphism(40).

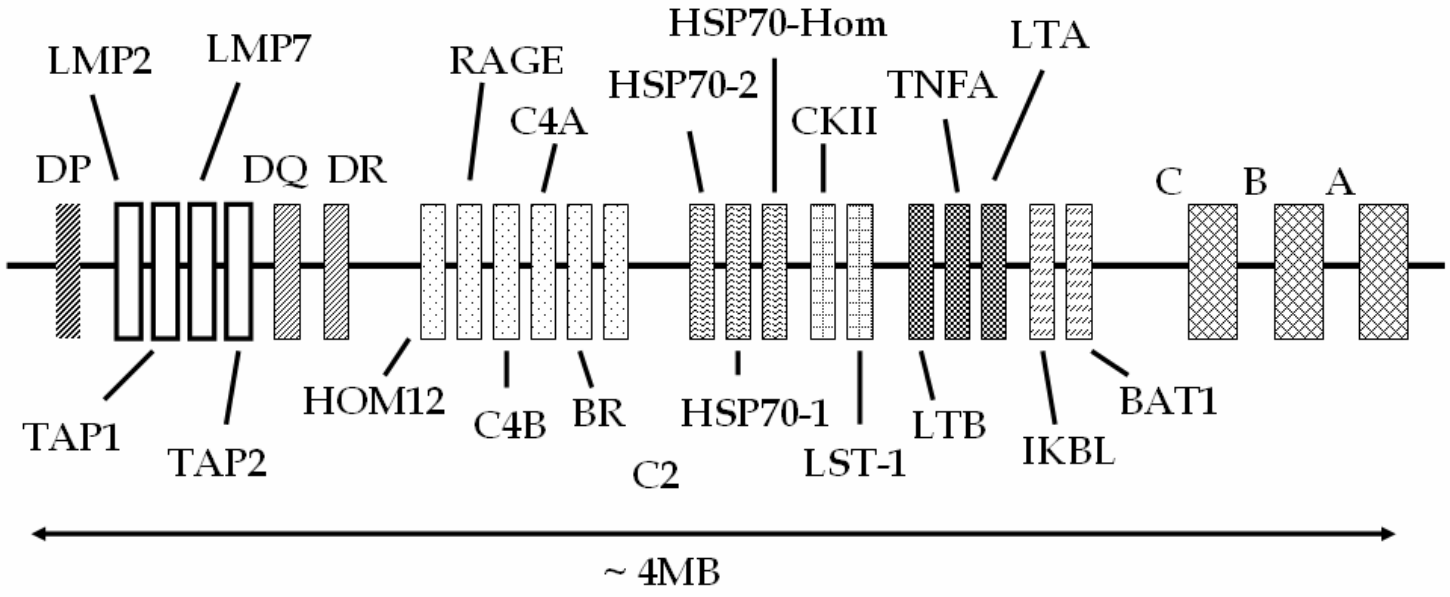


Figure 3. Area short arm in chromosome 6, demonstrating the close proximity of many genes that are involved in inflammatory responses within the human leukocyte antigen(HLA) locous. adapted from 34

2.3. Inflammatory markers and CAD

2.3.1. TNF-α , TNF-β and CAD

Tumor necrosis factor (TNF)-α and TNF-β are mediators of inflammatory responses and have been postulated to contribute to the etiopathogenesis of autoimmune diseases (41,42), molulate groeth and celluar differentiation, and activate blood coagulation(43). Because it proved to be highly toxic in animals and humans, TNF did not fulfill initial expectations that it would be useful in the treatment of cancer. However, considerable evidence suggests that overproduction or inappropriate production of TNF may play a part in various chronic inflammatory diseases(44). Immune pathway is probably involved in the pathogenesis of atherosclerosis . Several reports describe interactions between endothelial cells and macrophages, platelers and T cells. T cells were found 10-fold more often in atherosclerotic thanin normal intima; 70% of these T cells were activated(13,14) and expressed cytokine such as interleukin-1, TNF-α and TNF-β (45,46) .
Schreyer SA et al. reported a reduction of atherosclerosis lesion in LTA knockout mice, but not in TNF-α knockout mice, suggesting that LTA may be more important in the proatherogenic response (47). TNF makes plaques more prone to rupture by stimulating macrophages to secrete metallo proteinases capable of weakening the plaque (48,49). In addition, TNF affects lipid metabolism and may lead to hypertriglyceridaemia by decreasing lipoprotein lipase activity in cultured adipocytes (50) and by increasing de novo fatty acid synthesis in liver (51) .

2.3.2 . Interleukin-6 (IL-6) and CAD

The cytokine, IL-6, has proinflammatory activity by itself and through increasing the levels of IL-1 and TNF-α; all of these have been implicated in atherogenesis. Importantly, IL-6 stimulates liver production of CRP (52).

It regulates humoral and cellular responses and plays a central role in inflammation and tissue injury (53). These effects of IL-6 appear to be mediated through the interaction of IL-6 with its receptor complex (IL-6R). This complex is composed of a ligand-

binding protein (IL-6Rα), and a signal-transducing subunit, (IL-6Rβ or gP 130) (54).

IL-6 can be produced by many vascular cells including endothelium, smooth muscle cells, lymphocytes and macrophages (53). Because of its central role in the inflammatory responses, it is extremely likely that IL-6 is linked to the pathogenesis of CAD. It is a major inducer of acute phase proteins, as well as other cytokines and growth factors (55). In addition, IL-6 itself can activate platelets (56) and has procoagulant activity (57-59) and mitogenic activity for smooth muscles (60).

Large quantities of IL-6 are found in human atherosclerotic plaques (61) and several studies have found a direct link between IL-6 levels and CAD (62). IL-6 levels appear to be predictive of future heart disease (63) and elevated in patients with unstable angina compared with those with stable angina (64).

2.3.3. C-Reactive Protein (CRP) and CAD

C-reactive protein is an acute phase reactant synthesized by the liver and consists of five identical, non-glycosylated peptide subunits linked to form a cyclic polymerase (65).

