The single nucleotide polymorphisms of the perilipin (PLIN) gene are associated with CAD and systemic inflammatory markers in Koreans

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많은 시간을 공유하고 소중한 10년 우정을 지켜준 FF Family 미의 기준 이쁜달 융혜윤이, 똑소리 나는 리더 혜봉혜영이, 진정한 선생님이 될 명뽕명현이, 머찐 사진작가 굴하혜정이, 같은 연애관을 가진 팔등신 써니유선이, 영원한 효리 미소천사 수달수미…하늘에서 우리를 지켜주고 있을 웃음이 참 예뻤던 민영이에게도 진심으로 감사의 마음을 전하며 그녀들의 앞날에 행복과 좋은 일만 가득하길 기원합니다. 가장 힘든 시기에 많은 힘이 되어주고 예쁜 추억을 만들어준 오꼬가족 매너 좋은 재광오빠, 이제는 외롭지 않은 찬용오빠, 엄마가 된 아기보다 더 아기 같은 은희언니, 나의 영원한 달링 수연이, 눈웃음이 너무 예쁜 혜은이, 따뜻하고 넉넉한 강명이 너무 너무 고맙고, 함께할 멋진 여행을 기대합니다. 멋진 교수의 길을 걷고 있는 지영언니, 현명하고 좋은 선생님이 될 보연이, 유쾌상쾌통쾌 MD가 될 현정이, 변함없이 챙겨주고 끈끈한 정이 무엇인지 알게 해준 나의 소중한 인연들 현주, 미영, 영욱오빠, 광현오빠, 준녕오빠에게도 감사의 마음을 전합니다. 이 자리에 있기까지 너무나 많은 것을 주고 큰 버팀목이 되어준 수인언니에게 표현할 수 없을 정도의 깊은 감사의 마음을 전하며 늘 변함없는 애정과 관심으로 든든한 후원자가 되어 주고 친구보다는 가족이라는 이름이 어울리는 나의 베스트 명아, 경수 또 다른 부모님이 되어주신 어머님, 아버님 진심으로 감사하고 영원히 사랑합니다.

마지막으로 이세상 누구보다 사랑하는 나의 가족에게 고마움과 감사함을 전합니다. 늘 내 의견을 존중해주고 양보해주고 언제 어디서든 큰 힘이 되어준 하나뿐인 우리 오빠에게 지면을 통해 감사의 마음을 전합니다. “부모님”이라는 이름으로 표현할 수 없을 정도의 무한한 사랑을 베풀어주시고 많은 것을 희생해 주신 아버지, 어머니께 죄송한 마음을 먼저 전하며 너무나도 예쁜 딸로 키워주셔서 진심으로 감사합니다. 존경합니다. 사랑합니다.
모든 일에 최선을 다하는 사람이 될 것을 약속 드리며 두 분을 향한 딸의 사랑을, 작고 부족하지만 2년 동안의 노력의 결실인 이 논문을 부모님께 바칩니다.

2006년 1월

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ABSTRACT

The single nucleotide polymorphisms of the perilipin (PLIN) gene are associated with coronary artery disease and systemic inflammatory markers in Koreans

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Background: We investigated the association of single-nucleotide polymorphisms (SNPs) at the PLIN locus with coronary artery disease (CAD) and systemic inflammatory markers because of the potential central role of perilipins in lipid metabolism and adipocyte lipolysis, which could be of pathogenic importance in CAD.

Methods & Results: Of 7 PLIN SNPs (6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T and 14995A>T), significant differences in genotype distribution were observed for SNPs at positions 10076C>G (P=0.02) and 10171A>T (P=0.03).

Because of the positive linkage disequilibrium (D´=0.974, R^2=0.944, P<0.001) between those two SNPs, we examined three major haplotype groups; CA/CA (C/C at 10076 and A/A at 10171), CA/GT and GT/GT haplotypes (G/G at 10076 and T/T at 10171) in CAD patients and control controls, respectively. The absence of CA homozygosity was associated with significantly higher risk of CAD (OR 1.48 [95% CI 1.13-1.94, P=0.004 after adjusting for age]. CAD patients with GT/GT (n=43) had 31% higher free fatty acid (FFA) levels than those with CA/CA (n=196), whereas CAD patients carrying CA/GT (n=197) had 14% higher fasting FFA. CAD patients with GT/GT haplotype showed higher concentrations of triglyceride, LDL- and total-cholesterol and TNF-α (CA/CA:1.5±0.1, CA/GT:1.8±0.1, GT/GT:2.0±0.3pg/ml, P=0.048) and lower levels of adiponectin (CA/CA:4.5±0.2, CA/GT:3.9±0.2, GT/GT:3.7±0.4μg/ml, P=0.025). CAD patients with CA/GT or GT/GT showed higher mean concentrations of IL-6 and CRP than those with the CA/CA haplotype. In the subset who underwent CT measurements, compared with controls CAD patients showed 29% higher visceral fat areas at L1 (P<0.005) and 15% higher ones at L4 (P<0.05).

Conclusion: PLIN locus, particularly SNP10076C>G/10171A>T haplotype, is a determinant of CAD risk in Koreans. This haplotype may be a significant genetic predictor for lipolysis. In addition, the PLIN gene may be involved in lipid metabolism and systemic inflammation in CAD patients with high visceral fat.

Key Word: PLIN variant, CAD, free fatty acid, TNF-α, IL-6, CRP and adiponectin

1. INTRODUCTION

Perilipin is the predominant protein associated with adipocyte lipid droplets. The key roles of perilipin in regulating lipid storage in adipocytes and the accumulation of body fat have been demonstrated in both in vitro and in vivo studies (1-5). Genetic variation at the perilipin gene (PLIN) has been associated with modulation of the perilipin content and lipolytic rate in humans (6). Consistent with those functional observations, we have found significant associations between genetic variants at this locus, body-weight and obesity risk in several ethnic groups (7-9); however, their relation to other cardiovascular disease (CVD) risk factors or to CVD risk itself were not analyzed.

The obese state, particularly visceral adiposity (6), is characterized by low-grade systemic inflammation and elevations of C-reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) (7) which have been suggested as one of the links between obesity and increased CVD risk. In fact, the visceral fat area in the patients with coronary artery disease (CAD) was significantly higher than in the controls with similar age and body mass index (BMI) (8,9).

Abnormal increases of visceral adipose tissue mass causes the dysregulation of adipokines and cytokines and the elevation of plasma nonesterified fatty acids (NEFA) by increased activity of HSL-mediated lipolysis (10,11). Production and release of a variety of adipokines and cytokines from adipose tissue have been found to interrelate with each other (12,13). The overexpression of perilipin in adipocytes

inhibits TNF-α mediated stimulation of lipolysis (1,14). Conversely, perilipin ablation leads to increased lipolysis in fat cells (15,16) resulting in a lean mouse (16). However, these mice also developed glucose intolerance and insulin resistance more readily, probably due to the elevated levels of NEFA (16).

In this study, we investigated the association of SNPs at the PLIN locus with CAD risk and we examined whether the potential effects were mediated through levels of adipokines, cytokines and other systemic inflammatory markers which are related to the pathology of CAD.

2. BACKGROUND

2.1. Perilipin in Lipid Metabolism and Adipocyte Lipolysis

The related disorders of obesity and diabetes are increasing to epidemic proportions. The role of neutral lipid storage and hydrolysis, and hence the adipocyte, is central to understanding this phenomenon. The adipocyte holds the major source of stored energy in the body in the form of triacylglycerols (TAG). However, beyond the initial signaling cascade, the mechanistic details of this lipolytic reaction have remained unclear. Work in recent years has revealed that both hormone-sensitive lipase (HSL), generally thought to be the ratelimiting enzyme, and perilipin, a lipid droplet surface protein, are required for optimal lipid storage and fatty acid release. There are multiple perilipin proteins encoded by mRNA splice variants of a single perilipin gene. The perilipin proteins are polyphosphorylated by protein kinase A and phosphorylation is necessary for translocation of HSL to the lipid droplet and enhanced lipolysis.

2.1.1. The Lipid Storage Droplet / The Perilipin

Perilipin A and B are identical through their amino terminal 406 amino acids, thereupon their sequences diverge. Concomitant with the phosphorylation of HSL by PKA is phosphorylation of the perilipins. Perilipin is likely to undergo a conformation change. This change is observable by fluorescence microscopy as both a 'fraying' of the lipid droplet surface and a fragmentation of larger droplets into smaller ones (24). These observations have prompted an exploration of the role the perilipins play in cAMP-regulated lipolysis. Two approaches have been employed to study this phenomenon: knockout mice and tissue culture models. This decrease resulted from constitutively high basal adipocyte lipolysis, which we attribute to the absence of the protective coating of perilipin at the lipid droplet surface.

