# The *APOA5*-1131C allele may contribute to the increased susceptibility to diabetes mellitus in association with plasma triglyceride in Korean women

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# The *APOA5*-1131C allele may contribute to the increased susceptibility to diabetes mellitus in association with plasma triglyceride in Korean women

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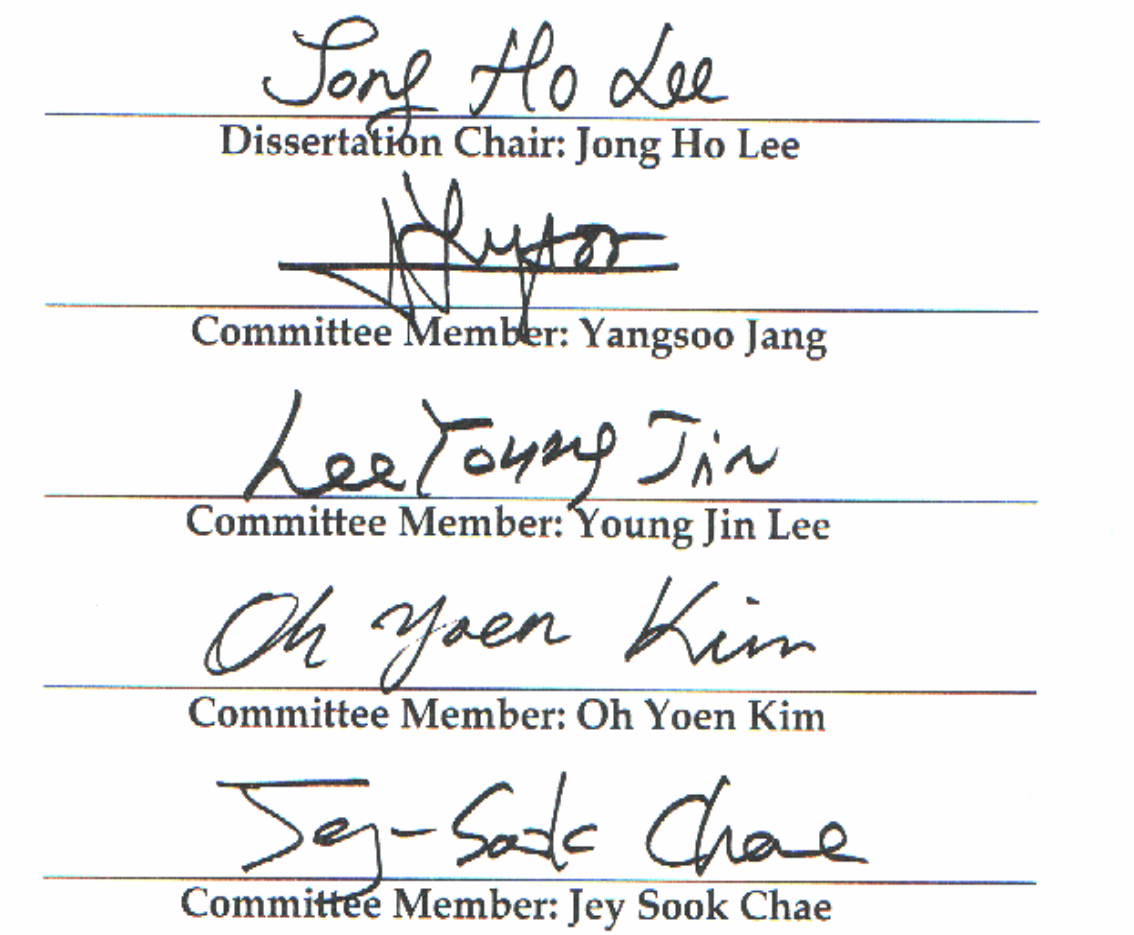
**Ki Ho Lee** is approved



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# 감사의 글

**서론**

이 감사의 글은 박사 과정과 논문에 도움을 주신 분들 뿐만 아니라 지금 저의 모습이 있을 수 있도록 도와 주신 모든 분들께 감사 드리기 위한 목적으로 작성 되었습니다.

**방법**

글을 다 쓰고 처음부터 읽어보니 이것이 한때는 문학 소년을 꿈꾸었던 사람의 글인가 싶을 정도로 유치하고 두서도 없네요. 아마도 정말로 감사하는 마음을 표현 할 때는 미사여구가 더 어색해 보인다는 생각이 듭니다. 그냥 생각 나는 대로 방법 없이 이 글을 씁니다.

**결과**

기쁨 반, 아쉬움 반. 어떤 일에 대한 끝맺음을 앞두고 있을 때마다 어김없이 느껴지는 감정인가 봅니다. 24년 가방 끈을 내려 놓는 시점이 되면 후련한 마음만 들것이라 생각 했는데, 막상 지금에 와서는 '이럴 때 좀더 잘할걸, 그 때 좀더 열심히 할 수 있었을 텐데' 하는 생각들과 아쉬움들만 꼬리를 물고 늘어집니다. 그러면서도 학위를 받게 된다는 것이 한편으로는 쑥스럽기도 하고, 대학원에 좀 더 필요한 사람이 되지 못한 것이 송구스럽기도 하네요.

석사 과정을 마치고 나서, 박사 과정은 좀 더 새롭고 실제적으로 저에게 도움이 될 수 있었으면 좋겠다 라고 생각하던 시기에 이종호 교수님을 처음 만났습니다. 교수님의 따뜻한 설명과 열정을 보고 주저 없이 입학을 하게 되었고, 학위를 받기 까지 논문의 완성 뿐만 아니라 앞으로 연구와 학문에 임하는 자세를 세워나가는 것에도 큰 도움을 주셨습니다. 진심으로 감사 드립니다.

대학원에 선생님이 계시다는 것만으로도 자랑이 되는 장양수 선생님과 항상 새로운 가르침을 주시는 정지형 선생님, 박사과정 내내 모든 방면에서 도움을 주신 채지숙 선생님께도 감사 드립니다. 그리고 김오연 선생님, 제 올해 운세에 5월에 서쪽에서 귀인이 나타난다고 했는데, 마침 올해 프랑스에서 돌아온 선생님이 그 귀인이었나 봅니다. 선생님 때문에 아직 결혼 안 한 제 선후배가 있는지 샅샅이 찾아보기도 했습니다. 논문에 대한

것은 물론이고 연구실 동료를 어우르며 열심히 연구하시는 선생님 모습과 인품에 배운 점이 많습니다. 고맙습니다. 선생님.

같이 졸업하면서도 선배처럼 자료도 주고 정보도 알려준 기특한 현양이와 박사 과정 동안을 함께 했던 김경철, 조석현, 오세연 선생님, 봉주르 봉준이, 정현이, 주희를 비롯한 노과 동기 선후배들, 지도 바쁘면서 틈날 때 마다 도와준 영민이, 혜원이, 영우, 식영과 식구들. 고맙습니다. 고마워 얘들아.

항상 부족한 저를 챙겨 주(시)는 재호형, 성철형, 지웅이형, 재훈이형, 충근형, 승철이, 병철이 감사 드립니다. 고맙다 친구들아..