The inflammatory process is complex and incompletely understood. The inflammatory response is initiated when inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)a are released from distressed tissue(66). These in turn lead to the release of IL-6, the cytokine that is primarily responsible for the induction of acute phase protein production by the liver (67). The panel of changes in protein synthesis involves dozens of proteins, including a large increase in the production and release of CRP, less dramatic increases in the synthesis of other proteins such as fibrinogen, and the inhibition of synthesis of others, such as albumin (68). The role of CRP in atherosclerosis remains to be defined. The presence of CRP immunoreactivity has been demonstrated in vulnerable and ruptured plaque. It is not clear yet whether it is locally expressed or absorbed from the blood stream. The possible mechanisms for direct involvement of CRP in atherosclerosis and thrombosis include complement activation through binding of damaged cell membranes and inducing tissue factor production by monocytes (69-71). The application of these assays during the last few years has made it possible to study CRP in a wide variety of atherosclerotic diseases and to explore its role in predicting cardiovascular events. CRP was first identified as a risk factor in the setting of preexisting cardiovascular

disease (72,73) and its levels were correlated with the presence and severity of coronary, cerebral and peripheral atherosclerosis (74).

2.3.4. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and CAD

Classic PGs, which are formed through the action of PGH synthase isozymes from free arachidonic acid,[7] F_2 isoprostanes are formed in situ from the fatty acid backbone esterified in membrane phospholipids. They are released in response to cellular activation, presumably through a phospholipase-mediated mechanism; they circulate in plasma and are excreted in the urine (75,76).

The urinary 8-iso-prostaglandin (PG) $F_{2\alpha}$ level was used as a measure of oxidative stress. Increased urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$, i$PF_{2\alpha}$-III, 15-F2t- IsoP), one of the more prominent isoprostanes, was foun delevated in several conditions linked to oxidative stress: smoking, pulmonary hypertension, type 1 and 2 diabetes, and obesity (77).

Enhanced urinary excretion of 8-iso-$PGF_{2\alpha}$ has been described in association with both type 1 and type 2 diabetes mellitus, and correlates with impaired glycemic control. Besides providing a likely noninvasive index of lipid peroxidation in this setting, measurements of specific F(2) isoprostanes in urine may provide a sensitive biochemical end point for dose-finding studies of natural and synthetic inhibitors of lipid peroxidation. Although the biological effects of 8-iso-$PGF_{2\alpha}$ in vitro suggest that it and other isoeicosanoids may modulate the functional consequences of lipid peroxidation in diabetes, evidence that this is likely in vivo remains inadequate at this time (78).

2.3.5. Adiponectin and CAD

Adiponectin, an adipocyte-specific adipokine,has been recently revealed to have anti-atherogenic and anti-inflammatory properties.

Decreased plasma adiponectin levels are observed in patients and animal models with obesity, coronary artery disease and type 2 diabetes .Plasma adiponectin concentrations are reported to be increased following weight loss and treatment of coronary artery diseases (79).

Overexpression of adiponectin reduces atherosclerotic lesions in mouse models , whereas adiponectin-deficient mice exhibit excessive intimal response to vascular injury and diet-induced insulin resistance (80).

Plasma levels of adiponectin are significantly decreased in obese patients, and the levels of adiponectin are strongly and negatively correlated with BMI (81). Intriguingly, in patients with type 2 diabetes, plasma adiponectin levels were shown to be prominently prominently lower in patients with CAD than in patients without CAD (82). Plasma levels of adiponectin were significantly decreased in patients with coronary artery disease (CAD) than in age- and BMI-adjusted control subjects (83). adiponectin levels may be particularly associated with macroangiopathy in patients with type 2 diabetes. A recent study reported that hypoadiponectinemia was significantly and independently correlated with CAD even after adjustment for several coronary risk factors (84).

2.4. Smoking status and CAD

Cigarette smoke is a complex mixture of more than 4700 chemical compounds, including benz(a)pyrene, cadmium, nicotine, hydroquinone and high concentrations of free radicals and other oxidants (85) .Oxidative stress plays a critical role in the inflammatory response to cigarette smoke throuth upregulation of redox-sensitive transcription factors and subsequently gene expression (86) .

Smoking is a one of the strongest cardiovascular risk factors for atherosclerotic iseases (87). Of inflammation was on the intermediate casual pathway in atherosclerosis, smoking is an important factor about inflammation. Macrophages are postulated to play an important role in driving the inflammatory response and can be activated by cigarette smoke (88). Smoking as a stress factor seems to lead to different inflammatory responses with presumably different cardiovascular morbidity and mortality (89).

Several studies have revealed increased plasma levels of TNF-α and of CRP in smokers as compared to non-smokers (90). This could indicate that genetic factors are important determinants of the biological pathways linking smoking with cardiovascular disease risk (87).

The specific mechanisms of oxidative injury are becoming increasingly clear. ROS

and specific proinflammatory cytokines such as tumour necrosis factor alpha (TNF-a) activate transcription factors including nuclear factor-kB (NF-kB) via specific intracellular signalling pathways. Translocation of transcription factors to the nucleus from the cytoplasm subsequently initiates transcription in genes with multiple inflammatory functions, including cytokines (TNF, IL-4, IL10, etc), and antioxidant enzymes such as glutathione peroxidase, superoxide dismutases, and catalase (90,91). Heavy, current smokers increased levels of 8-epi-$PGF_{2\alpha}$, which is currencly comsidered the most reliable marker of oxidative stress in vivo (92). Since smoking is also associated with altered levels of IL6, CRP (93) leukocyte count (94), and increased monocyte adhesiveness (95), it appears to be essential to take the smoking status into account with respect to the genotype-phenotype association (88).

3. Subjects and Methods

3.1. Subjects

Four hundred eighty healthy male subjects were recruited either from the Health Service Center during routine check-up visits or by newspaper advertisements. The inclusion criteria were 40≤age<60 years, no history or diagnosis of atherosclerosis, vascular disease, diabetes mellitus, cancer (clinically or by anamnesis) or renal disease and no pathological EKG patterns. None of the subjects were taking medication. Written informed consent was obtained from all subjects and the protocol was approved by 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=272), and subjects who were currently smoking >3 cigarettes per day as 'regular smokers' (n=208)

3.2. Materials and Methods

For laboratory assay, all measurements were done in a single batch at the end of the Study, and the laboratory staff was blinded to the clinical data.