The deletion of perilipin was also able to reverse the obesity found in db/db mice (25), and to prevent the weight gain associated with feeding with a high fat diet (26) but it may not have reversed diabetes in these animals. Further, fed glucose and insulin values of perilipin-null mice revealed that nearly twice the amount of insulin was required to maintain euglycemia. This insulin resistance may not be solely due to fatty acids from adipose tissue. Plasma fatty acid levels were paradoxically lower in perilipin-null animals than in their wild type littermates. A graph of gonadal fat pad mass vs. plasma leptin revealed a curve that was nearly four times steeper for perilipin-null compared to wild type mice. This ameliorative effect of leptin has been observed in other lean lipodystrophic animals as well (27, 28).

Perilipin A appears to block lipolysis by restricting the ability of HSL to associate

with the droplet surface (29). Fig. 1 shows our current model for PKA mediated adipocyte lipolysis. By contrast, phosphorylated perilipin A is required for the normal interaction of HSL with the lipid droplet surface. This requirement for perilipin-coated lipid droplets to facilitate HSL translocation was demonstrated in cultured embryonic fibroblasts that had been differentiated into adipocytes. In contrast to cultured adipocytes from wild type animals, those from perilipin-null animals failed to exhibit translocation of HSL under stimulated conditions.

This finding was reinforced by a reconstruction of the HSL translocation reaction in CHO fibroblast expressing perilipin A plus GFP-HSL. GFP-HSL only migrated to lipid droplets upon elevation of PKA activity in cells that also expressed perilipin A on their lipid droplets. In the absence of perilipin A or in the presence of perilipin A in which the 3 most Nterminal PKA sites had been mutated, HSL translocation did not occur. Collectively this work and the gene mutation studies discussed above put forth a new mechanism of lipolysis.

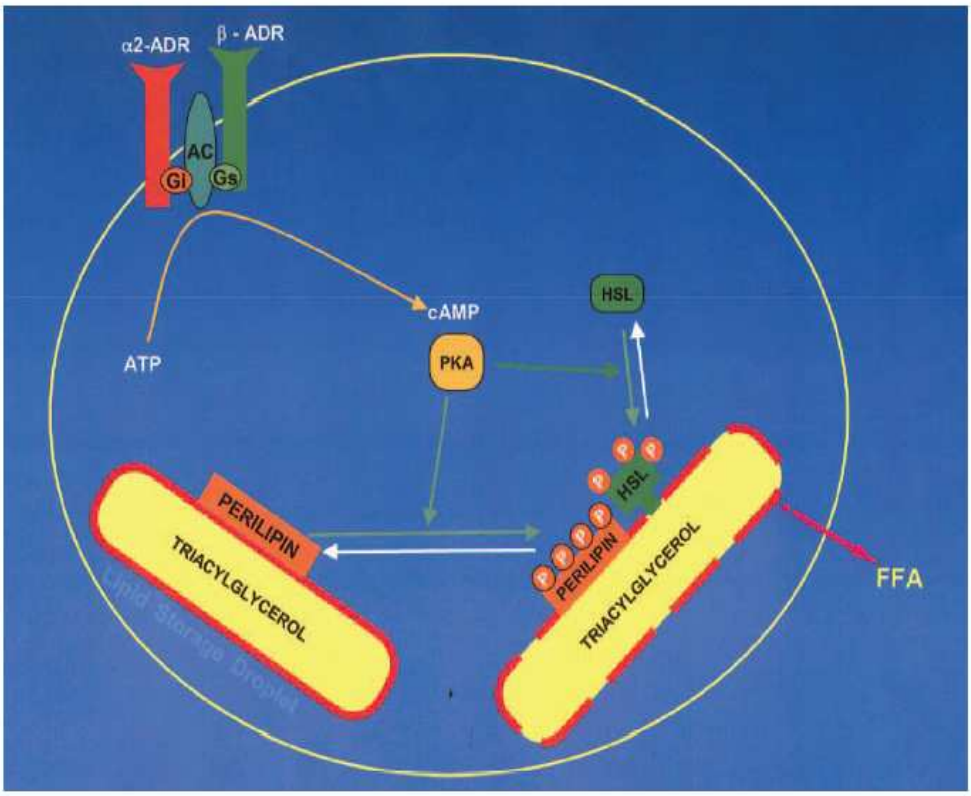

Figure 1. Model of PKA-stimulated lipolysis in adipocytes.

Lipolysis is initiated by adrenergic stimulation at b adrenergic receptors and activation of adenylyl cyclase (AC), leading to elevation of cAMP and activation of protein kinase A that phosphorylates both HSL and perilipin A. HSL then migrates to the lipid droplet surface and encounters its TAG substrate. The phosphorylated perilipin Aundergoes a change in conformation that accommodates the attachment of HSL to the droplet surface.

2.2. The Genetics of Perilipin in Obesity

Perilipin is a lipid droplet surface protein present in adipocytes and steroidogenic cells (30-31). Functional data suggest that perilipin acts as a barrier against the hormonesensitive lipase mediated lipolysis of triacylglycerols in intracellular lipid droplets (33,34). The role of perilipin on body fat accumulation has been demonstrated in vivo using knockout mice models. The data from animal models have consistently shown that targeted disruption of the perilipin (PLIN) gene results in mice that are leaner than controls and are resistant to diet-induced obesity (35,36). In humans a recent report has shown an association between genetic variation in the PLIN gene and both adipocyte perilipin content and lipolysis rate in 117 Swedish obese women (37). Moreover, we have demonstrated for the first time that common variants at the PLIN locus are associated with body mass index (BMI) and obesity risk in women from two different studies carried out in Spain and the United States (38,39), suggesting that PLIN variation may play a role in human obesity.

However, to confirm this role replication of our previous results in other populations is needed. It has long been noted that different race/ethnic groups experience dramatic differences in their susceptibility to obesity. Although differences in life-style factors might account for some of the observed differences, genetic variability could play a major role in the interpopulation difference, as shown in twin studies (40,41). In this report we focus on a multiethnic Asian population from Singapore, where there are substantial ethnically related differences in the

prevalence of overweight and obesity (41): Malays have the highest prevalence, followed by Indians and Chinese, in whom the prevalence of obesity is very low.

2.3. Obesity and Coronary Artery Disease

Obesity is an independent risk factor for the development of coronary artery disease (CAD). Obesity also increases risk for CAD indirectly through its association with insulin resistance, hyperlipidemia, and hypertension. An increased accumulation of fat in the intraabdominal cavity, termed visceral adiposity, is highly correlated with an adverse coronary risk profile. In patients at risk for coronary artery disease, the treatment of obesity results in an improved coronary risk profile. The prevalence of obesity is extremely high in coronary populations, yet the effect of weight loss on cardiovascular outcomes in CAD patients has received relatively little attention. Observational studies in the cardiac rehabilitation setting showed that patients who lose weight and exercise show an improvement in coronary risk profile.

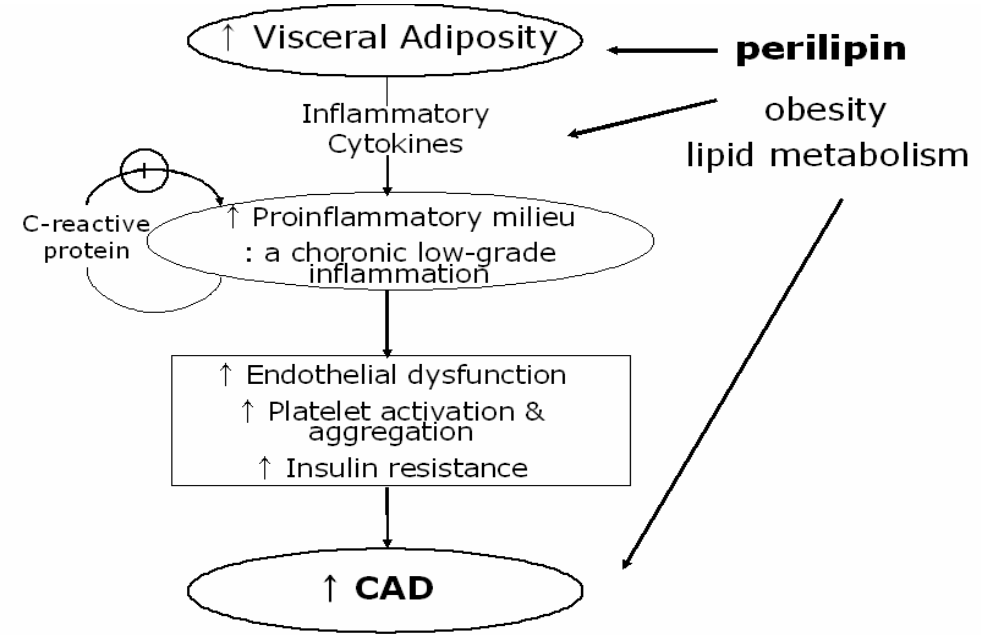

Figure 2. Visceral adiposity and Atherosclerosis

2.3.1. Cardiovascular Disease

Obese subjects have increased risk of cardiovascular disease (CVD), and this is true especially for the abdominal or central type obesity. Obese individuals with central fat distribution are at higher risk for heart disease than those with peripheral type of obesity. Classically, weight cycling has been linked to a higher cardiovascular risk.