가정의학과 의사들 사이에는 오래 전부터 학교에 구름을 타고 지팡이를 짚고 다니셨다는 전설적인 분이 계신데, 그 분과 함께 근무하고 있다는 것만으로도 영광으로 생각합니다. 제자가 어려운 결정을 내려야 되기 전에 미리 방향을 제시해 주시고, 가능성과 열정이 있으면 언제나 기회를 만들어주시는 이영진 선생님께 존경하는 마음과 감사의 말씀을 전하고 싶습니다. 영원한 스승 이득주 선생님, 김상만 선생님, 저희 가정의학과 전체의 중심을 잡아주시는 김문종 선생님, 제 대학원 선배님이시라는 죄(?)로 대답하시기 어려운 제 질문에도 자세히 말씀을 해주신 신경균 선생님, 항상 상대방을 유쾌하게 만들어 주시는 박경채 선생님께도 감사의 마음을 전합니다. 사심 없는 학자로서, 항상 세상에 부끄럼 없는 삶을 사시는 최준영 선생님. 선생님과 함께 하면서 항상 반성하고 남의 충고에 귀기울이는 태도를 조금씩 배우게 되었고 제가 지침이 필요할 때마다 자문을 얻을 수 있는 분이 생긴점에 대해서 언제나 감사하게 생각하고 있습니다. 교수로서 학자로서 또 삶을 살아가는 한 사람으로서 필요한 점들을 형처럼 조언해 주시는 김범택 선생님께도 감사함을 전하고 싶습니다. 제가 가진 모든 단점을 장점으로 가지고 있는 요철 같은 제 동료, 최범희 선생님. 범샘. 나 샘이 옆에 있어서 좋아..인제 골프 연습좀 그만하고 나 좀 가르쳐줘..

항상 인자하신 모습으로 바쁘신 와중에도 격려의 말씀을 전해주시는 전세일 원장님과, 생활의 여유와 편안함을 만들어 갈수 있는 덕담으로 따뜻함을 주시는 조세현 원장님께 감사함을 전합니다. 가족 같은 이애영 간호사, 동생 같은 이정실 간호사, 차병원 식구들 항상 감사 드립니다.

건강하게 있는 것 만으로도 든든한 우리 가족 인옥, 인정, 인영 누나와 매형들, 어머님이 전원 주택 생활을 시작 하시면서 조카들과도 함께 만나는

기회가 전보다는 늘었지만, 소중한 마음에 비하면 아직도 부족한 듯 싶습니다.  특히 부모님들 만큼이나 동생 뒷바라지에 신경 많이 써줬던 큰누나, 큰매형.. 항상 고맙게 생각하고 있고. 기운 내세요.

정헌형. 돌이켜보면 형님을 몰랐다면 제가 알고 싶어하는 어느 부분들에 대해서 제 주변에는 자문을 구할 사람이 없는 것들이 많은 것 같습니다. 항상 도와주시려는 형님께 감사하는 마음 가지고 있구요, 세분 처형들과 재민형, 현기형 항상 만날 때마다 '오늘은 어떤 일이 벌어질까'하는 기대와 유쾌함을 주셔서 행복합니다.

우리 삼촌. 인쇄소를 운영하시지만 요즈음은 학위 논문 제본을 하지 않으시면서도 흔쾌히 연락도 자주 못 드리는 조카의 논문을 만들어 주셨습니다. 선비 같고 신사 같은 삼촌이 이제 제 옆에 있는 가장 든든한 남자 어른이세요. 항상 삼촌 안부에 소홀한 조카를 먼저 챙겨 주셔서 감사 드립니다.

어머님 못지 않은 사랑으로 저를 오랫동안 보살펴 주셨던 할머님, 병상에 계셨던 아버님을 신경 쓰다가 정작 할머니가 제 곁을 떠나실 수 있었다는 것을 깨닫지 못했던 것 같습니다. 남에게 이야기 하시던 화제의 대부분의 제 자랑이셨던 할머님, 제 학위 소식에도 눈물을 흘리면서 기뻐하셨을 할머님께 너무나 죄송스럽고 송구스러운 마음이 떠나지 않습니다. 할머님 생전 교회에서 별명이 "천사" 셨던 것처럼 계신 곳에서 기뻐해 주시고 저를 용서해 주세요.

아버님. 석사 졸업식 때도 사실 아버님이 오신다고 하셔서 참석을 했었는데, 그 때 찍어 드린 아버님 사진이 웃으시는 듯 어쩐지 슬퍼 보이셔서 마음이 더 아픕니다. 아버님께서 병상에 누우셨던 그 때가 제 인생에 가장 힘든 시기였던 것은 사실이었고, 이런 저런 이유 때문에 병상에 계신 아버님을 더 자주 뵙지 못했다고 위안도 해보지만, 정작 아버님은 인생에서 가장 힘든 시기에도 저를 먼저 생각해 주셨고, 항상 저에게만은 저희 형편보다 조금 더 사치스럽게 생활할 수 있도록 아버님이 희생하셨던 것을 잘 알고 있기 때문에 더욱 죄스럽고 눈물이 앞을 가립니다. 얼마나 불효 자식이라고 생각이 드시면 그렇게 기도를 드려도 꿈에도 한 번 나타나 주시지 않는 아버님 영전에 용서를 빌고 너무나 감사 드리는 마음으로 이 작은 영광을 바칩니다.

어찌 보면 아버님과 할머님에 대한 뉘우침으로 어머님 건강은 좀 더 신경을 쓰게 되는 것 같습니다. 그래 봐야 혈압약 처방해서 보내드리고

하는 것 뿐인데, 어머님께서는 하고 싶으셨던 전원 생활 하신다고, 큰일 날뻔 하기 전에 종양 치료를 빨리 받으셨다고 누굴 만날 때마다 저에게 고맙다고 하십니다. 어머님 이젠 저에게, 제 인생의 처음부터 지금까지 모든 시간 동안, 저에 대한 아무런 평가 없이도 무조건 저를 사랑해 주어 왔던 사람은 어머님 밖에 안 계세요. 어머님이 오랫동안 행복하신 것이 어머님이 저에게 고마운 것이 아니라 제가 어머님께 감사드릴 따름인 것이고 제 박사 과정 동안에도 제가 실제 힘든 것 보다 더 큰 어려움을 겪는 줄 알고 저보다 더 많이 걱정해 주셨던 마음에 감사 드립니다.

상투적인 이야기 같지만, 눈에 넣어도 아프지 않다는 말이 실감이 나지 않았는데, 무엇과도 바꿀 수 없을 만큼에 소중한 마음을 제게 선물해준 제 딸 채율이. 지금은 뭐를 축하해 줘야 하는지도 모를 것이고 조만간 있을 축하 파티에도 '생일 축하 합니다'를 불러줄 것이지만, 오늘도 " 아빠 양말에 커다란 짱구가 났어요 " 라는 한마디 말로 제 스트레스를 한번에 날려주는, 저를 닮았지만 묘하게 장점만 닮아 엄마 만큼 예쁘게 생긴 제 딸래미와 기쁨을 나누고 싶습니다.

끝으로, 사람들이 팔불출이라고 할까봐, 또 안 믿을까봐 최준영 선생님께만 이야기 했었는데, 다시 태어나서 또 결혼을 하게 되더라도 저는 제 아내와 결혼할 마음입니다. 아버님 덕택에 6개월 군복무를 하게 되면서도 짧은 4주 훈련 동안 보고 싶어 탈영을 생각했을 정도로 저에겐 너무나 소중한 사람입니다. 항상 좋은 남편이 되려고 누구보다 노력은 한다고 자부하지만 그래도 언제나 조금씩 부족한 마음이 드는 건, 제가 아내에게 받는 기쁨이 더 크기 때문일 것입니다. 임의 조제 해도 될텐데, 집안에서도 의약 분업을 철저히 지켜서 약을 꼭 저에게 물어보고 먹는 큰 딸 같은 제 아내에게 너무나 사랑하는 마음 전하고 싶고, 이 소중한 기쁨을 누구 보다도 먼저 함께 하고 싶습니다.