3.2.1. Anthropometric parameters, blood pressure, cigarette smoking and alcohol consumption

Body weight and height were measured in the morning, light clothed without shoes. The body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Waist and hip circumference were measured and waist to hip ratio (WHR) was computed as an indication of the index of body fat distribution. Waist circumference was measured with a flexible tape midway between the lower rib margin and the iliac crest, and the hip girth was measured at the widest part of the hip. Both circumferences were measured in the standing position after normal expiration.

Blood pressure was measured from the left arm while the subjects remained seated. An average of three measurements was recorded for each subject. When the systolic blood pressure was greater than or equal to 140mmHg, or the systolic blood pressure was greater than or equal to 90mmHg, they were classified as having hypertension according to the 6th report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure.

Current cigarette smokers were defined as subjects reporting at least one cigarette per day

Total alcohol intake was expressed as the sum of milliliters of alcohol per week by questionnaires.

3.2.2. Fasting blood collection

To reduce the influence of circadian variation, all blood specimens were collected between 08:00 and 10:00 after the subjects had fasted overnight. Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-hour fast and stored at –70 ℃ until analysis after plasma and serum were separated.

3.2.3. Serum lipid profiles

Fasting serum concentrations of total cholesterol and TG were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd. Tokyo, Japan). After precipitation of serum chylomicron, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol left in the supernatant was measured by an enzymatic method. LDL cholesterol was estimated indirectly using the Friedewald formula for subjects with serum TG concentrations <4.52 mol/l (400mg/ml).

3.2.4. Serum glucose

Glucose was measured by a glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Insulin was measured by radioimmunoassays with commercial kits from Immuno Nucleo Corporation (Stillwater, MN, USA).

3.2.5. Plasma Adiponectin

Plasma adiponectin concentration was measured using 'B-Bridge Human Adiponectin ELISA kit'(B-Bridge International, Inc. 639 North Pastoria avenue sunnyvale, CA 94085 USA). Pre-treated samples readded to an appropriate number of wells of the microtiter plate and incubated. Adiponectin in the sample will be bound by primary anti adiponectin monoclonal antibody immobilized in the well. After washing, the secondary rabbit anti-adiponectin antibody is added to each well and allowed to incubate. The secondary rabbit anti-adiponectin polyclonal antibody will bind to the adiponectin trapped in the well in the 1st reaction. After washing conjugate of horseradish peroxidase and goat anti-rabbit IgG is added to each well and allowed to incubate. The detection antibody will recognize and bind to the rabbit anti-adiponectin antibody trapped in the well in the 2nd reaction. After washing, the colorimetric substrate for the enzyme is added to all wells and incubated. The color development is terminated by the addition of stop solution. The resultant color reaction was read using Victor2 (Perkin Elmer life sciences, Turk, Finland)t 450mn.

3.2.6. Serum Tumor Necrosis Factor-α and interleukin-6

Serum tumor necrosis factor-α (TNF-α) was measured using an enzyme immunoassay (R&D systems, MN, USA). Sample was added to a monoclonal anti-TNF-α antibody which was precoated onto a microplate. After washing away any

unbound substances, an enzymelinked polyclonal anti- TNF-α antibody was added. A substrate solution was added to develop color in proportion to the amount of TNF-αbound. The resultant color reaction was read using a Victor2 (Perkin Elmer life sciences, Turka, Finland) at 450nm and wavelength correction was set to 540nm. Quantification of TNF-α was performed with using the peak area ratio.

Serum interleukin-6 (IL-6) was measured using an enzyme immunoassay (R&D systems, MN, USA). Sample was added to a monoclonal anti-IL-6 antibody which was pre-coated onto a microplate. After washing away any unbound substances, an enzyme-linked polyclonal anti-IL-6 antibody was added. A substrate solution was added to develop color in proportion to the amount of IL-6 bound. The resultant color reaction was read using a Victor2 (Perkin Elmer life sciences, Turka, Finland) at 450nm and wavelength correction was set to 540nm. Quantification of IL-6 was performed with using the peak area ratio.

3.2.7. Serum high-sensitivity C-reactive Protein

Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured using a commercially available high-sensitivity kit, CRP-Latex (II) X2 supplied by Seiken Laboratories Ltd. (Tokyo, Japan) that allowed detection of CRP levels as low as 0.001mg/dL and as high as 32mg/dL. The assay principle is that latex microparticles coated with monoclonal antibodies against CRP reacts with the CRP in the added samples for immunoagglutination reactions. The measurements of these

immunoagglutination reactions were performed on Express Plus auto-analyzer (Chiron Diagnostics Co., MA, USA) using reaction buffer. The absorbance change was calculated at 572nm for 3 min. Because the absorbance change is proportional to the CRP concentrations in samples, CRP values of samples were automatically calculated from a calibration curve prepared with the 5-points CRP (II) H standard (Seiken Laboratories Ltd., Tokyo, Japan) consisted of 5 different concentrations (5, 2, 4, 16, 32mg/dL) of CRP. The normal reference value was less than 3mg/dL.

3.2.8. Urine collection and urinary 8-epi-prostaglandin $F_{2\alpha}$

Urine was collected after 12 hour fast in polyethylene bottles containing 1% butylated hydroxytoluene (BHT) before blood collection. The tubes were immediately covered with aluminum foil and stored at –70 ℃ until extraction. 8-epi-prostaglandin F2α (8-epi-PGF2α) was measured using an enzyme immunoassay (Bioxytech 8-IsoprostaneTM Assay kit, OXIS Internationl Inc., OR, USA). The resultant color reaction was read using Victor2 (Perkin Elmer life sciences, Turka, Finland) at 650nm. Quantification of 8-epi-PGF2α was performed with using the peak area ratio. Urinary creatinine concentrations were determined by the alkaline picrate (Jaffe) reaction, and urinary 8-epi-PGF2α levels were expressed as picograms per milligram creatinine.

3.3.9. LDL particle size

1) Sample preparation of LDL (1.019-1.063g/ml)

After mixing 80ul plasma with 6.4ul solution at d 1.182g/ml, 33ul d 1.019g/ml dilution was over layered. After overnight centrifugation at 42k, 10℃ for 18h using Beckman Ti 42.2 pancake rotor, IDL was removed in the top 40ul. The remaining infranant was adjusted to density by adding 29.4ul solution at d 1.182g/ml and overlayered with 10.6ul solution at d 1.063g/ml. After an overnight centrifugation(42k, 18h, 10℃), LDL was obtained in the top 40ul.