However, it appears that weight cycling in obese subjects of both genders is not associated with cardiovascular risk factors in an independent manner(42) and in middleaged men does not directly increase the risk of death. The connection between

obesity and CVD is not simple.(43) The atherosclerotic process is apparently regulated by inflammatory mechanisms(44) and systemic inflammation has been linked to insulin resistance in a large number of human population studies.

2.3.2. Heart Disease

Obesity is associated with several heart abnormalities. In a study of the American population, there was a significant increase in the prevalence of heart disease, diabetes, hypertension and hypercholesterolemia with increasing body weight in all gender, racial and socioeconomic groups(45). Obesity is associated with accelerated coronary atherosclerosis and long-term longitudinal studies demonstrate that obesity predicts coronary atherosclerosis in an independent manner.

The risk for developing coronary artery disease is increased 3.3-fold in American women with a BMI over 29 kg/m2, in comparison with women with a BMI below 21 kg/m2.(46) Moreover, a WHR of R0.92 is associated with a 3-fold increased risk of coronary heart disease.(47) IL-6, a cytokine produced by adipose tissue, seems to play a key role in the development of coronary heart disease associated with obesity through different metabolic, endothelial and procoagulant mechanisms.(48) Left ventricular hypertrophy is frequent in obesity, and is not just related to concomitant hypertension: increases in stroke volume, cardiac output and diastolic dysfunction are seen in obese subjects without hypertension. The changes affecting the left ventricle have been related to sudden deaths in these patients. Not only the left

ventricle but also the right ventricle presents some changes. As a consequence of left ventricular dysfunction or the coexistence of OSA and/or obesity hypoventilation syndrome, pulmonale can occur.

Therefore, for all these reasons heart failure in obesity is frequently biventricular. Obesity can also be the cause of arrhythmias, and the prolonged QT interval also seen in obesity might be a predisposing factor for sudden death.(49) Also, in patients with morbid obesity, we have demonstrated that although the cardiopulmonary capacity appears to be normal, the exercise capacity is significantly reduced, as compared to control subjects.

2.4. Adipose tissue, Inflammation, and Cardiovascular Disease

Hypercholesterolemia was, for many years, the predominating pathogenic theory of atherosclerosis. Although some aspects of this model still hold, we now know that the connections between obesity, fatty arteries, and a vulnerable heart are far more complicated and that inflammation plays a major part in disease progression. Cardiovascular disease (CVD) is associated with elevated markers of systemic inflammation, including C-reactive protein (CRP) and members of the coagulation cascades.(50) Elevated levels of these proteins were also associated with infarction risk factors, such as obesity, diabetes mellitus, and angina pectoris.(51) Despite these intriguing associations, the increase in systemic inflammation was generally thought to be a result of local atheromatous inflammation.

The pathogenic significance of systemic inflammation was mostly eclipsed by the vigorous advances in lipid research. Subsequent studies have demonstrated the importance of systemic inflammation and demonstrated that this systemic inflammation is not merely a reactive sequelae of atherogen- esis but is instead an important contributor.

Obese hypertrophic adipocytes and stromal cells within adipose tissue directly augment systemic inflammation. This increase in systemic inflammation mediates multiple pathogenic mechanisms in the well-known but poorly understood associations between obesity, cardiovascular pathology, and the comorbidities such

as dyslipidemia, type 2 diabetes mellitus, hypertension, and the metabolic syndrome.

2.4.1. Systemic Inflammation and Increased Cardiovascular Risk

Systemic inflammation has been reported to be present before any evidence of myocardial infarction. This was first interpreted as an inflammatory response to the developing atheromatous vascular damage. However, an alternative explanation could be offered by suggesting that systemic inflammation is causing atherosclerosis rather than being the result of it. This interpretation is supported by the observation that patients with pre-existing inflammatory diseases have a dramatically increased risk of CVD at younger ages. Patients with autoimmune diseases such as rheumatoid arthritis and lupus have accelerated rates of atherosclerosis.(52)

Systemic inflammation resulting from untreated indolent infections like periodontal disease is also correlated with increased cardiovascular risk.(53) One of these studies found that serological evidence of past or ongoing chlamydial infection had increased amounts of carotid arteriosclerosis and demonstrated that empiric antichlamydial antibiotic treatment in these patients actually slowed their carotid arteriosclerotic narrowing over the next 3 years compared with untreated controls.(54) Because there was no evidence of direct infectious infiltration of these patients' atheromatous lesions, these studies made conclusions similar to the studies on rheumatoid arthritis.(55)

2.4.2. C-Reactive Protein (CRP) and Cardiovascular Disease

The first observations of the well-known association between CRP and cardiac risk was in 1954, when it was found that after myocardial infarction, there was a dramatic rise in circulating CRP levels and the amplitude of this rise correlated with poor prognosis.(56,57) Subsequently, it was also found that preinfarctelevated CRP levels correlated with an increased risk of future cardiac events as well. CVD. There is currently great excitement surrounding the acutephase reactant CRP for the insights it is providing into the etiologic relationships between inflammation and clinical vasculopathic syndromes, its usefulness as a clinical marker, and evidence for a direct pathogenic involvement. Elevated CRP levels are unquestionably associated with obesity and increased risk of CVD.

Numerous additional studies have further strengthened the association of elevated CRP levels with nearly all the important cardiovascular risk factors, including insulin resistance and diabetes, metabolic syndrome, hypertension, smoking, and dyslipidemia.(58) Numerous ongoing studies have demonstrated a linear relationship between circulating levels of CRP and CVD risk.(59) They revealed that elevated CRP levels in obese patients are not only prognostic for the development of CVD but also predictive of the risk of progression to type 2 diabetes mellitus.(60)

Elevated CRP levels in obesity and the decreases associated with weight loss provide another suggestive link between CRP and obesity-associated risks for CVD and diabetes. (61,62,63,64) In addition to epidemiological and mouse model data

demonstrating CRP to be a useful clinical marker for CVD risk, there is in vitro evidence demonstrating that it also may be an active mediator of inflammatory vasculopathy. After myocardial infarction, CRP accumulates within damaged myocardium, and it is thought to participate in the opsonization of necrotic tissue.(65)Because of the correlation between circulating CRP levels and postinfarction prognosis, it was proposed that the complement activating and opsonizing activities of CRP actually participate in the postinfarction pathology.(65,66)

However, even before infarction, circulating CRP has atherogenic activities on vascular endothelium and smooth muscle. Elevated CRP levels have been associated with endothelial dysfunction in the form of inappropriate vascular constriction/relaxation. Other effects include increased smooth muscle cell migration, proliferation, and vascular remodeling.(67) These multiple molecular mechanisms for vasculopathic effects by CRP may explain the central position of CRP within the context of cardiovascular risk factors.

2.4.3 Tumer Necrosis Factor-α (TNF-α) and Cardiovascular Disease

The contribution of TNF-α to vasculopathy is complex and controversial. Obesity-associated TNF-α is primarily secreted from macrophages accumulated in obese adipose tissue, whereas the adipocytes, predominantly produce unsecreted, membrane-bound TNF-α.(68,69) The secreted adipose tissue TNF-α is specifically

increased in visceral adipose depots.(70) The resulting systemic rise in circulating TNF-α has been implicated in causing adipocyte insulin resistance.(71,72) Thus, some of the contribution of TNF-α to vasculopathic processes may be mostly through its involvement in the development of insulin-resistant diabetes and the ensuing hyperglycemia.(73)

Circulating TNF-α may also contribute by its induction of CRP production and general systemic inflammation, which, in turn, impacts on the vasculature. In vitro experiments have also shown that TNF-α increases activation of endothelial and smooth muscle NF-κB, which, in turn, induces vascular adhesion molecules and cytokines, resulting in inflammatory and foam cell accumulation.(74)

Despite these intriguing in vitro data, the animal studies on TNF-α and development of atherosclerosis have produced mixed results. Although reducing TNF-α levels in apoE deficient mice resulted in significant decrease of atheromatous lesions,(75) in a wild-type background, it produced no improvements.(76) although TNF-α is thought to play a role in the progression of ischemia-related congestive heart failure, anti-TNF therapy has shown no benefits for congestive heart failure progression in patients.(78) these conflicting results, there remains a great interest in testing anti–TNF-α therapies for cardioprotective effects.

2.4.4. Interleukin-6 (IL-6) and Cardiovascular Disease

Like TNF-α, the data as to the significance of increased systemic IL-6 levels in obese states are mixed and controversial. Obesity associated induction of adipose IL-6 production induces CRP secretion, and there are data that suggest IL-6 decreases lipoprotein lipase activity, which results in increased macrophage uptake of lipids. In young atheromatous lesions, macrophage foam cells and smooth muscle cells express IL-6, suggesting a role for this cytokine in the earliest stages of atherosclerosis. Furthermore, circulating IL-6 stimulates the hypothalamic pituitary adrenal axis, activation of which is associated with central obesity, hypertension, and insulin resistance.(66)the association of increased IL-6 levels with vasculopathic disease states, and data demonstrating possible specific provasculopathic activities by IL-6, there is growing evidence hat it also has roles in inducing lipolysis and decreasing appetite and weight gain, thus controlling obesity-associated pathology.(79,80)

Understanding of the roles of IL-6 and TNF-α in the context of obesity and vascular pathology is ambiguous. Although these cytokines may play an important role in the beneficial control of metabolic functions in lean, physically active individuals,(81)or overall effects on vasculopathy in obese states is still unknown. It remains highly likely that they contribute to obesity-associated systemic inflammation and its sequelae and remain potentially important targets for prevention of inflammation-induced insulin resistance or vasculopathy.