**결론**

여기에 일일이 글로 적지는 못하였으나 그 동안 음으로 양으로 도움을 주시고 격려해주신 모든 분들께 다시 한번 감사드리며, 앞으로 더욱더 학문 정진에 최선을 다 하겠습니다.

2009년 7월 이 기호

# CONTENTS

**List of Figures** ..... iii

**List of Tables** ..... iv

**ABSTRACT** ..... v

**1. INTRODUCTION** ..... 1

**2. SUBJECTS AND METHODS** ..... 2

**2.1. Study population** ..... 2

**2.2. Anthropometric parameters, blood pressure measurements, and blood collection** ..... 4

**2.3. *APOA5* -1131T>C genotyping** ..... 5

**2.4. Serum lipid profiles, apolipoproteins A1 and B and glucose** . 5

**2.5. Plasma apolipoprotein A5** ..... 6

**2.6. Plasma LDL particle size** ..... 6

**2.7. Statistical analyses** ..... 7

**3. RESULTS** ..... 8

**3.1. Characteristics of study subjects** ..... 8

**3.2. Distribution of *APOA5*-1131T>C polymorphism** ..... 12

**3.3. Association of *APOA5*-1131T>C polymorphism with lipid profiles, lipoprotein and LDL particle size** ..... 14

**3.4. Relative risk of diabetes mellitus according to *APOA5*-1131T>C genotype** ..... 19

**3.5. The associative effect of *APOA5*-1131T>C and fasting glucose on plasma triglyceride** ..... 21

**4. DISCUSSION** ..... 25

**REFERENCES** ..... 30

**ABSTRACT (KOREAN)** ..... 35

# List of Figures

**Figure 1. Plasma levels of triglyceride, HDL-cholesterol and apolipoprotein A5 according to *APOA5*-1131T>C polymorphism** ..... 18

**Figure 2. Synergistic effect of *APOA5*-1131T>C genotype and fasting glucose on plasma triglyceride in diabetes mellitus** ..... 24

# List of Tables

**Table 1. Demographic and metabolic parameters of study** ..... 11

**Table 2. Genotype distribution and allele frequency of *APOA5*-1131T>C polymorphism in study population** ..... 13

**Table 3. Association of *APOA5*-1131T>C polymorphism and lipid profiles, lipoprotein and LDL particle size in Korean women (n=3450)** ..... 17

**Table 4. Odds ratio (OR) for diabetes mellitus according to *APO*A5-1131T>C genotype** ..... 20

**Table 5. Pearson or partial correlation coefficients between fasting glucose concentration and plasma TG level in total or diabetic women according to APOA5-1131T>G genotype** ..... 23

# ABSTRACT

## The *APOA5*-1131C allele may contribute to the increased susceptibility to diabetes mellitus in association with plasma triglyceride in Korean women

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**Background and objective:** *APOA5*-1131C allele is associated with higher plasma triglyceride concentration and contributes to higher cardiovascular disease (CVD) risk in Koreans. Hypertriglyceridemia is not only a classical risk factor for CVD but also one of the commonly recognized lipid abnormalities in diabetes mellitus

(DM), furthermore it is an independent risk factor for atherosclerosis in type 2 DM. Therefore, our study aimed to investigate the association of *APOA5*-1131T>C and the relative risk of DM in Korean, and their relationship with plasma lipid profile in Korean.

**Design and methods:** Totally 3450 unrelated Korean women were included in this study [healthy control, n=2033; diabetes mellitus (DM), n=304, and metabolic abnormality (MA) (metabolic syndrome or impaired fasting glucose), n=1113]. Association of *APOA5*-1131T>C and DM risk were calculated by logistic regression analysis (odds ratio, OR; 95%confidence interval, CI) with adjustment for compounding factors. Differences in basic characteristics and biochemical parameters among groups were tested by one-way analysis of variance (ANOVA) followed by Bonferroni method with adjustment or not

**Results:** The frequency of *APOA5*-1131C allele is higher in DM patients than non diabetic subjects. C carriers showed significantly higher risk of DM than TT genotype [$OR_0$ 1.39 (95% CI, 1.09–1.77), P=0.008] and the significance of the association still remained after the adjustment for age, BMI, menopause, smoking

and drinking [$OR_1$ 1.61 (95% CI, 1.23-2.10), P<0.001]. Particularly, CC genotype showed a much higher increased risk of DM than TT genotype [$OR_0$ 1.93 (95% CI, 1.27-2.92), P=0.002, $OR_1$ 2.35 (95% CI, 1.46-3.77), P<0.001]. When adjusted for metabolic syndrome, C carriers still have a significantly high risk of DM [$OR_2$ 1.98 (95% CI, 1.09-3.59, p=0.024]. CC homozygote also showed high risk, but it turned to a tendency [$OR_2$ 2.51 (95% CI, 0.90-6.96, p=0.078]. Regarding lipid profiles, C carriers, particularly CC homozygote, had higher levels of TG, and lower levels of HDL-cholesterol in all three subjects groups. Apoplipoprotein A5 levels were also significantly associated with genotypes in healthy control and MA group, but not in DM patients. Fasting glucose levels were significantly positively correlated with plasma TG levels in whole or in each genotype of total population On the other hand, DM patients show the positive correlation in C carriers (r=0.165, p=0.035), which was much higher in CC genotype (r=0.395, p=0.021), but not in TT genotype (r=0.139, p=0.102). These patterns still retained after adjusted for age, BMI, menopause, smoking and drinking. In DM patients particularly with higher fasting glucose levels, CC homozygote showed a dramatically higher level of TG than TT

genotype before and after the adjustment.

**Conclusion:** Our new findings suggest that *APOA5*-1131C allele may contribute to the increased susceptibility of diabetes mellitus in association with plasma triglyceride in Korean women

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**Key words:** *APOA5*-1131C allele, diabetes mellitus, plasma triglyceride, Korean women

# 1. INTRODUCTION

Apolipoprotein A5 gene (*APOA5*) is involved in triglyceride metabolism in both fasting and postprandial states in human (1-3). Among several common single nucleotide polymorphisms (SNPs) in *APOA5*, the C allele in the promoter region at position 1131 (*APOA5*-1131T>C) showed a strong association with hypertriglyceridemia and the increased risk of CAD in multiple ethnic population. The frequency of the C allele at *APOA5*-1131SNP is diverse among population, which is relatively high in East Asian (33% in Chinese and Japanese and 28~30% in Korean) compared to Western people (9% in Caucasian and 16% in Hispanics) (2,4-9). In our previous studies, *APOA5*-1131C allele was associated with higher plasma triglyceride concentration and turned out to be a significant factor contributing to higher cardiovascular disease (CVD) risk in Koreans, independently of common environmental factors (2,10).