2) Gradient gel electrophoresis

The particle size distribution of LDL by sequential floatation ultracentrifugation was examined non-denaturing gradient gel electrophoresis as described by Nichols et al. Electrophoresis was performed by a pore gradient lipoprotein system (CBS Scientific, CA, USA) using commercially available pre-poured polyacrylamide slab gel containing a linear gradient 2-16% (Alamo Gels Inc., San Antonio, TX USA). The buffer system used within the electrophoresis apparatus (GE-4, Pharmacia) was TRIS Base (90mM), boric acid (80mM), and EDTA (2.5mM), pH 8.3. The gels were Pre-equilibrated for 20minutes at 70 volts. Samples of LDL (d 1.019-1.063) were mixed in a 2:1 ratio with tracking dye made up of 4g sucrose and 1mg bromophenol blue in 10ml of electrophoresis buffer. Approximately 5-10ul of sample containing 5-10ul of protein was applied to the top of the gel and electrophoresed at 20volts for

20minutes and than 70volts for 30minutes and finally 120volts for 24hours. Standards of HMW Beads were used to estimate the relative migration (Rf) rates of each band.

Gels were fixed in 10% (w/v) sulphosalicylic acid for 30minutes, stained with 0.1%

(w/v). Coomassie Brilliant Blue 250 in methanol : acetic aicd : water (4:1:5 v/v/v) for 1hour and destained in 7.5% acetic acid (v/v) in 5% methanol (v/v). The gels were scanned by computer assisted densitometry in a GS-800 Calibrated Imaging Densitometer (Bio-Rad Laboratories, CA, USA). LDL size was calculated with reference to the Rf value of the standards.

3.2.10. Genetic analysis

TNF-β analyzed according to the following method; Genomic DNA was prepared from peripheral blood samples using a Puregene® DNA purification kit (Gentra, Minneapolis, MN, USA), following the manufacturer's protocol. SNP genotyping was performed by SNP-IT™ assays using SNP stream 25K® System (Orchid Biosciences, Princeton, NJ, USA). Briefly, the genomic DNA region spanning the polymorphic site was PCR-amplified using one phosphothiolated primer and one regular PCR primer. The amplified PCR products were then digested with exonuclease (Amersham Biosciences, Uppsala, Sweden). The 5´ phosphothiolates were used in this study to protect one strand of the PCR-product from exonuclease digestion. DNA amplified

by PCR forward primer TTTGGTTTTGGTTTCCTTCTC and sequence PCR reverse primer AAGATGCAGTCAGAGAAACCC, choosen. Genotyping primer is GAGACAGGAAGGGAACAGAGAGGAA. The single-stranded PCR template generated from exnuclease digestion was overlaid onto a 384 well plate that precoated covalently with the primer extension primers, SNP-IT™ primers. These SNP-IT™ primers were designed to hybridize immediately adjacent to the polymorphic site. After hybridization of template strands, SNP-IT™ primers were then extended by a single base with DNA polymerase at the polymorphic site of interest. The extension mixtures contained two labeled terminating nucleotides (one FITC, one biotin) and two unlabeled terminating nucleotides. The final single base incorporated was identified with serial colorimetric reactions with anti-Fluorescein-AP (Roche, Basel, Switzerland) and streptavidin-HRP (Pierce, Rockford, IL, USA), respectively. The results of blue and/or yellow color developments were analyzed with an ELISA reader and the final genotyping (allele) calls were made with the QCR eview™ program.

3.2.11. Statistical analysis

Statistical analyzed 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 with adjustments for age. One-way analysis of covariance (ANCOVA) with a general linear model for adjustment of age was also used, followed by a Bonferroni test to compare the differences in biomarkers among genotype groups in nonsmokers and one-way analysis of variance (ANOVA) with Kruskal-Wallis test in smokers were used. Associations between genotypes and levels of inflammatory markers were determined in smokers and nonsmokers. The null hypothesis was that smokers and nonsmokers with different TNF and LTA genotypes do not differ in circulating levels of inflammatory markers. Using the general linear models to assess whether the LTA genotypes, smoking status and the interaction between LTA 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), gene variant, and an interaction term between smoking status and gene variant were elucidated. Each variable was examined for normal distribution patterns. In the case of an analysis of the possible interaction between the polymorphism and smoking status, both gene variant and smoking status for main effect were eliminated from the corresponding model (97). 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 two tailed value of $P<0.05$ was considered statistically significant.

4. Results

4.1. **Main characteristics of the study subjects**

Table 1 presents the main characteristics of the study population, which included 208 smokers with an average consumption of 18±1 cigarettes/d. There were no significant differences between smokers and nonsmokers (n=272) in body mass index(BMI), waist to hip ratio (WHR), blood pressure, serum glucose, lipid profiles, and alcohol intake. However, smokers were older than nonsmokers (52±0.5y vs 54±0.5y, P=0.003). After adjustment for age, smokers still had higher levels of TNF-α (P=0.002), IL-6 (P=0.023), and CRP (P<0.001) compared to nonsmokers. Moreover, smokers had higher urinary excretion of 8-epi $PGF_{2\alpha}$ than nonsmokers (P=0.04).

Table 1. Main characteristics of the study subjects

	Nonsmokers (n=272)	Smokers (n=208)
Age (years)	51.8 ± 0.49	54.0± 0.55**
Body mass index (kg/m^2)	24.3 ± 0.17	24.4± 0.19
Waist-hip ratio	0.89 ± 0.02	0.89± 0.03
Blood Pressure (BP)		
Systolic BP (mmHg)	125.6 ± 1.00	127.7± 1.16
Diastolic BP (mmHg)	80.6 ± 0.66	81.2± 0.71
Glucose (mg/dL)	90.9 ± 0.66	91.9± 0.92
Triglyceride (mg/dL)	142.4 ± 4.56	141.4± 5.46
LDL-cholesterol (mg/dL)	124.1 ± 2.40	124.1± 5.46
HDL-cholesterol (mg/dL)	47.7 ± 0.71	49.5± 0.96
Total cholesterol (mg/dL)	198.0 ± 2.18	204.4± 2.46
Alcohol intake (g/day)	26.8 ± 2.47	22.5± 2.83
Tobacco (cigarettes/day)	-	18.3± 1.00
TNF-α (ρg/mL)	1.63 ± 0.58	2.06± 0.20**
IL-6 (ρg/mL)	3.12 ± 0.26	4.19± 0.45*
CRP (mg/dL)	0.76 ± 0.06	0.77± 0.66***
Adiponectin (μg/mL)	5.00 ± 0.17	4.80± 0.18

$PGF_{2\alpha}$ (ρg/mg creatinine)	861 ± 29	927± 35*

Mean ±S.E.