2.4.5. Adiponectin and Cardiovascular Disease

Adiponectin is an adipocyte secretory protein, the circulating levels of which are decreased in obese and diabetic states. This protein has been shown to play a role in liver insulin sensitivity and whole-body metabolism.(82) Adiponectin has been implicated in cardiovascular health as well, at the very least as a highly sensitive serum marker for the prediction of future cardiovascular events. Retrospective case-control studies demonstrate that patients with the highest levels of adiponectin have a dramatically reduced 6-year risk of myocardial infarction comparedwith case controls with the lowest adiponectin levels, and this relationship persists even when controlling for family history, BMI, alcohol, history of diabetes and hypertension, hemoglobin, CRP, and lipoprotein levels.(83) Animal models also corroborate these observations, showing that adiponectin is particularly important for preventing diet-induced progression of atherosclerosis.(85,86)

In addition to its possible anti-inflammatory properties and their implications for CVD, it is important to mention the recent demonstration that adiponectin may also have an important role in protecting against cardiac hypertrophy in cardiac overload states such as hypertension, hypertrophic cardiomyopathy, and schemic heart disease. Adiponectin was shown in mice to protect against overload-induced and adrenergically induced cardiac myocyte hypertrophy, specifically by inhibiting hyper trophic signals via AMPK.(84)

3. SUBJECT AND METHOD

3.1. SUBJECT

Study participants were divided into two groups; subjects with CAD (n=438) and control subjects (n=659). The CAD inclusion criteria were: angiographic evidence with > 50% occlusion of one or more major coronary arteries, old myocardial infarction (MI), or angina pectoris, but any possible non-atherogenic occlusions such as osteal stenosis and spasm were excluded.

Patients with orthopedic limitations, weight loss/gain over the previous 6 months or any diagnosis of diabetes mellitus, liver disease, renal disease, thyroid or pituitary disease were excluded. Control subjects were recruited concomitantly from participants in a prospective human genetic study, supported by a Genome Research Development Project on Health and Medicine (project #: HMG-00-GN-01-0001), Ministry of Health & Welfare. None of them were taking any medication or had diagnosis of coronary vascular disease and any disease mentioned above.

Written informed consent was obtained from all subjects and the protocol was approved by the Institute of Review Board of Yonsei University.

3.2. METERIAL AND METHOD

3.2.1 Blood Collection

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 stored at -70°C until analysis.

3.2.2. Genotyping

Genomic DNA was extracted from 5ml of whole blood using a commercially available DNA isolation kit (WIZARD^{R} Genomic DNA purification kit, Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. We genotyped 7 site of PLIN SNPs already reported (6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T and 14995A>T) both in 438 CAD patients and 659 controls. Each genotyping was performed by SNP-IT™ assays using single primer extension technology (SNPstream 25K™ System, Orchid Biosystems, NJ, USA). The DNA fragments were visualized by UV illumination using Image Analyzer (AlphaImager™ 1220, Alpha Innotech Corp., California, USA) respectively.

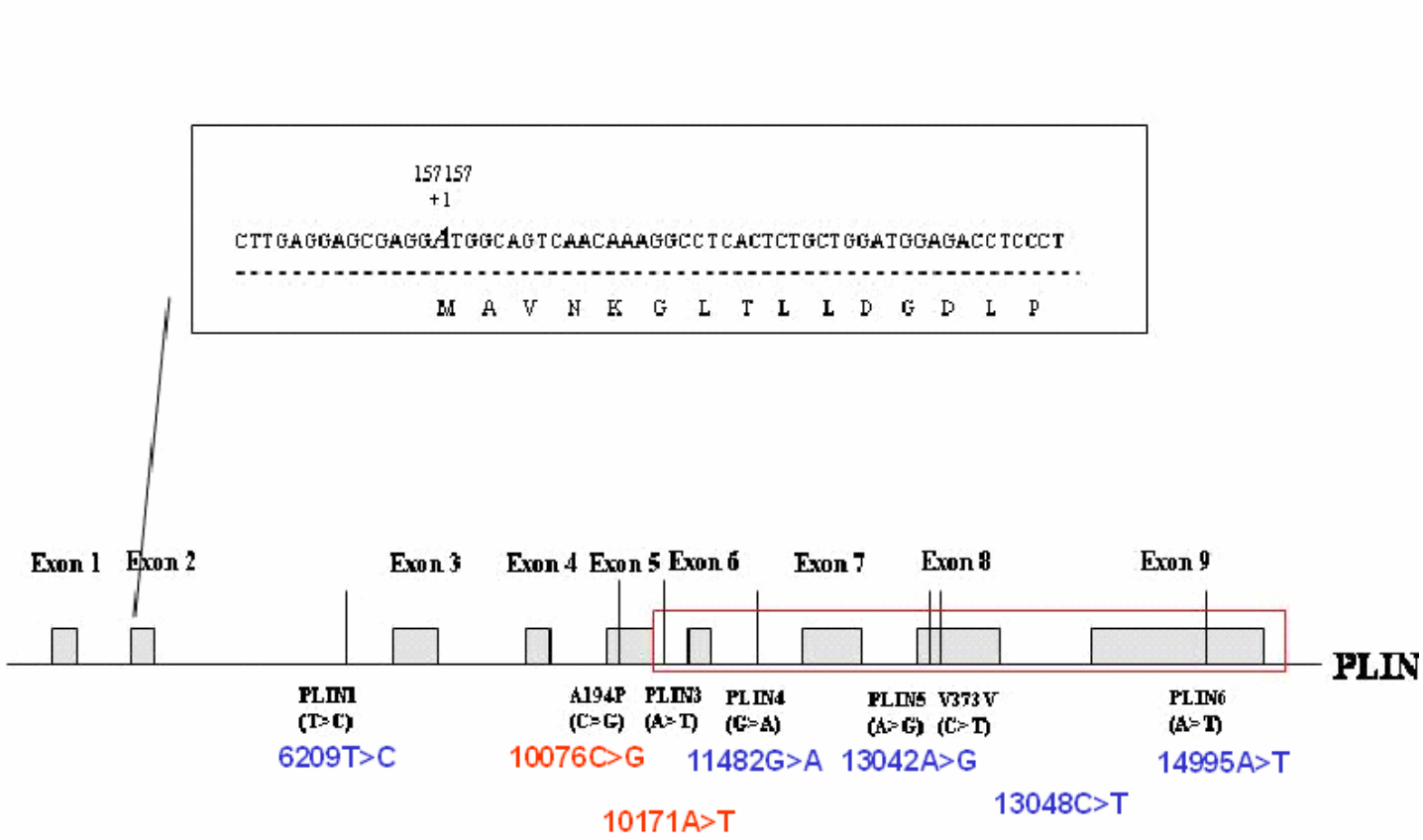

Figure3. Positions of the PLIN polymorphisms

3.2.3 Anthropometrical and Blood Pressure Measurements

Body weight and height were measured in the morning, unclothed and without shoes. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m^2). Circumferences of waist and hip were measured in the standing position after normal expiration and waist to hip ratio (WHR) was also computed Blood pressure was read from the left arm with automatic blood pressure monitor (TM-2654, Japan), while subjects remained seated after 20 minutes rest. An average of three measurements was recorded for each subject.

3.2.4. Abdominal fat distribution at levels of L1 and L4 using CT scanner

Abdominal fat areas were measured by computerized tomography (CT) scanning using a General Electric (GE) High Speed Advantage 9800 scanner (Milwaukee, WI). Two cross-sectional images were made for each subjects; abdomen at the levels of 1^{st} lumbar (L1) vertebra and 4^{th} lumbar (L4) vertebra. Each CT slice was analyzed for the cross-sectional area of fat using a density control program available in the standard GE computer software. Parameters for total abdominal fat density at the levels of L1 and L4 were selected between the range of -150 and -50 Hounsfield Units (HU). Total abdominal fat area was divided into visceral and subcutaneous fat areas to calculate specific fat areas.

3.2.5. Serum Lipid Profile

Fasting serum concentrations of total cholesterol and TAG 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 very low density lipoprotein (VLDL) with dextran sulfate-magnesium, 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 TAG concentrations <4.52 mol/l (400mg/ml) and directly measured for subjects with serum TAG concentration ≥4.52 mol/l. Serum apolipoprotein A-I and B were determined by turbidometry at 340nm using a specific anti serum (Roche, Switzerland).