Hypertriglyceridemia is not only a classical risk factor for CVD but also one of the commonly recognized lipid abnormalities in diabetes mellitus (DM), furthermore, it is an independent risk factor for atherosclerosis in type 2 DM (11-12). Consequently, the

*APOA5* has become an interest as a good candidate for the development of vascular complications in type 2 DM or of DM directly. However, the results are still discrepancy. Zhai et al reported that the frequency of *APOA5*-1131C allele in DM patients was significantly higher than that of non diabetic subjects (13). When compared with TT genotype, CC homozygotes had a significantly increased DM risk in 3.75 times higher. *APOA5*-1131C carriers of DM patients contributed to the increments of triglyceride, total-cholesterol and LDL-cholesterol in plasma, but no effect on insulin resistance. Yan et al. showed the association of *APOA5*-1131C allele with elevated plasma triglyceride in type 2 DM in Chinese population (14). He also found that C carriers had two fold increased risk of type 2 DM with coronary heart disease compared with TT subjects. However, the statistical significances disappeared after adjusted for well known risk factors such as sex, low HDL-cholesterol, and higher triglyceride and smoking. On the other hand, there are no studies on the relationship of *APOA5*-1131T>C polymorphism with DM in Korean population.

Therefore, our study aimed to investigate the association of *APOA5*-1131T>C and the relative risk of DM in Korean, and their relationship with plasma lipid profile in Korean.

# 2. SUBJECTS AND METHODS

## 2.1. Study population

Study participants (women) were recruited from the Health Service Center in the course of a routine checkup visit or by a newspaper announcement for health examination. To begin with, were excluded subjects with orthopedic limitations, weight loss/gain over the previous 6 months, or any diagnosis of vascular disease, cancer (clinically or by anamnesis), renal disease, liver disease, thyroid disease, or acute or chronic inflammatory disease. Healthy controls have no history or diagnosis of metabolic syndrome (MS), impaired fasting glucose tolerance (IFG), diabetes mellitus (DM) and the diseases mentioned above. None of them were taking any medications (antihypertensive, antidyslipidemic, antithrombotic and antidiabetic drugs). Definition of MS was followed by modification of National Cholesterol Education program-Adult Treatment Panel III guideline, Asian-Pacific guideline and American Diabetes Association guide line. It included at least three components of waist circumference>80cm (female), triglyceride (TG) ≥150mg/dl, HDL-cholesterol<50mg/dl (female), blood pressure≥130/≥85mmHg, or fasting glucose≥100mg/dl, but in fact fasting glucoses in between 100 and 126 mg/dl

were classified to IFG. Diabetes mellitus (DM) includes people who got diagnosis for DM from hospital and take hypoglycemic agents prescribed by medical doctor, or fasting glucose≥126mg/ml.

Finally, in this study we included 3450 unrelated Korean women; 2033 healthy control, 304 DM patients, and additionally 1113 people with metabolic abnormality (MS or IFG). Written informed consent was obtained from all subjects, and the protocol was approved by the Institute of Review Board of Yonsei University.

## 2.2. Anthropometric parameters, blood pressure measurements, and blood collection

Body weight and height were measured unclothed and without shoes in the morning. Blood pressure was obtained from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 min of rest. After an overnight fasting period, venous blood specimens were collected in EDTA-treated or plain tubes and centrifuged into plasma or serum then, stored at −70 °C until analysis.

## 2.3. *APOA5* -1131T>C genotyping

Genomic DNA was extracted from 5 ml of whole blood using a commercially available DNA isolation kit (WIZARDR Genomic DNA purification kit, Promega Corp., Madison, WI) according to the manufacturer's protocol. -1131T>C genotyping was performed by SNP-IT™ assays using single primer extension technology (SNPstream 25K™ System, Orchid Biosystems, NJ). The results of yellow and/or blue color developments were analyzed with ELISA reader and the final genotype calls were made with QCReview™ program.

## 2.4. Serum lipid profiles, apolipoproteins A1 and B and glucose

Serum concentrations of total cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd. Tokyo, Japan). After using dextran sulfate magnesium to precipitate serum chylomicron, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol from the supernatant were measured by an enzymatic method. LDL cholesterol was indirectly estimated in subjects with serum triglyceride concentrations <4.52 mol/l (400 mg/ml) using the

Friedewald formula. In subjects with serum triglyceride concentrations ≥4.52 mol/l, LDL cholesterol was measured by an enzymatic method on a Hitachi 7150 Analyzer directly. Serum apolipoprotein (apo) A1 and B were determined by turbidometry at 340 nm using a specific anti-serum (Roche, Switzerland). Glucose was measured by using a glucose oxidase method with the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA).

## 2.5. Plasma apolipoprotein A5

Plasma apolipoprotein A5 (Apo5) concentration was measured using an enzyme immunoassay (Human Apolipoprotein A ELISA Kit, Millipore, MO). The resultant color reaction was read at 450 nm using a Victor$^2$ (Perkin Elmer life sciences, Turka, Finland). The intra- and inter-assay CVs were 2.46% and 7.45%, respectively.

## 2.6. Plasma LDL particle size

Particle size distribution of LDL (1.019–1.063 g/ml) isolated by sequential flotation ultracentrifugation was examined by a pore-gradient lipoprotein system (CBS Scientific, CA) using commercially available non-denaturing polyacrylamide slab gels containing a

linear gradient of 2–16% acrylamide (Alamo Gels Inc., San Antonio, TX). Standards of latex beads (34 nm), thyroglobulin (17 nm), apoferritin (12.2 nm) and catalase (10.4 nm) were used to estimate the relative migration (Rf) rates of each band. The gels were scanned by GS-800 Calibrated Imaging Densitometer (Bio-Rad Laboratories, Graz, Austria). LDL particle size was calculated with reference to the Rf value of the standards.

## 2.7. Statistical analyses

Statistical analyses were performed with SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Inc, Chicago, Ill). Sample size was checked with PS version 2.1 (power and sample size calculation, Nashville, Tenn). Hardy-Weinberg Equilibrium was examined using the Executive SNP Analyzer 1.0 (http://www.istech.info/SilicoSNP/index.html). Genotype distributions and allele frequencies were compared between healthy controls and MS/IGT or diabetic patients by $\chi^2$ test. The association of DM with genotype was calculated using the odds ratio (OR) [95% confidence intervals (CIs)] of a $\chi^2$ test with adjustment for compounding factors. One-way analysis of variance (ANOVA) followed by Bonferroni method was used to compare the

differences among subjects groups [healthy control, metabolic abnormality (MA) and DM, respectively] or among genotype groups of each subject group with adjustment or not. Pearson correlation and partial correlation test were performed to examine the relationship between fasting glucose concentration and plasma triglyceride in association with *APOA5*-1131T>C. Each variable was examined for normal distribution patterns, and the 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 or percentage, and a two-tailed value of $p<0.05$ was considered statistically significant.

# 3. RESULTS

## 3.1. Characteristics of study subjects

Table 1 shows general characteristics of 3450 study subjects. Mean values of age and BMI, and proportion of post-menopause were higher in subjects with MA (n=1133) and DM (n=304) compared to healthy control (N=2033). Proportion of cigarette smoking and alcohol consumption were not significantly different among the three groups. Systolic blood pressures were higher in subjects with MA and DM than healthy control ($p<0.001$), and diastolic blood pressures and total cholesterol levels were higher in MA group than the other two groups ($p<0.001$). Plasma levels of TG and ApoB were highest in MA women and lowest in healthy control ($p<0.001$). HDL-cholesterol and ApoA1 levels were highest in healthy control and lowest in MA women ($p<0.001$). ApoA5 levels were lower in people with MA and DM than healthy control ($p<0.001$) and LDL particle sizes were lower in MA group than the other two groups. As expected, fasting glucose levels were highest in DM patients, and higher in MA group compared with healthy control ($p<0.001$). These patterns still retained after the adjustment for age

BMI and menopausal status. On the other hand, significant difference in LDL cholesterol levels among groups disappeared after the adjustment (p0=0.006, p1=0.072).