*p<0.05, **p<0.01, ***p<0.001 compared to nonsmokers.

4.2. Genotypes and allele frequencies of the LTA 252 A>G polymorphisms

Genotype distributions were in Hardy-Weinberg equilibrium in the population as a whole or in nonsmokers and smokers separated. In the whole population, 32% of A/A, 48% of A/G and 20% of G/G was at LTA 252 polymorphism. The allele frequency of the A allele of LTA 252 was 0.564, corresponding to the allele frequency of G allele was 0.436 in the whole population. The frequency of the LTA mutant allele in the present study is higher than those reported in European Caucasians (ranging form 0.30 to 0.32) (13). Nonsmokers and smokers had similar genotype distribution at the 252A>G polymorphism. In nonsmokers, the genotype distribution was 31% for A/A, 52% for A/G, and 17% for G/G. In smokers, the genotype distribution was 33% for A/A, 46% for A/G, and 21% for G/G.

4.3. Clinical characteristics according to LTA 252 A>G polymorphism

In smokers, there were no significant genotype-related differences among LTA 252A>G genotypes with respect to age, BMI, WHR, alcohol intake, blood pressure, serum glucose, lipid profiles, cigarette and alcohol consumption in smokers (data not shown). Similarly, in nonsmokers, differences between genotype groups were not found in BMI, WHR, alcohol intake, blood pressure, serum glucose, lipid profiles, cigarette and alcohol consumption (data not shown). However, there was a significant association between age and 252A>G genotypes in nonsmokers (A/A: 50±0.5 y, A/G: 53±1 y, G/G: 52±1 y, P=0.041). Nonsmokers with A/G were older than those with A/A (Table2).

Table 2. Clinical characteristics and lipid levels in response to smoking status and the LTA252 polymorphism in healthy men

	Nonsmokers				Smokers			
	A/A (n=90)	A/G (n=124)	G/G (n=58)	P	A/A (n=65)	A/G (n=107)	G/G (n=36)	P
Age (years)	50.3±0.94[b]	53.1±0.68[ab]	51.5±0.94[a]	0.041	52.2±0.11	54.8±0.71	54.6±1.15	0.095
Body mass index (kg/m^2)	2.79±0.44	3.51±0.50	3.63±0.68	0.683	24.7±0.38	24.3±0.27	24.3±0.33	0.678
Waist-hip ratio	0.89±0.00	0.90±0.00	0.89±0.01	0.893	0.89±0.01	0.90±0.00	0.90±0.01	0.424
Systolic BP (mmHg)	126.8±1.56	126.4±1.56	122.3±2.21	0.219	126.9±1.99	127.5±1.59	129.7±3.10	0.703
Diastolic BP (mmHg)	81.5±1.10	80.4±1.03	79.7±1.45	0.582	81.0±1.34	80.7±0.93	83.2±1.88	0.444
Glucose (mg/dL)	91.8±1.13	90.6±1.03	90.2±1.30	0.637	91.5±1.50	91.9±1.46	92.4±1.17	0.948
Triglyceride (mg/dL)	147.7±7.87	135.3±6.99	148.9±9.17	0.532	140.7±9.08	137.6±7.01	154.5±17.6	0.540
LDL-cholesterol(mg/dL)	121.9±4.12	119.2±2.97	120.4±4.87	0.904	124.5±5.00	125.9±2.83	118.0±6.38	0.499
HDL-cholesterol (mg/dL)	47.7±1.30	48.6±1.05	45.4±1.41	0.253	51.3±1.70	49.7±1.41	46.1±1.98	0.199
Total cholesterol (mg/dL)	200.5±3.96	195.9±3.02	198.3±5.02	0.789	206.0±4.90	206.2±3.11	196.1±6.24	0.311
Alcohol (g/day)	27.0±4.76	27.5±3.40	24.5±5.38	0.902	27.0±4.76	27.5±3.40	24.5±5.38	0.902
Tobacco (cigarettes/day)		—			14.7±0.98	15.7±1.04	17.7±0.98	0.196

Data are presented as mean±S.E.

There were no significant differences according to LTA 252A>G genotypes in each group tested by one-way analysis of covariance with a general linear model for an adjustment of age followed by a Bonferroni test.

4-4. Relationship of genotypes of LTA 252 A>G polymorphism and inflammatory markers with oxidative stress

After adjustment for age, in nonsmokers, the 252A>G genotypes in the LTA gene were associated with circulating levels of TNF-α (P=0.041) and urinary excretion of 8-epi $PGF_{2\alpha}$ (P=0.003) (Fig. 4). Nonsmokers with the G/G genotype had significantly higher concentrations of TNF-α and 8-epi $PGF_{2\alpha}$ than those with A/A or A/G genotype. TNF-α concentrations were significantly higher in smokers than nonsmokers of the same genotype (Fig.4). Smokers with the G/G genotype showed higher concentrations of TNF-α than those with the A/A genotype. In smokers, LTA 252 polymorphism was associated with of TNF-α (P=0.029), IL-6 (P=0.013), adiponectin (P=0.040), and 8-epi $PGF_{2\alpha}$ (P<0.001). Smokers with G/G had higher levels of circulating IL-6 and urinary 8-epi $PGF_{2\alpha}$ than those with A/G or A/A (Table 3, Fig.4). Furthermore, smokers with the A/G or G/G genotype of the LTA 252 polymorphism showed lower adiponectin concentrations than those with the A/A genotype(Table 3)