3.2.6. Glucose, Insulin and Free fatty acids and HOMA-IR

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). Free fatty acids (FFA) were analyzed with a Hitachi 7150 autoanalyzer (Hitachi Ltd, Tokyo, Japan). IR was calculated with the homeostasis model assessment (HOMA) using the following equation: IR = {fasting insulin (μIU/ml) × fasting glucose (mmol/l)} / 22.5.

3.2.7. Inflammatory Markers and Serum Adiponectin

Plasma CRP was measured by an Express Plus autoanalyzer (Chiron Diagnostics Co., MA, USA) using a commercially available high-sensitivity kit, CRP-Latex (II) X2 (Seiken Laboratories Ltd., Tokyo, Japan) (17). TNF-α and Interleukin-6 were measured by WALLAC Victor² (Perkin Elmer life sciences, Turka, Finland)) at 450nm with Quantikine ELISA kit (Human TNF-α and Human IL-6, R& D system). Plasma adiponectin concentration was measured using an enzyme immunoassay (Human Adiponectin ELISA kit, B-Bridge International Inc., CA, USA). The resultant color reaction was read using a Victor² (Perkin Elmer life sciences, Turka, Finland) at 450nm.

3.3. Statistical Analysis

We used SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, USA) for all our statistical analyses. linkage disequilibrium (LD), was examined using Executive SNP Analyzer 1.0 (http://www.istech.info/SilicoSNP/index.html). We selected SNPs showing strongly positive LD (D'>0.9, R^2>0.9) from LD test and made haplotype. The associations between genetic variants and continuous variables were examined using analysis of covariance and a general linear model followed by the Bonferroni test with adjustment for covariate if differences were shown in baseline clinical characteristics among haplotype groups.

The association between CAD and genotypes was analyzed by a χ^2 test. Odds ratios (ORs) were calculated along with 95% confidence intervals (CIs) with adjustment for covariate. Each variable was examined for normal distribution and significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented on untransformed and unadjusted variables. Results are expressed as mean±SE. A two tailed value of P<0.05 was considered statistically significant.

4. RESULTS

Compared with controls, CAD patients were older (47.9±0.5 vs 55.2±0.4 yrs, P<0.001). Clinical features of CAD patients and control subjects are shown in Table 1. There were no significant differences in sex distribution, smoking, drinking alcohol, BMI and WHR between CAD patients and controls. CAD patients were more frequently treated with antihypertensive, antidyslipidemic and antiplatelet drugs. In the subset who underwent CT measurements (n=143), those who were in the CAD group (n=54) had showed higher visceral fat areas at L1 (122±5 vs 157±10 cm^2, P<0.005) and L4 (101±4 vs 116±7 cm^2, P<0.05) but lower subcutaneous fat areas at L1 (116±5 vs 82±10 cm^2, P<0.005) and L4 (175±6 vs 130±12 cm^2, P<0.001) as compared with controls (n=89).

Table 1. General characteristics of CAD patients and controls subjects (age-adjusted means)

	Controls (n=527)		**CAD patients (n=438)**	
Men/Women	477 / 50		410 / 28	
Smokers (%)	67.8		76.4	
Alcohol drinkers (%)	73.9		62.3	
BMI (kg/m^2)	25.2	± 0.12	25.2	± 0.14
Weight (kg)	70.6	± 0.37	71.9	± 0.47
Height (cm)	167.4	± 0.26	168.4	± 0.32
Waist (cm)	89.2	± 0.32	89.9	± 0.40
Hip (cm)	98.5	± 0.24	99.2	± 0.30
Waist/hip	0.91	± 0.00	0.91	± 0.00
Antihypertensive therapy	-		356 (81.3)	
Antidyslipidemic therapy	-		242 (55.3)	
Antiplatelet therapy	-		347 (79.2)	

Data are means±S.E. or percent.

CAD: patients with either stenosis>50% in epicardial coronary artery at coronary angiography (n=268) or with previous AMI (n=170).
Control: Normal subjects with normal resting ECG and nondiabetics.

4.1. Perilipin Polymorphisms and CAD

None of the PLIN polymorphisms examined deviated from Hardy-Weinberg equilibrium in the population as a whole or in cases and controls separately. CAD and control subjects had similar genotype distribution at the 6209T>C, 11482G>A, 13042A>G, 13048C>T and 14995A>T polymorphisms. By contrast, significant differences in genotype distribution were observed for SNPs at positions 10076C>G (P=0.02) and 10171A>T (P=0.03) with homozygotes for each of the most common alleles being more frequent among the control group (Table 2). We found the positive linkage disequilibrium (D´=0.974, R^2=0.944, P<0.001) between those two SNPs. Table 3 shows distribution of PLIN 10076C>G/10171A>T haplotypes. We examined three major haplotype groups; CA/CA haplotype (i.e., individuals with C/C at PLIN10076 and A/A at PLIN10171), CA/GT haplotype (i.e., individuals with C/G at PLIN10076 and A/T at PLIN10171) and GT/GT haplotype (i.e., individuals with G/G at PLIN10076 and T/T at PLIN10171) in CAD patients and controls, respectively. 13 subjects with minor haplotypes were not included in the final analysis since their inclusion or exclusion did not affect the results. Our data revealed significant differences in 10076C>G/10171A>T haplotype distribution with the CA/CA combination (C/C at 10076 and A/A at 10171) being more frequent in the control group as compared with the CAD group (P=0.024) (Table 3). The absence of CA homozygosity was associated with significantly higher risk of CAD (OR 1.48 [95% CI 1.13-1.94, P=0.004 after adjusting for age] (Table 4).

Table 2. Distribution of genotypes at 10076 and 10171 of the PLIN gene in CAD cases and controls

	CAD (n=438)	Control (n=527)	p
10076 C/C	197 (45.0)	281 (53.3)	
10076 C/G	198 (45.2)	204 (38.7)	0.035
10076 G/G	43 (9.8)	42 (8.0)	
10171 A/A	197 (45.0)	281 (53.3)	
10171 A/T	198 (45.2)	203 (38.5)	0.036
10171 T/T	43 (9.8)	43 (8.2)	

Table 3. Distribution of PLIN 10076C>G/10171A>T haplotypes

	CAD	Control	P
n	438	527	
CA/CA	196 (44.7)	279 (52.9)	
CA/GT	197 (45.0)	201 (38.1)	0.028**
GT/GT	43 (9.8)	42 (8.0)	
CA/GA	1 (0.2)	2 (0.4)	
CA/CT	1 (0.2)	2 (0.4)	
Others*	0	1 (0.2)	

Data are n(%).
*Others : CT/CT, GA/GA, CT/GT, GA/GT. **CA/CA vs CA/GT vs GT/GT (2 degrees of freedom)

Table 4. Prevalence of carriers of the minor allele in 10076C>G, PLIN 10171 A>T genotypes or PLIN 10076-10171 haplotype in CAD patients and controls and risk (OR and 95% CI) of CAD in carriers of minor allele as compared with homozygotes for the common allele

Gene variant	CAD	Healthy	Unadjusted		Age-adjusted	
	N (%)	n (%)	OR (95%CI)	p	OR (95%CI)	p
10076 C>G						
CC	197 (45.0)	281 (53.3)	1		1	
CG+GG	241 (55.0)	246 (46.7)	1.397 (1.084-1.802)	0.010	1.496 (1.141-1.961)	0.005
10171 A>T						
AA	197 (45.0)	281 (53.3)	1		1	
AT+TT	241 (55.0)	246 (46.7)	1.397 (1.084-1.802)	0.010	1.494 (1.139-1.959)	0.004
10076+10171						
CA/CA	196 (45.0)	279 (53.4)	1		1	
CA/GT+GT/GT	240 (55.0)	243 (46.6)	1.406 (1.089-1.815)	0.009	1.511 (1.151-1.983)	0.003

4.2. Clinical characteristics, serum lipids and HOMA-IR according to PLIN haplotypes

Table 5 and 6 show the clinical characteristics, serum lipid profiles and HOMA-IR in controls and CAD patients, respectively, according to the PLIN 10076C>G /10171A>T haplotypes. In controls no differences between haplotype groups were found for sex distribution, age, BMI, smoking and alcohol consumption, HDL-C, LDL-C and total cholesterol, glucose, insulin and HOMA-IR.

Conversely, CAD patients with the GT/GT haplotype showed significantly higher levels of LDL-C and total cholesterol, apo B and atherogenic index than those with the CA/CA (Table 6). In CAD patients, differences between three haplotype groups were not found in sex distribution, age, BMI, smoking and alcohol consumption and pharmacological interventions.