**Table 1. Demographic and metabolic parameters of study population (n=3450 women)**

| | Healthy control (n=2033) | MA (n=1113) | DM (n=304) | $p_0$ | $p_1$ |
|---|---|---|---|---|---|
| Age (year) | 54.6 ± 0.3 | 59.7 ± 0.3 | 62.1 ± 0.5 | <0.001 | - |
| Body mass index (kg/m$^2$) | 23.4 ± 0.1 | 25.4 ± 0.1 | 25.1 ± 0.2 | <0.001 | - |
| Post-menopause (%) | 64.7 | 82.8 | 89.4 | <0.05 | - |
| Current smoking (%) | 2.4 | 1.8 | 2.0 | n.s. | - |
| Current drinking (%) | 31.9 | 24.2 | 16.2 | n.s. | - |
| Blood Pressure (BP) | | | | | |
| Systolic BP (mmHg) | 120.8 ± 0.3$^b$ | 133.0 ± 0.5$^a$ | 132.1 ± 1.1$^a$ | <0.001 | <0.001 |
| Diastolic BP (mmHg) | 73.8 ± 0.2$^b$ | 79.9 ± 0.3$^a$ | 76.9 ± 0.6$^b$ | <0.001 | <0.001 |
| Triglyceride (mg/dL)[1] | 94.5 ± 0.8$^c$ | 170.7 ± 2.5$^a$ | 151.6 ± 5.9$^b$ | <0.001 | <0.001 |
| Total-cholesterol (mg/dL) | 194.3 ± 0.8$^b$ | 200.1 ± 1.1$^a$ | 194.9 ± 2.3$^b$ | <0.001 | 0.033 |
| HDL-cholesterol (mg/dL) | 60.3 ± 0.3$^a$ | 47.1 ± 0.4$^c$ | 51.3 ± 0.7$^b$ | <0.001 | <0.001 |
| LDL-cholesterol (mg/dL) | 115.0 ± 0.7 | 118.7 ± 1.1 | 114.2 ± 2.2 | 0.006 | 0.072 |
| Apolipoprotein A1 (mg/dL) | 149.4 ± 0.7$^a$ | 135.6 ± 0.8$^c$ | 140.8 ± 1.4$^b$ | <0.001 | <0.001 |
| Apolipoprotein B (mg/dL) | 81.8 ± 0.5$^c$ | 94.3 ± 0.7$^a$ | 88.5 ± 1.4$^b$ | <0.001 | <0.001 |
| Apolipoprotein A5 (mg/dL)[1] | 250.4 ± 7.0$^a$ | 213.8 ± 8.2$^b$ | 187.1 ± 6.6$^b$ | <0.001 | <0.001 |
| LDL size (nm)[1] | 24.3 ± 0.03$^a$ | 23.9 ± 0.04$^b$ | 24.2 ± 0.09$^a$ | <0.001 | <0.001 |
| Glucose (mg/dL)[1] | 83.6 ± 0.2$^c$ | 89.5 ± 0.4$^b$ | 127.1 ± 2.1$^a$ | <0.001 | <0.001 |

Mean±S.E. MA: metabolic abnormality such as metabolic syndrome or impaired fasting glucose, DM: diabetes mellitus,

[1] tested by log-transformed, tested by one-way analysis of variance (ANOVA) followed by Bonferroni method with adjustment or not

Sharing the same alphabet at the same row indicates no significant difference between two groups after adjustment for age and body mass index.

n.s.: no significant, $p_0$: unadjusted p-value, $p_1$: p-value adjusted for age, BMI and menopausal status

## 3.2. Distribution of *APOA5*-1131T>C polymorphism

Genotype distribution was in Hardy–Weinberg equilibrium in the entire population as well as in healthy controls, MA, and DM, separately. The minor allele frequency of the -1131T>C was 0.29 in whole population which was consistent with previous observations in a Korean population (2,10). The genotypic distribution of the -1131T>C polymorphism in healthy controls (0.27) was significantly different from those in the other two groups (MA: 0.32, DM: 0.33) ($P<0.05$) (Table 2).

**Table 2. Genotype distribution and allele frequency of *APOA5*-1131T>C polymorphism in study population**

| *APOA5*-1131T>C | Healthy control (n=2033) | MA (n=1113) | DM (n=304) | p-value |
|---|---|---|---|---|
| TT | 1096 (53.9) | 514 (46.2) | 139 (45.7) | |
| TC | 798 (39.3) | 489 (43.9) | 131 (43.1) | <0.05 |
| CC | 139 (6.8) | 110 (9.9) | 34 (11.2) | |
| C allele frequency | 26.5 % | 31.9 % | 32.7 % | <0.05 |

MA: metabolic abnormality such as metabolic syndrome or impaired fasting glucose, DM: diabetes mellitus

Genotype distributions and allele frequencies were compared between healthy controls and MA or DM by $\chi^2$ test.

### 3.3. Association of *APOA5*-1131T>C polymorphism with lipid profiles, lipoprotein and LDL particle size

General characteristics such as age, BMI, systolic and diastolic blood pressure were not associated with the *APOA5*-1131T>C genotype in each subject group (healthy control, MA and DM, respectively) (Table 3). Neither significant difference was observed for the proportions of post-menopause, current smoking and alcohol consumption according to genotypes (data not shown).

Plasma triglyceride levels were higher in C carriers, particularly in CC homozygote than TT genotype in both Healthy control and MA women (p0), which still retained after the adjustment for age, BMI, menopause, smoking and drinking (p1) (Figure 1). However, these significances disappeared after further adjustment for plasma ApoA5 (p2) and lipid drug consumption (p3). Oh the other hand, DM patients consistently showed higher levels of triglyceride in CC genotype than T carriers (p0, p1 and p3). Plasma HDL-cholesterol levels were lower in CC homozygotes in all three subject groups before (p0) and after the adjustment for age, BMI, menopause, cigarette smoking and alcohol drinking (p1) (Figure 1). However, the significant differences disappeared after further adjustment for plasma

ApoA5 (p2) and lipid drug consumption (p3). Plasma ApoA5 levels were significantly lower in C carrier of healthy control, and in CC homozygotes of MA women before (p0) and after the adjustment for age, BMI, menopause, smoking and drinking (p1). However, the significance disappeared after further adjustment for lipid drug consumption in MA women. On the other hand, no significant differences were observed in DM patient before (p0) and after the adjustment (p1 and p4) (Figure 1).