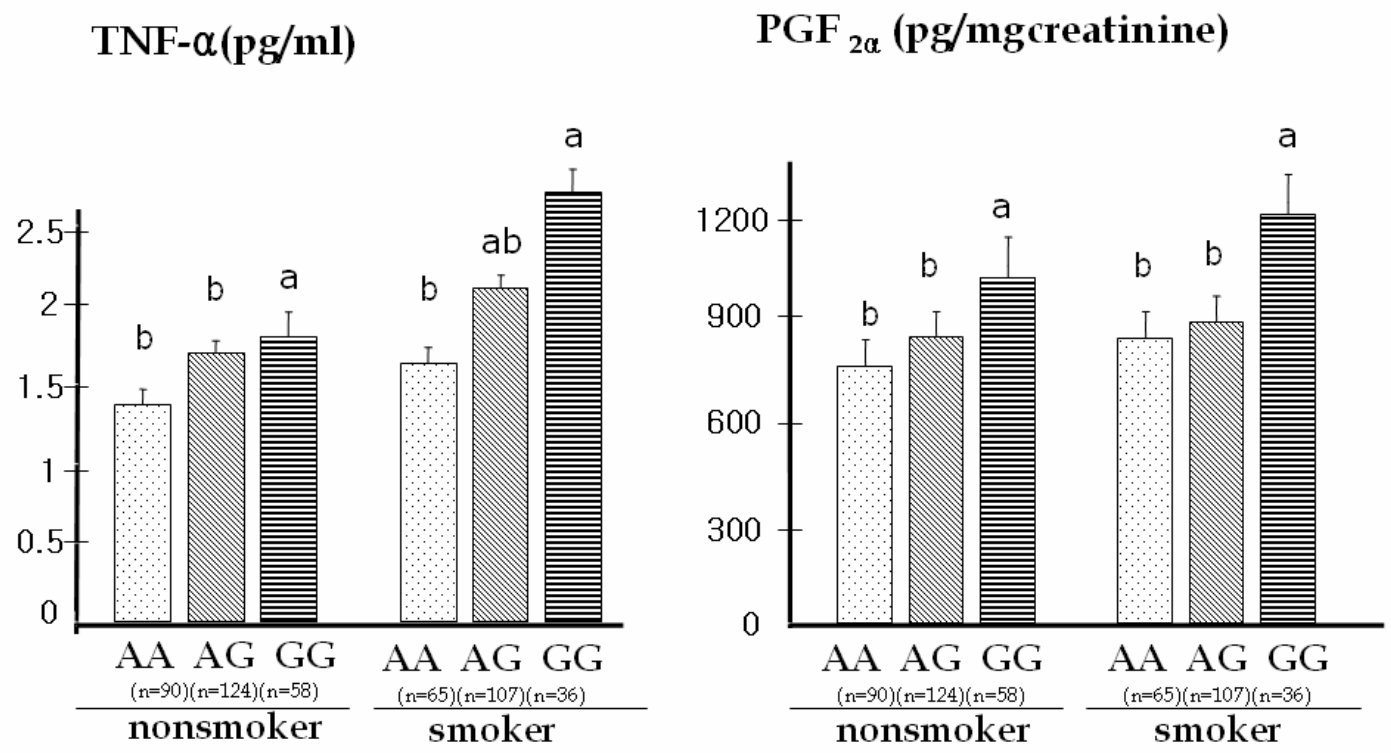


Figure 4. TNF- α concentrations and urinary 8-epi $PGF_{2\alpha}$ concentrations in relation to smoking status and the LTA 252A>G polymorphism in 480 healthy men

Table 3. Levels of inflammatory markers and oxidative stress in response to smoking status and the LTA252 polymorphism in healthy men

	Nonsmokers			Smokers		
	A/A (n=90)	A/G (n=124)	G/G (n=58)	A/A (n=65)	A/G (n=107)	G/G (n=36)
IL-6 (ρg/mL)*	2.51±0.34	3.28±0.38	3.63±0.68	3.42±0.65^{b}	3.66±0.45^{ab}	6.99 ±1.77^{a}
CRP (mg/dL)*	0.62±0.09	0.80±0.10	0.89±0.14	0.67±0.10	0.77±0.10	0.96 ±0.16
Adiponectin (μg/mL)*	5.16±0.32	5.08±0.26	4.73±0.35	5.45±0.37^{a}	4.64±0.25^{ab}	4.19 ±0.31^{b}

Mean±S.E.

Values significantly different ($p<0.05$) within the same subjects group are indicated by different letters. Differences was determined by one-way analysis of covariance with a general linear model for an adjustment of age followed by a Bonferroni test in nonsmokers and by one-way ANOVA with Kruskal-Wallis in smokers, respectively.

*log-transformed.

4.5. Genotypes of LTA 252, smoking status and levels of inflammatory markers

The adjusted analysis regressing log-transformed TNF-α levels against smoking status, LTA genotype, and smoking status-genotype interaction revealed a main effect for smoking status (F=4.897, P=0.028), and for genotype (F=7.240, P=0.001). The interaction-term between genotype and smoking status showed to gain significance (F=3.882, P=0.001), indicating that the effect of smoking on serum levels of TNF-α is affected by the LTA 252 polymorphism (Fig.4). The adjusted model with serum levels of IL-6 failed to show significant main effects for genotype (F=2.671, P=0.070) and for smoking (F=3.266, P=0.072), but did show significant smoking status-genotype interaction (F=2.634, P=0.016). The adjusted model with urinary excretion of 8-epi $PGF_{2\alpha}$ also showed significant main effects of genotype (F=6.748, P=0.010) and for smoking (F=3.684, P=0.049), as well as the smoking status-genotype interaction (F=5.438, P<0.001).

4.6. Relation of TNF-α concentrations to IL-6 and adiponectin levels in all subjects

Pearson correlation test showed that TNF-α concentrations had a positive relation with serum concentrations of IL-6 ($r=0.385$, $P<0.001$) and a negative relation with adiponectin ($r=-0.123$, $P<0.05$) (Fig. 5).