Table 5. Characteristics, serum lipid profiles and HOMA-IR according to PLIN10076C>G/10171A>T haplotypes in control subjects

	CA/CA (n=279)	**CA/GT** (n=201)	**GT/GT** (n=42)
Women/Men	27 / 252	19 / 182	3 / 39
BMI (kg/m^2)	24.9 ± 0.16	25.6 ± 0.18	25.1 ± 0.30
Age (years)	52.9 ± 0.61	50.4 ± 0.75	47.9 ± 1.66
Tobacco (cigarettes/day)	21.4 ± 0.64	20.6 ± 0.66	21.0 ± 1.59
Alcohol (g/day)	22.2 ± 1.66	26.9 ± 1.64	28.8 ± 3.72
Total cholesterol (mg/dl)	199.6 ± 2.27	204.2 ± 2.41	204.8 ± 4.56
HDL cholesterol (mg/dl)	47.5 ± 0.74	47.1 ± 0.78	47.0 ± 2.11
LDL cholesterol (mg/dl)	122.8 ± 2.11	124.5 ± 2.30	129.6 ± 5.23
Apolipoprotein B (mg/dl)	97.2 ± 2.41	98.0 ± 2.48	92.7 ± 3.72
Atherogenic index[1]	3.46 ± 0.08	3.55 ± 0.09	3.67 ± 0.21
Glucose (mg/dl)	93.2 ± 0.79	92.0 ± 0.88	93.1 ± 1.84
Insulin (μU/ml)	8.95 ± 0.55	8.89 ± 0.30	8.63 ± 0.53
HOMA-IR[2]	2.07 ± 0.13	2.03 ± 0.07	2.03 ± 0.14

Mean±S.E. There were no significant differences among 3 haplotype groups based on one-way analysis of variance (ANOVA) with Bonferroni test.

[1]AI=(total cholesterol-HDL cholesterol)/HDL cholesterol

[2]Insulin resistance = {fasting insulin(μIU/ml) × fasting glucose(mmol/l)}/22.5

Table 6. Characteristics, serum lipid profiles and HOMA-IR according to PLIN 10076C>G /10171A>T haplotypes in CAD patients

	CA/CA (n=196)	CA/GT (n=197)	GT/GT (n=43)	$P^{\dagger}$
Women/Men	13 / 183	12 / 185	3 / 40	0.964
BMI (kg/m^2)	25.1 ± 0.18	25.1 ± 0.21	25.6 ± 0.47	0.421
Age (years)	55.7 ± 0.55	54.7 ± 0.60	55.1 ± 1.22	0.441
Tobacco (cigarettes/day)	22.2 ± 1.12	20.7 ± 0.96	19.5 ± 1.93	0.388
Alcohol (g/day)	22.5 ± 2.39	22.5 ± 2.20	21.7 ± 3.01	0.985
Total cholesterol (mg/dl)	174.9 ± 2.60^{b}	184.1 ± 2.69^{ab}	189.3 ± 6.87^{a}	0.015
HDL cholesterol (mg/dl)	43.0 ± 0.86	42.4 ± 0.79	38.8 ± 1.55	0.092
LDL cholesterol (mg/dl)	101.9 ± 2.31^{b}	109.9 ± 2.48^{ab}	112.1 ± 6.14^{a}	0.038
Apolipoprotein B (mg/dl)	80.0 ± 1.48^{b}	86.6 ± 1.58^{a}	91.5 ± 4.08^{a}	0.001
Atherogenic index[1]	3.32 ± 0.10^{b}	3.63 ± 0.11^{b}	4.21 ± 0.28^{a}	0.001
Glucose (mg/dl)	93.5 ± 1.57	94.2 ± 1.31	92.3 ± 2.93	0.834
Insulin (μU/ml)	11.2 ± 0.67	10.8 ± 0.42	11.1 ± 0.82	0.710
HOMA-IR[2]	2.53 ± 0.15	2.49 ± 0.11	2.50 ± 0.21	0.949
Antihypertensive therapy	163 (83.2)	156 (79.2)	37 (86.0)	0.438
Antidyslipidemic therapy	110 (56.1)	106 (52.8)	26 (60.5)	0.602
Antiplatelet therapy	162 (82.7)	149 (74.6)	36 (83.7)	0.108

Mean±S.E. †Significance tests were based on comparison of three haplotype groups. Significantly different($p<0.05$) in the same row with different superscripts from each other at $p<0.05$ based on one-way analysis of variance(ANOVA) with Bonferroni test.

[1]AI=(total cholesterol-HDL cholesterol)/HDL cholesterol

[2]Insulin resistance = {fasting insulin(μIU/ml) × fasting glucose(mmol/l)}/22.5

4.3. Serum FFA and triglyceride according to the perilipin(PLIN) 10171A>T/10076C>G haplotypes

A marked haplotype influence on fasting FFA concentrations was observed in CAD patients (P<0.001) (Fig. 4). CAD patients with the GT/GT and CA/GT haplotypes had 31% (P<0.001) and 14% (P<0.05) higher fasting FFA than those with the CA/CA haplotype. Similarly, PLIN 10076C>G/10171A>T haplotypes were associated with serum fasting FFA in controls (P=0.033). Control subjects with CA/GT haplotype had 11% higher fasting FFA levels than those with CA/CA haplotype (P<0.05). PLIN 10076C>G/10171A>T haplotypes were also associated with serum triglyceride in CAD patients (P=0.001). Mean concentrations of triglyceride in CAD patients with CA/GT and GT/GT were higher than those in the CA/CA haplotype group (P<0.05). However, these haplotype effects on triglyceride concentrations were not observed in controls.

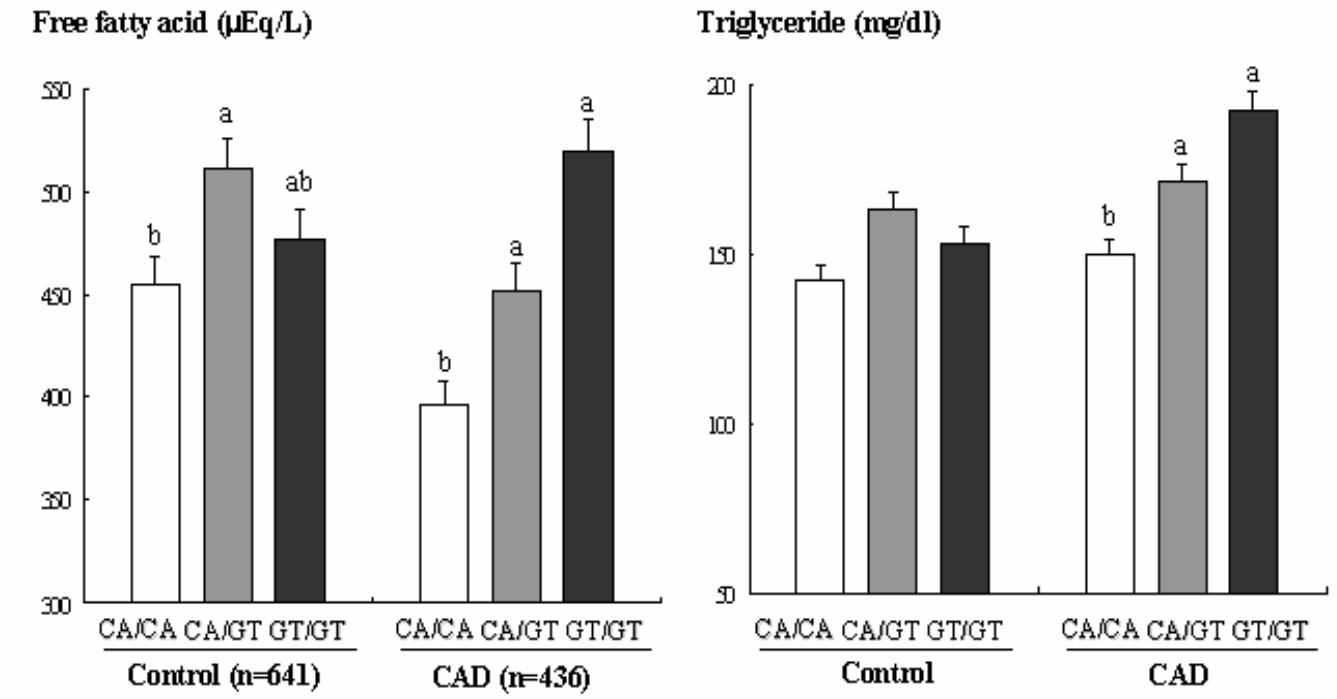

Fig 4. Influence of PLIN 10076C>G/10171A>T haplotypes on serum concentrations of free fatty acid and triglyceride in controls and CAD patients

Values significantly different ($p<0.05$) within the same group are indicated by different alphabets by one-way ANOVA with Bonferroni test.

4.4. Circulating levels of adiponectin and inflammatory markers according to the PLIN 10076C>G /10171A>T haplotypes

PLIN 10076C>G/10171A>T haplotype associations with circulating levels of adiponectin (p=0.025) and TNF-α (p=0.048) were observed in CAD patients (Fig. 5). GT/GT patients showed lower adiponectin but higher TNF-α levels than CA/CA patients (P<0.05). Moreover, we found significant haplotype associations with serum IL-6 (P=0.009) and CRP (P=0.026) in CAD patients. CAD patients with CA/GT or GT/GT haplotypes showed higher mean concentrations than those with the CA/CA haplotype (P<0.05) (Fig 6). However, none of these associations were observed in controls.