In addition, Table 3 also presents the genotype associated levels of total cholesterol, LDL cholesterol, ApoA1, ApoB and LDL particle size in each subject population. Plasma levels of total cholesterol, LDL cholesterol and ApoB were not significantly different among genotypes in both healthy control and DM patients. On the other hand, MA group shows significant higher levels of total cholesterol and ApoB in TC genotype and lower levels of LDL cholesterol in CC genotype. Lower levels of ApoA1 were observed in CC genotype of healthy control and MA women. LDL particle sizes were higher in TT genotype than CC genotype among healthy control. Regarding fasting glucose, there were no significant genotype associated differences in all subject groups (TT:83.7±0.2, TC:83.5±0.3, CC:83.6±0.8 mg/dL in healthy control, TT:89.5±0.6, TC:89.8±0.6,

CC:88.6±1.1 mg/dL in MA, TT:129.0±3.0, TC:125.7±3.2, CC:124.6±7.5 mg/dL in DM patients). In addition, proportions of medications such as antihypertensive, lipid-lowering and oral hypoglycemic agents were not significantly different according to genotype in the MA group and DM group respectively (data not shown).

**Table 3. Association of *APOA5*-1131T>C polymorphism and lipid profiles, lipoprotein and LDL particle size in Korean women (n=3450)**

| | | TT | | | TC | | | CC | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Healthy control | Age (year) | 55.1 | ± | 0.4 | 54.2 | ± | 0.4 | 52.9 | ± | 1.1 |
| | Body mass index (kg/m$^2$) | 23.6 | ± | 0.1 | 23.3 | ± | 0.1 | 22.9 | ± | 0.2 |
| TT (n=1096) | Systolic blood pressure (mmHg) | 121.7 | ± | 0.5 | 120.0 | ± | 0.6 | 117.8 | ± | 1.3 |
| TC (n=798) | Diastolic blood pressure (mmHg) | 74.3 | ± | 0.3 | 73.3 | ± | 0.4 | 72.0 | ± | 0.8 |
| CC (n=139) | Total-cholesterol (mg/dL) | 194.4 | ± | 1.1 | 194.5 | ± | 1.2 | 192.5 | ± | 3.2 |
| | LDL-cholesterol (mg/dL) | 114.9 | ± | 1.0 | 114.9 | ± | 1.1 | 115.9 | ± | 2.8 |
| | Apolipoprotein A1 (mg/dL) | 150.9 | ± | 0.9$^a$ | 149.2 | ± | 1.1$^a$ | 140.6 | ± | 2.8$^b$ |
| | Apolipoprotein B (mg/dL) | 81.1 | ± | 0.7 | 82.1 | ± | 0.8 | 85.3 | ± | 2.1 |
| | LDL particle size (nm)[1] | 24.3 | ± | 0.04$^a$ | 24.3 | ± | 0.04$^{ab}$ | 24.1 | ± | 0.09$^b$ |
| MA | Age (year) | 60.5 | ± | 0.5 | 58.9 | ± | 0.5 | 59.6 | ± | 1.1 |
| | Body mass index (kg/m$^2$) | 25.4 | ± | 0.1 | 25.3 | ± | 0.1 | 25.8 | ± | 0.3 |
| TT (n=514) | Systolic blood pressure (mmHg) | 133.0 | ± | 0.7 | 132.4 | ± | 0.7 | 135.1 | ± | 1.7 |
| TC (n=489) | Diastolic blood pressure (mmHg) | 79.9 | ± | 0.4 | 79.6 | ± | 0.5 | 81.2 | ± | 1.1 |
| CC (n=110) | Total-cholesterol (mg/dL) | 197.4 | ± | 1.7$^b$ | 204.3 | ± | 1.7$^a$ | 194.5 | ± | 3.7$^b$ |
| | LDL-cholesterol (mg/dL) | 118.6 | ± | 1.5$^a$ | 121.0 | ± | 1.6$^a$ | 109.6 | ± | 3.7$^b$ |
| | Apolipoprotein A1 (mg/dL) | 135.2 | ± | 1.1$^a$ | 137.8 | ± | 1.2$^a$ | 128.3 | ± | 2.3$^b$ |
| | Apolipoprotein B (mg/dL) | 92.2 | ± | 1.1$^b$ | 96.2 | ± | 1.1$^a$ | 95.6 | ± | 2.4$^{ab}$ |
| | LDL particle size (nm)[1] | 23.9 | ± | 0.06 | 23.9 | ± | 0.06 | 23.7 | ± | 0.15 |
| DM | Age (year) | 62.9 | ± | 0.7 | 61.7 | ± | 0.9 | 60.4 | ± | 1.8 |
| | Body mass index (kg/m$^2$) | 25.0 | ± | 0.3 | 25.3 | ± | 0.3 | 24.6 | ± | 0.4 |
| TT (n=139) | Systolic blood pressure (mmHg) | 132.8 | ± | 1.7 | 131.6 | ± | 1.6 | 131.2 | ± | 2.5 |
| TC (n=131) | Diastolic blood pressure (mmHg) | 76.9 | ± | 1.0 | 77.0 | ± | 0.9 | 76.3 | ± | 1.4 |
| CC (n=34) | Total-cholesterol (mg/dL) | 196.8 | ± | 3.3 | 192.8 | ± | 3.5 | 195.3 | ± | 6.8 |
| | LDL-cholesterol (mg/dL) | 115.4 | ± | 3.2 | 113.9 | ± | 3.4 | 110.7 | ± | 6.2 |
| | Apolipoprotein A1 (mg/dL) | 143.1 | ± | 2.2 | 139.9 | ± | 2.0 | 134.6 | ± | 4.4 |
| | Apolipoprotein B (mg/dL) | 88.4 | ± | 2.1 | 87.6 | ± | 2.2 | 91.8 | ± | 4.5 |
| | LDL particle size (nm)[1] | 24.3 | ± | 0.14 | 24.1 | ± | 0.14 | 24.1 | ± | 0.25 |

Mean±S.E. MA: metabolic abnormality such as metabolic syndrome or impaired fasting glucose, DM: diabetes mellitus,

[1] Tested by log-transformed, tested by one-way analysis of variance (ANOVA) followed by Bonferroni method in each subject group

Sharing the same alphabet at the same row indicates no significant difference between two groups.