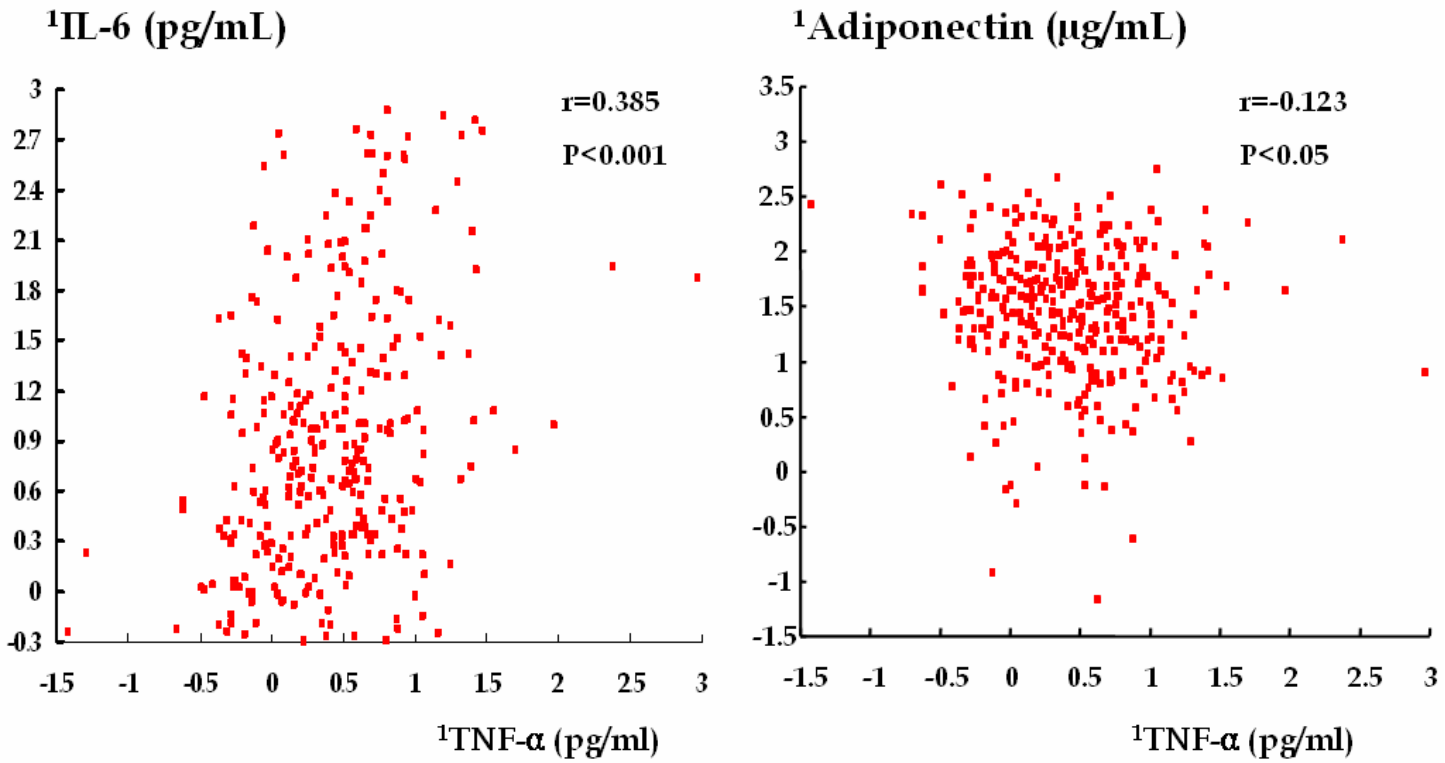


Fig 5. Relation of TNF- α concentrations to IL-6 and adiponectin levels in all subjects

[1]log transformed

5. Discussion

This study investigated the effect of a possible interactive effect between smoking and the LTA 252A>G polymorphism on circulating levels of inflammatory markers. There was a higher absolute difference in the mean estimates of TNF-α levels between the A/A and G/G genotypes in smokers as compared to nonsmokers. This effect of smoking on circulating TNF-α levels in men of genotype G/G may suggest a genotype-specific interactive effect with smoking. Environmental genotype interaction in this study support previous studies (17, 98), which found an interaction between smoking and polymorphisms of genes participating in the inflammatory response. Also, there were the main effects for the LTA252 polymorphism and for smoking status in terms of circulating levels of TNF-α. The difference in circulating TNF-α levels across LTA genotypes is in agreement with previous findings of higher TNF-α secretary capacity in 252G allele than 252A allele (13), as well as higher circulating levels of TNF-α (14).

TNF-α is central to acute smoke-induced inflammation and the powerful proinflammatory cytokine that is a key mediator of inflammation (15). In general, increased TNF-α plasma levels and activity are associated to increased production of interleukins (18). In this study, we also observed a strongly positive correlation between circulating levels of TNF-α and IL-6. In addition, we found an interaction of the LTA 252A>G genotypes with cigarette smoke on IL-6 concentrations (98). Specifically, the studied LTA 252A>G polymorphism was associated with increased

IL-6 in smokers but showed no association in nonsmokers. Age, body mass index (BMI), smoking status, blood pressure, alcohol intake, and presence of diabetes are known to be all independent correlates of circulating concentrations of IL-6 (99). However, smokers of the present study did not show the genotype-related differences in these variables. Therefore, higher levels of IL-6 in smokers with the G/G genotype as compared to those with A/A or A/G might relate to the modulation of TNF-α on IL-6 concentrations through the regulation of IL-6 synthesis (100).

IL-6 induces the hepatic synthesis of CRP, a known proinflammatory marker of atherothrombotic vascular disease, but circulating concentrations of IL-6 and CRP have not been always found to track one another (100). Thus, IL-6 and CRP have independently demonstrated associations with cardiovascular events and mortality among apparently healthy individuals (100-103). Although we found slightly increased CRP levels in smokers as compared to nonsmokers, there was no main effect for the LTA 252 polymorphism or no interaction between smoking and polymorphism on circulating CRP levels. This observation might be partly explained by no significant relation between TNF-α and CRP levels in this study.

The negative correlation between circulating levels of TNF-α and adiponectin in this study support the previous finding (104,105) that TNFα might downregulate adiponectin production. Smokers with the G/G genotype showed the lowest adiponectin concentration with the highest TNF-α levels, even though there was no significant interaction of the LTA 252A>G genotypes with cigarette smoke on

adiponectin concentrations. Therefore, our results suggest that the effect of LTA polymorphism on adiponectin may be mediated by enhanced TNF-α levels in this case smoking.