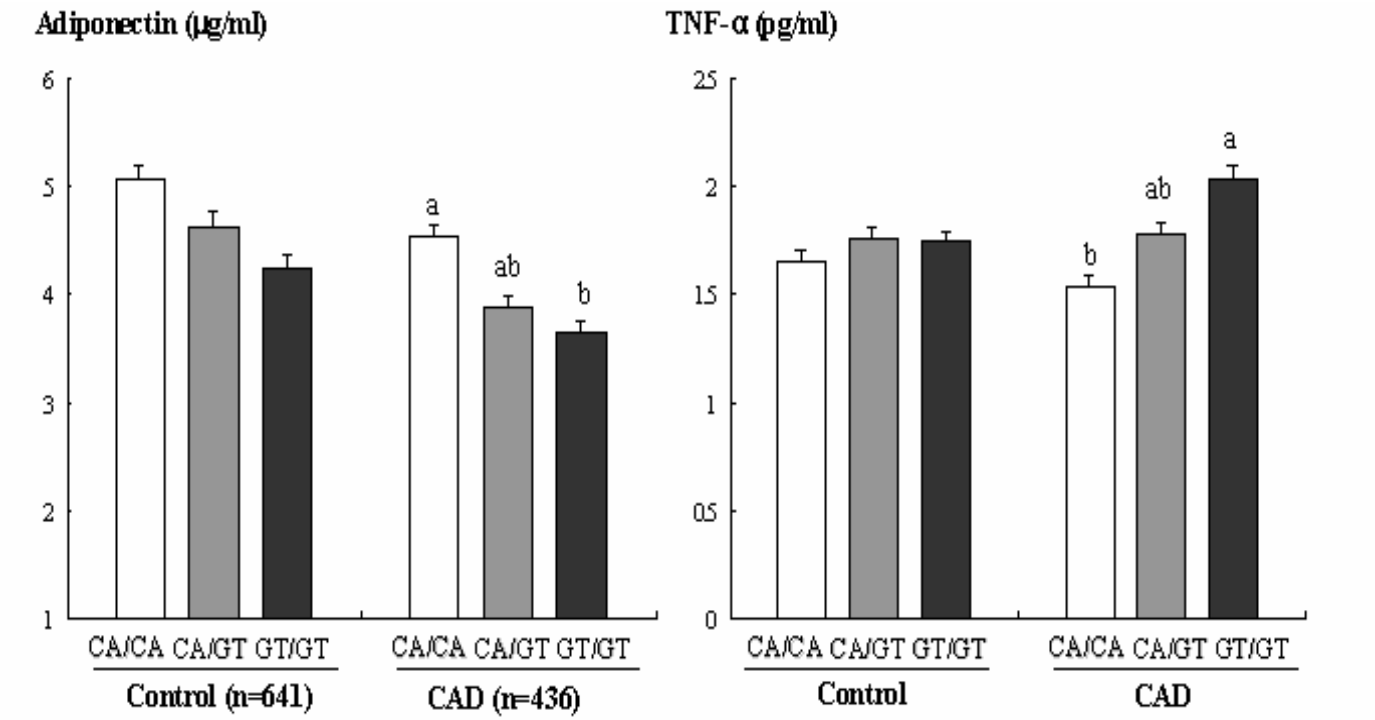

Fig 5. Influence of PLIN 10076C>G/10171A>T haplotypes on circulating concentrations of adiponectin and TNF-α in controls and CAD patients

Values significantly different ($p<0.05$) within the same group are indicated by different alphabets by one-way ANOVA with Bonferroni test.

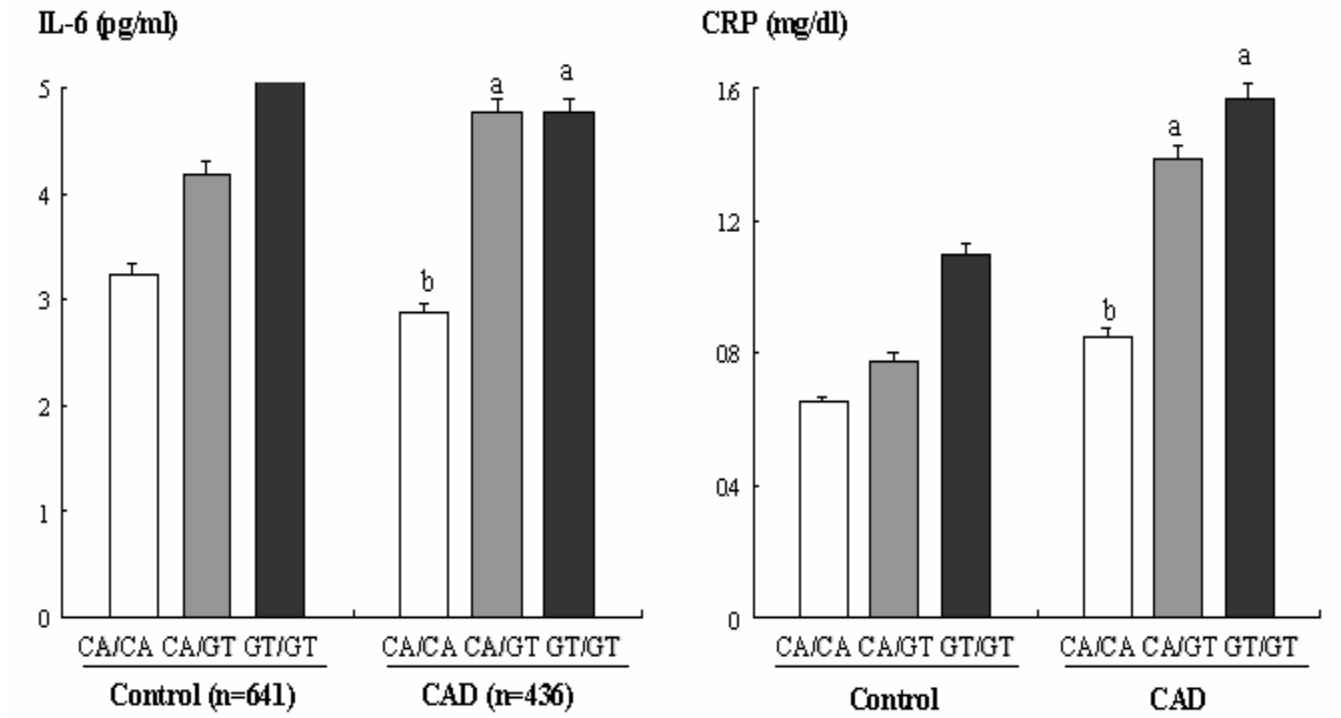

Fig 6. Influence of PLIN 10076C>G/10171A>T haplotypes on circulating concentrations of IL-6 and CRP in controls and CAD patients

Values significantly different ($p<0.05$) within the same group are indicated by different alphabets by one-way ANOVA with Bonferroni test.

5. DISCUSSION

Genetic variation at the PLIN locus has been previously implicated with anthropometric variables and obesity risk. The goal of the current study was to examine using a case-control design whether the previously reported associations could be extended to associations with CAD risk and other intermediate CAD risk factors. Our data show that certain haplotypes inferred using the PLIN 10076C>G and PLIN 10171A>T SNPs were associated with an increased risk of CAD for the CA/GT (i.e., individuals with C/G at 10076 and A/T at 10171) and GT/GT haplotype (i.e., individuals with G/G at 10076 and T/T at 10171) in comparison with CA/CA haplotype (i.e., individuals with C/C at PLIN10076 and A/A at PLIN10171).

Recently, genetic variation at the PLIN locus has been associated with decreased perilipin protein expression in human adipocytes and increased circulating concentrations of FFA, which indirectly mirror in vivo lipolytic activity (3). Therefore, our data would be consistent with some of the effects expected from an increased basal lipolysis in carriers of the GT haplotypes.

CAD patients with the GT/GT haplotype had about 31% higher fasting FFA levels whereas heterozygotes had intermediate levels as compared with CA/CA subjects. The effects of PLIN 10076C>G/10171A>T haplotype on fasting FFA were also observed in controls with 11% higher fasting levels in CA/GT as compared with CA/CA subjects. Of the 7 SNPs, we found that two SNPs (PLIN 10076C>G and PLIN 10171A>T) located in intron 5 and exon 5, respectively, were associated with

increased lipolysis. These 2 SNPs may alter the transcription product by affecting splicing (4), thus the rare allele at these loci could be associated with lower expression of the PLIN gene or impaired perilipin activity (2). Perilipin null mice also showed elevated basal lipolysis because of the loss of the protective function of perilipin as a key regulator of lipolysis in adipocytes (15).

Mottagui-Tabar et al. (3) have reported that the PLIN 11482G>A was associated with enhanced basal and norardrenaline induced lipolysis in obese white women. However, an association between the PLIN 11482G>A and fasting FFA concentrations was not found in our subjects. This discrepancy might relate to differences in gender distribution (93% males in our study) and ethnicity between two studies. In fact, variations in expression levels of perilipin have been reported between men and women and between obese and nonobese individuals (13). Furthermore, another study has reported different associations between genetic variants at the PLIN locus across ethnic groups (5).