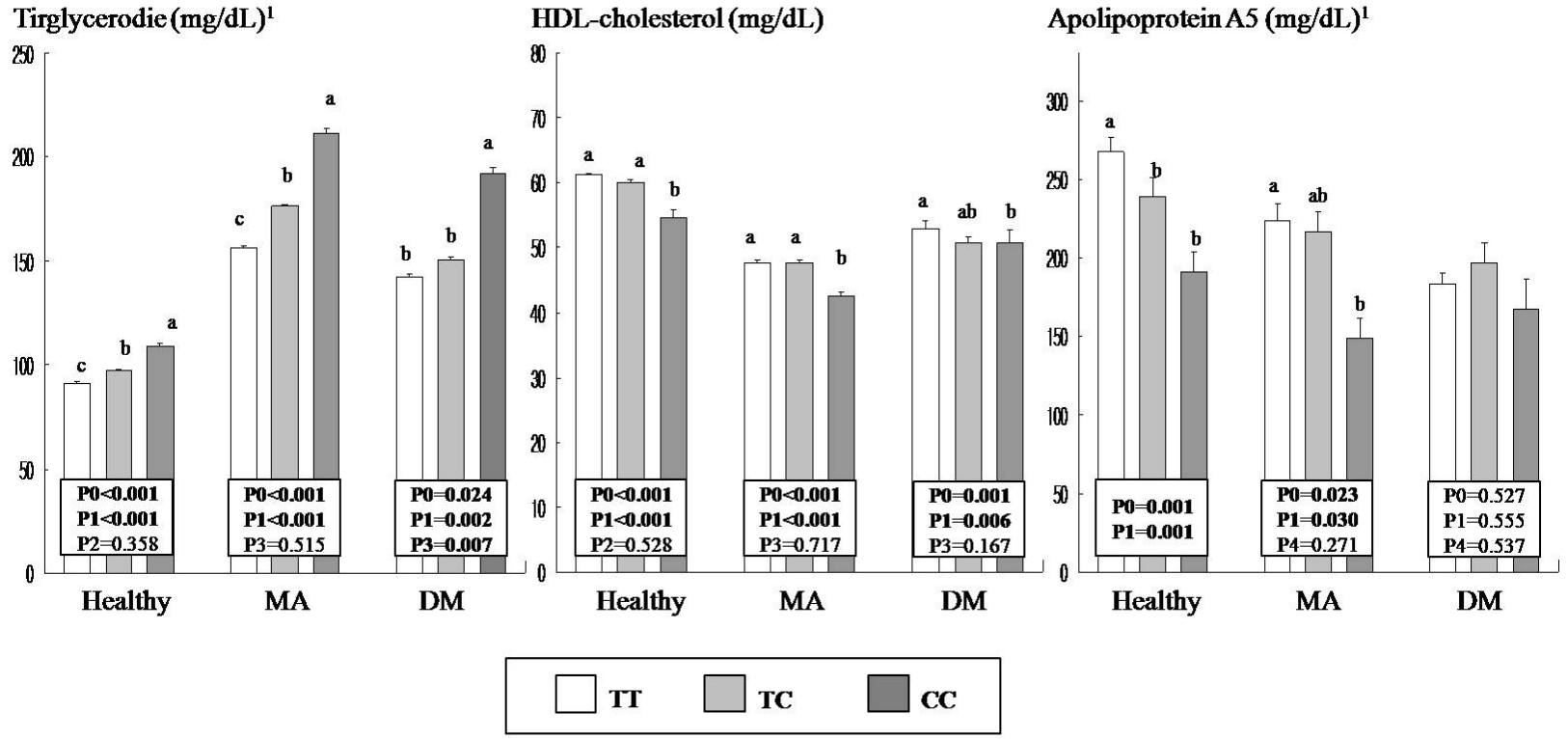


**Figure 1. Plasma levels of triglyceride, HDL-choletserol and apolipoproteinA5 according to *APOA5*-1131T>C polymorphism**

Mean±S.E. [1] tested by log-transformed, tested by one-way analysis of variance (ANOVA) followed by Bonferroni method with or without adjustment in each subject group

Sharing the same alphabet at the same row indicates no significant difference between two groups after the adjustment.

P0: unadjusted, P1: adjusted for age, body mass index, menopause, smoking and drinking,, P2: adjusted for age, body mass index, menopause, smoking, drinking and ApoA5, P3: adjusted for age, body mass index, menopause, smoking, drinking, ApoA 5 and lipid drug,, P4: adjusted for age, body mass index, menopause, smoking, drinking and lipid drug

Healthy control (TT: n=1096, TC: n=798, CC: n=139), MA (TT: n=514, TC: n=489, CC: n=110), DM (TT: n=139, TC n=131, CC n=34)

MA: metabolic abnormality such as metabolic syndrome or impaired fasting glucose, DM: diabetes mellitus

## 3.4. Relative risk of diabetes mellitus according to *APOA5*-1131T>C genotype

Based on the result shown in Table 2, carriers of minor C allele were compared with TT homozygotes (Reference group) (Table 4). C carriers showed significantly higher risk of DM than TT genotype [$OR_0$ 1.39 (95% CI, 1.09–1.77), P=0.008] and the significance of the association still remained after the adjustment for age, BMI, menopause, smoking and drinking [$OR_1$ 1.61 (95% CI, 1.23-2.10), P<0.001]. Particularly, CC genotype showed a much higher increased risk of DM than TT genotype [$OR_0$ 1.93 (95% CI, 1.27-2.92), P=0.002, $OR_1$ 2.35 (95% CI, 1.46-3.77), P<0.001]. These patterns were weaker but similar for the risk of MA. When adjusted for metabolic syndrome, C carriers still have a significantly high risk of DM [$OR_2$ 1.98 (95% CI, 1.09-3.59, p=0.024]. CC homozygote also showed high risk, but it turned to a tendency [$OR_2$ 2.51 (95% CI, 0.90-6.96, p=0.078].

**Table 4. Odds ratio (OR) for diabetes mellitus according to *APO*A5-1131T>C genotype**

| | | Unadjusted ($OR_0$) | | adjusted[1] ($OR_1$) | | adjusted ($OR_2$) |
|---|---|---|---|---|---|---|
| | | MA | DM | MA | DM | DM |
| TT[1] | | 1 | 1 | 1 | 1 | 1 |
| C carrier (TC+CC) | OR | 1.363 | 1.388 | 1.466 | 1.608 | 1.983 |
| | (95% CI[2]) | (1.177- 1.578) | (1.090- 1.768) | (1.248-1.721) | (1.233-2.098) | (1.094-3.593) |
| | p-value | <0.001 | 0.008 | <0.001 | <0.001 | 0.024 |
| CC | OR | 1.687 | 1.929 | 1.913 | 2.348 | 2.511 |
| | (95% CI[2]) | (1.287-2.213) | (1.274- 2.919) | (1.423-2.573) | (1.463-3.769) | (0.903-6.958) |
| | p-value | <0.001 | 0.002 | <0.001 | <0.001 | 0.078 |

[1] Reference, [2]Confidence interval

MA: metabolic abnormality such as metabolic syndrome or impaired fasting glucose, DM: diabetes mellitus

The association between genotype and MA, or DM was calculated using the odds ratio (OR) [95% confidence intervals (CIs)] of a $\chi^2$ test with adjustment or not.

1: adjusted for age, BMI, menopause, smoking and drinking

2: adjusted for age, BMI, menopause, smoking, drinking and metabolic syndrome

## 3.5. The associative effect of *APOA5*-1131T>C and fasting glucose on plasma triglyceride

Table 5 shows the relationship between fasting glucose concentration and plasma TG concentration in association with *APOA5*-1131T>C genotype. In all subjects, fasting glucose concentrations were positively correlated with plasma TG levels (R0: r=0.142, p<0.001). This relationship was also shown in DM patients (R0: r=0.148, p=0.010). When subdivided into *APOA5*-1131T>C genotype, total population shows significant positive correlations between these two variables in each genotype (R0). On the other hand, DM patients show the positive correlation in C carriers (R0: r=0.165, p=0.035), which was much higher in CC homozygote (R0: r=0.395, p=0.021), but not in TT genotype (R0: r=0.139, p=0.102). These patterns still retained after adjusted for age, BMI, menopause, smoking and drinking (R1).

Based on the results in Table 5, plasma TG levels were compared among genotypes according to fasting glucose concentration (median value of each subject group: 84mg/dl in healthy control, 87mg/dl in MA: and 122mg/dl in DM) (Figure 2). In healthy control, plasma TG levels were higher in CC homozygote as well as C carriers

than TT genotype regardless of glucose levels, before and after adjusted for age, BMI, menopause, smoking, drinking and/or plasma ApoA5. These patterns were similar shown din MA group. In DM patients, no significant association between TG levels and genotypes were observed when fasting glucose levels were less than 122mg/dl, but dramatically higher TG levels were observed in CC genotype than TT genotype when fasting glucose levels were 122mg/dl and higher before and after the adjustment.