In the inflammatory response to cigarette smoke through proinflammatory gene expression, oxidative stress has been suggested to play a critical role (106). 8-epi $PGF_{2\alpha}$, the golden standard for quantification of in vivo oxidative stress, is a potent vasoconstrictor and has been shown to be elevated in urine in smokers (107-109). These previous findings were confirmed in our study with healthy men, where LTA252 polymorphism and smoking status had effects on urinary 8-epi $PGF_{2\alpha}$. Levels of 8-epi $PGF_{2\alpha}$ were observed to be associated with several risk factors for atherosclerosis including diabetes, obesity and hypercholesterolemia (110). However, the subjects of the present study did not show the genotype-related differences in these variables. Therefore, higher levels of 8-epi $PGF_{2\alpha}$ in smokers with the G/G genotype as compared to those with A/A or A/G might relate to the synergistic effects of cigarette smoking and the inflammatory roles of the two cytokines, TNF-α and IL-6. Our results suggest that interaction of genetic background with cigarette smoke is the important component in inflammatory responses and oxidative stress, a possible contributor to atherogenesis. In fact, Padovani et al. (111) observed that smokers carrying the LTA 252 A→G mutation had about 2.5 times higher risk of myocardial infarction than smokers with the AA genotype.

Although values for TNF-α, IL6, CRP, adiponectin and oxidative stress were available in this study, one important study weakness was the lack of TNF-β

measurements. The strength of this study is its homogenous populations, consisting of subjects with the same gender, race and nationality and health status. Thus, this study group was controlled for more environmental factors. However, translation of the results to a general population or other race is uncertain and we were examined only one site of LTA gene so required other sites examined.

This study showed a relation between gene variant, smoking and circulating TNF-α levels among healthy men. LTA polymorphism along the inflammatory pathway may account for the differential effect of tobacco consumption on the cardiovascular risk such as inflammatory markers and oxidative stress in individuals who smoke. These results could adopt a clinical practice that would counsel smokers to quit smoking based on a particular gene polymorphism. The adverse effect of smoking in relationship to the LTA genotype on cardiovascular morbidity and mortality must be determined in long-term studies.

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국문요약

건강한 한국남성에게서 흡연과 Lymphotoxin-α

유전자 다형성이 염증성 지표에 미치는 영향

흡연은 복합적인 유독 화합물의 총체로서 사람에게서 산화적 스트레스를 유발시키는 환경 인자로 알려져 있다. 흡연으로 인해 신체 내에서 광범위하고 해로운 활성을 가진 활성산소 종(reactive oxygen species,ROS) 농도가 비정상적으로 높아지면, 체내의 항산화계의 방어 한계를 넘어서게 되고 그 결과 체내에서 산화반응이 강하게 일어난다. 이런 때에 항산화 시스템의 균형이 깨지면 산화적 스트레스 현상이 일어나게 된다. 이러한 현상이 진행되면 노화, 암을 비롯한 여러 질병의 발병 및 진행에 매우 큰 영향을 주게 된다. 또한 lymphotoxin-α의 유전자 다형성은 TNF-α의 농도상승과 관련이 깊은 것으로 잘 알려져 있다.

본 논문은 Lymphotoxin-α 유전자의 252A>G 단일염기다형성(SNP)과 흡연습관이 혈중 염증성 지표의 농도와 심혈관계질환의 위험과의 관련성을 살펴보고자 하였다.

480 명의 40~60 세의 건강한 성인 남성을 대상으로 흡연의 여부에 따라서 비흡연군(272 명)과 흡연군(208)명으로 분류하였다.

Genotype의 결과를 보면 흡연자, 비흡연자간의 genotype별 차이는 나타나지 않았다. 그러나 흡연자, 비흡연자 그룹간에 TNF-α, IL-6, CRP, $PGF_{2\alpha}$ 의 농도가 비흡연자에 비하여 흡연자에서 유의적으로 높게 나타났다.($p<0.05$).

Genotype별로 살펴보면 TNF-α와 $PGF_{2\alpha}$ 의 농도가 두 그룹 모두에게서 G/G genotype을 가진 그룹에서 유의적으로 높게 나타났다.

또한 IL-6와 adiponectin은 비 흡연자 그룹에서는 genotype별로 차이가 나타나지 않았지만 흡연자 그룹에서는 IL-6 의 경우 G/G genotype을 가진 경우 유의적으로 높게 측정되었고, adiponectin의 경우에는 G/G genotype을 가진 경우에 비하여 A allele을 가진 경우 유의적으로 높게 측정되었다. 반면 CRP의 농도는 두 그룹 모두에서 genotype별 차이가 나타나지 않았다.

그리고 모든 대상자들을 대상으로 TNF-α농도를 기준으로 보았을 때 adioponectin

과는 음의 상관관계(r=-0.123, P<0.05)를 얻을 수 있었고, IL-6와는 양의 상관관계 (r=0.385, P<0.001)를 갖는 것으로 조사되었다.

흡연과 genotype, 흡연-genotype interactive effect로 나누어 보았을 때, TNF-α는 흡연에 대해서 (F=4.897,P=0.028), genotype(F=7.240,P=0.001), 흡연-interactive effect (F=3.882,P=0,001)로 유의적인 효과를 보였고 8-epi $PGF_{2\alpha}$를 보면 genotype (F=6.748, P-0,010), 흡연(P=3.684, P=0.049), 흡연-genotype interactive effect(F=5.438, P<0.001)로 결과가 나왔다. 반면 IL-6의 경우 genotype(P=2.671, P=0.070)이나 흡연습관(P=3.266, P=0.072) 을 단독으로 보았을 때는 IL-6의 농도 상승에 끼치는 영향이 유의적이지 않았지만, genotype과 흡연 습관의 interactive effect를 보니 영향력이 유의적으로 미치는 것(F=2.634, P=0,016)으로 나타났다.

본 연구의 결과를 통해 보았을 때 건강한 한국인 성인 남성에게서 흡연여부와 Lymphotoxin-α 유전자의 252A>G 단일염기다형성이 혈장 TNF-α, IL-6, adiponectin 농도와 소변 내 $PGF_{2\alpha}$ 의 농도와 관련이 있으며 이러한 염증성 지표에 대한 영향력으로 보았을 때 Lymphotoxin-α 유전자의 252A>G 단일염기 다형성과 흡연은 심혈관계질환 위험요소와 관련이 있다고 사료된다.

핵심되는 말 : Lymphotoxin-α, 252A>G, 흡연, TNF-α, IL-6, $PGF_{2\alpha}$, adiponectin, 심혈관계 질환