An enhanced hepatic flux of FFAs from adipocytes can cause an increase in absolute reesterification levels of FFAs, resulting in overproduction of triglyceride and increased secretion to the blood compartment (18). The higher triglyceride levels in CAD patients with the GT/GT haplotype compared with those carrying CA/CA might relate to higher fasting FFA concentrations. CAD patients with the GT/GT haplotype also showed high concentrations of LDL and total cholesterol. This association between PLIN SNPs and lipid profiles supports the involvement of the PLIN gene in human lipid metabolism. This notion is also supported by the perilipin

null mice weighing more than 30g showed modest increases in the plasma cholesterol and triglyceride (16).

Studies in humans have reported a negative association between fasting concentrations of triglyceride and adiponectin, an adipocyte secretory protein (19,20). In this study, GT/GT CAD patients with high levels of triglyceride showed low concentrations of circulating adiponectin in comparison with those carrying CA/CA. This haplotype effect of PLIN 10076C>G/10171A>T on adiponectin might also implicate the possible interrelationship between productions and releases of proteins in adipocytes. In fact, perilipins in adipocytes that are not secreted have been suggested participate in adipokine release, thus affect circulating adipokines such as leptin and adiponectin (16,21).

Adiponectin, a potential antiatherogenic and antiinflammatory adipokine (12,19), has been reported to reduce the production and activity of TNF-α and IL-6 (7,12). Elevated production of TNF-α or other cytokines increases basal lipolysis possibly through its effects on perilipin expression or activity (13). Furthermore, perilipin downregulation in adipocytes is a key event in TNF-α stimulation of lipolysis (1,22), while the overexpression inhibits TNF-α mediated lipolysis (1). In this study, CAD patients with GT/GT showed higher TNF-α levels and those carrying GT haplotypes had higher IL-6 concentrations in comparison with CA/CA homozygotes. IL-6, derived from visceral adipose tissue draining directly into the portal system, causes the rise of liver CRP production (7). Similar to results of IL-6, CAD patients carrying GT haplotypes had higher CRP concentrations in comparison with CA/CA

homozygotes in this study. Elevated levels of CRP are known to be prognostic for the development of CVD (7).

Increase in fasting FFA, blood lipid profiles and TNF-α and decrease in circulating adiponectin are typical features in insulin-resistant state and obesity. A number of pharmacological intervention aimed improving insulin sensitivity, hypertension or cholesterol biosynthesis have been shown to reduce indicators of systemic inflammation such as TNF-α, IL-6 and CRP. However, serum insulin concentrations, HOMA-IR, BMI and pharmacological interventions were not different between haplotype groups in present CAD patients. We can suggest that the effects of PLIN 10076C>G/10171A>T haplotypes on FFA, lipid profiles and systemic inflammatory markers are probably related to decreased perilipin contents or activities in CAD individuals with variant allele rather than insulin resistance, obesity and pharmacological intervention. Alternatively, it is possible that gene-diet interactions modulate the association between the PLIN gene and HOMA-IR since CAD patients in this study consumed a therapeutic diet (<15-20% of total calorie from fat and <6% from saturated fat) that may prevent the expression of the negative effects associated with the GT haplotype.

Unsimilar to CAD patients, controls in this study did not show the haplotype effects of PLIN 10076C>G/10171A>T on serum lipids, antiinflammatory and inflammatory markers except free fatty acids. High visceral fat accumulation in CAD patients could explain this difference. In a recent report, expansion of fat cell size with obesity was not accompanied by a proportionate increase in perilipin

concentrations. Wang et al. (13) found that perilipin protein was 2-fold lower in obese than non-obese subjects. Since increased transcription of perilipin genes is probably related to removal of fatty acids from circulation and their storage in white adipose tissue (23), low perilipin levels in CAD patients with variant allele might show increased fasting lipolysis and dysregulation of adipokines and cytokines from high visceral fat. Secretion of inflammatory factors from visceral adipose tissue into the portal system and resulting effects on the liver and systemic inflammation have been suggested to be the cause of the tight correlation between increased visceral fat and CAD (7).

In conclusion, PLIN locus, particularly the 10076C>G/10171A>T haplotype, is a determinant of CAD risk in Koreans. This haplotype may be a significant genetic predictor for lipolysis. In addition, the PLIN gene may be involved in lipid metabolism and systemic inflammation in CAD patients who have high visceral fat accumulation.

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국문요약

한국인에서 perlilpin 유전자 다형성이 관상동맥질환과 체내 염증성 지표에 미치는 영향

비만은 단순한 체중증가나 체지방의 증가 외에도 지질대사나 당대사 이상을 초래하는데 특히 복부비만은 당뇨병이나 고혈압, 이상지혈증, 심혈관계질환 등 대사증후군과 밀접한 관계가 있다. 체내 지방 조직 내에서 중성지방구를 싸고 있는 단백질인 perilipin(PLIN)의 유전자 다형성은 지질대사와 비만위험도를 조절한다고 밝혀지고 있다. 하지만 아직 perilipi(PLIN)의 유전자 다형성과 심장혈관질환의 주요 위험인자나 심장혈관질환 자체의 위험도에 대한 관계에 대해서는 연구되어지지 않았다. 비만의 대표적인 지표는 낮은 수치의 체내 염증성 지표와 비만과 심장혈관질환(cardiovascular disease,CVD)의 증가와 관계가 있는 C-reactive protein(CRP), interleukin 6(IL-6), tumor necrosis factor-α(TNF-α)의 증가로 특징 되어 질 수 있다.

실제로 관상동맥질환(coronary artery disease,CAD)환자들의 내장지방 분포는 비슷한 나이와 BMI를 가진 정상인에 비해 현저히 높은 것을 알 수 있었다. 따라서 본 연구에서는 perilipin이 지질대사와 지방분해에 중요한 역할을 하기 때문에 PLIN 유전자 다형성이 CAD와 염증성지표와 어떠한 관계가 있는지에 대해 알아

보고자 한다.

CAD환자 438명과 건강한 성인 659명을 대상으로 7개의 PLIN SNPs(6209T>C, 10076C>G, 10171A>T, 11482G>A, 13042A>G, 13048C>T and 14995A>T), 중 에서 10076C>G (P=0.02)과 10171A>T (P=0.03)의 유전자형을 살펴보았다. 이 두 개의 SNP사이에서 positive linkage disequilibrium (D´=0.974, R^2=0.944, P<0.001)을 보였기 때문에 CAD환자와 대조군에서 가장 주력한 세 개의 site인 CA/CA (C/C at 10076 and A/A at 10171), CA/GT 그리고 GT/GT haplotypes (G/G at 10076 and T/T at 10171)에 대해 살펴보았다. CA homozygosity의 부재는 CAD위험지표를 현저히 증가시켰다(OR 1.48) [95% CI 1.13−1.94, P=0.004 after adjusting for age]. GT/GT (n=43)를 가진 CAD환자들은 CA/CA (n=196)를 가진 사람들에 비해 free fatty acid (FFA)농도가 31%높았던 반면에 CA/GT (n=197)를 가진 CAD환자들에서는 FFA농도가 14% 증가된 것을 알 수 있었다. GT/GT haplotype을 가진 CAD환자들에서는 중성지방(triglyceride,TG),LDL−cholesterol, total−cholesterol 그리고 TNF−α (CA/CA:1.5±0.1, CA/GT:1.8±0.1, GT/GT:2.0±0.3pg/ml, P=0.048)의 수치가 증가됨을 알 수 있었으며 adiponectin (CA/CA:4.5±0.2, CA/GT:3.9±0.2, GT/GT:3.7±0.4 μg/ml, P=0.025)의 수치는 낮았음을 알 수 있었다. CA/GT 또는 GT/GT를 가지고 있는 CAD환자들에서 IL−6 와 CRP의 분포가 CA/CA haplotype을 가지고 있는 환자들에 비해 증가되어 있음을 알 수 있었다.. 컴퓨터 단층촬영을 통한 체지방 분포를 보았을 때 CAD 환자들은 대조군과 비교하여 L1(P<0.005)의 내장지방분포가 29%나 높았으며 L4(P<0.05)의 내장지방 분포

는 15%정도 높았다.

PLIN유전자 다형성 중 특히 SNP10076C>G/10171A>T haplotype은 한국인에게 있어 CAD 위험인자의 결정적인 요소가 될 것으로 사료된다. 이 haplotype은 lipolysis를 가장 잘 나타내주는 유전적 기호가 될 수 있다. 그와 더불어 PlLIN 유전자는 심혈관 질환 환자의 지방대사, 체내 염증성 지표와 높은 내장지방과 높은 연관성을 가진다.

핵심이 되는 말 : PLIN유전자 다형성, 관상동맥질환(CAD), 유리지방산, TNF-α, IL-6, CRP, adiponectin