**Table 5. Pearson or partial correlation coefficients between fasting glucose concentration and plasma TG level in total or diabetic women according to APOA5-1131T>G genotype**

| | | According to APOAT-1131T>C genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total | | TT | | C carrier (TC+CC) | | CC | |
| | | R0 | R1 | R0 | R1 | R0 | R1 | R0 | R1 |
| Total population (n=3450) | *r* | 0.142 | 0.130 | 0.144 | 0.105 | 0.139 | 0.096 | 0.183 | 0.150 |
| | *p* | <0.001 | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | 0.002 | 0.013 |
| DM (n=304) | *r* | 0.148 | 0.146 | 0.139 | 0.113 | 0.165 | 0.168 | 0.395 | 0.451 |
| | *P* | 0.010 | 0.015 | 0.102 | 0.219 | 0.035 | 0.039 | 0.021 | 0.027 |

*r*: correlation coefficient, *p*: p-value

R0: non-adjusted, R1: adjusted for age, BMI, menopause, smoking and drinking

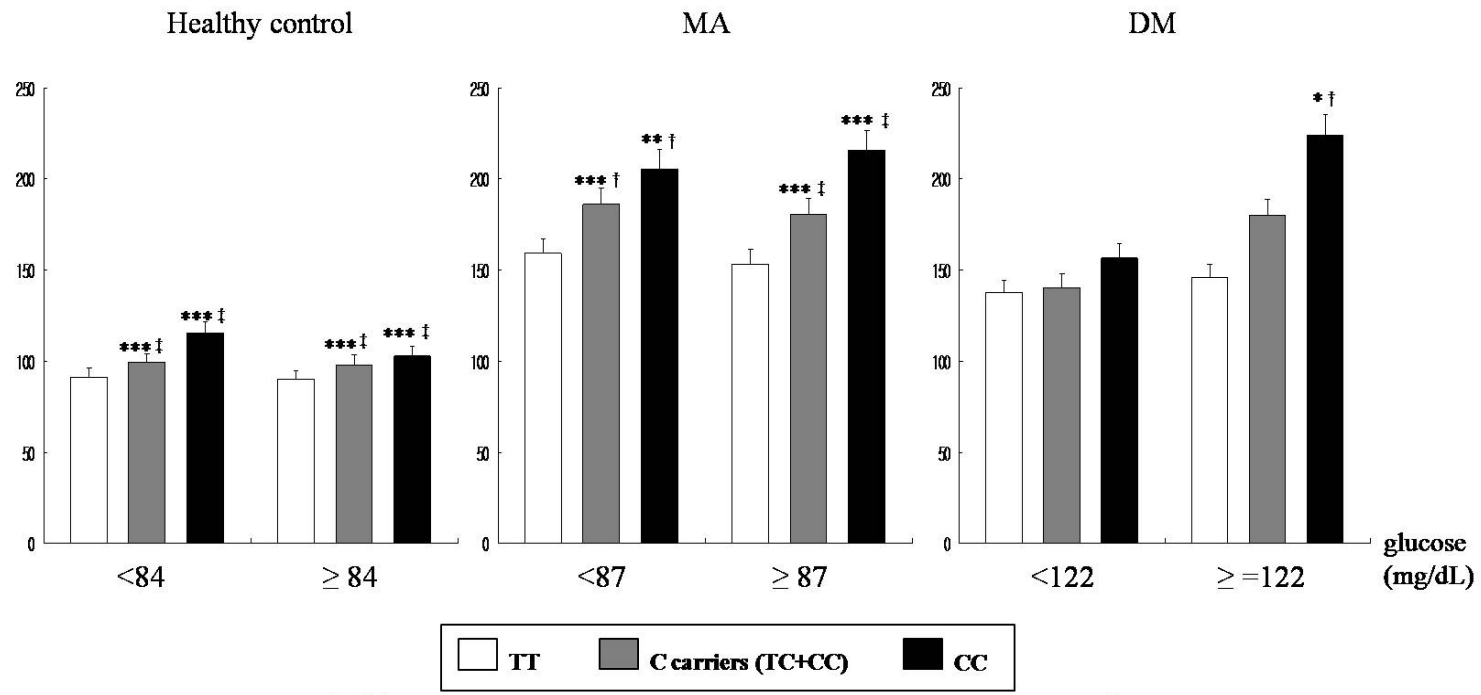


**Figure 2. Synergistic effect of *APOA5*-1131T>C genotype and fasting glucose on plasma triglyceride[1] (mg/ml) in diabetes mellitus**

Mean±S.E. [1]tested by log-transformed,

*p<0.05, **p<0.01, ***p<0.01, compared with TT subjects in each subject group after adjustment for age and body mass index, menopause, smoking and drinking

†p<0.01, ‡p<0.001, compared with TT subjects in each subject group after adjustment for age, body mass index, menopause, smoking, drinking, ApoA5 (healthy control) and lipid drug (MA, DM)

84, 87 and 122mg/dl indicate median values of fasting glucose in healthy control, MS or IGT and DM women, respectively.

MA: metabolic abnormality such as metabolic syndrome or impaired fasting glucose, DM: diabetes mellitus

# 4. Discussion

Our present study shows that C allele frequency is higher in diabetic patients than non diabetic subjects and that C carriers, particularly CC homozygote had the increased risk for diabetes mellitus (DM). This significant association retained even after the adjustment for age, BMI, smoking, drinking, menopausal status and metabolic syndrome. Our results for the first time suggest the contribution of *APOA5*-1131C allele to the increased susceptibility of DM in Korean. It may be in line with the studies of Zhai et al. (13) and Yan et al (14) reporting the higher risk of DM in C carriers (2~3.75 times). However, they lost the significant association after adjusted for well known risk factors (sex, low HDL-cholesterol, higher triglyceride, smoking etc). This discrepancy with our results may be due to the differences in subject characteristics and ethnicity. In our study, subjects were all Korean women and the diabetic patients did not have cardiovascular disease, whereas the other studies included both men and women (13,14) and in part, diabetic patients carried coronary heart disease (14). It is well known that frequency of the C allele at *APOA5*-1131SNP is higher in East Asian (28~30%) than Western people (9~16%) (2,4-9), but it might be possible that minor allele frequency are different among populations even

in the same Asian region. The minor C allele frequency of healthy subjects in our study was 0.265, but 0.296 in Zhai's and 0.277 in Yan's works (13,14) which were a little bit higher than ours.

*APOA5*-1131T>C is strongly associated with hypertriglyceridemia, a classical CVD risk factor and the increased risk of CVD in multiple ethnic population (2,4,10,15). Hypertriglyceridemia is also commonly observed in diabetes mellitus (DM) and an independent risk factor for cardiovascular complication in type 2 DM (11-12). In all of our subjects, C carriers particularly CC homozygote had higher levels of TG and lower levels of HDL-cholesterol. The significances still retained after the adjustment for confounders such as age, BMI, smoking, drinking and menopause, and further adjustment for plasma ApoA5 levels still maintained the significance in diabetic patients, but attenuated the significant association with genotype in healthy control and MA people.

ApoA5 is involved in the regulation of circulating TG levels by directly interacting with lipoprotein lipase (LPL) and inducing LPL-mediated hydrolysis of plasma TG (16, 17). Several studies reported that ApoA5 levels are lower in CAD or DM patients than healthy people (18,19,20). Our results also show the lower levels of ApoA5 in DM

patients than healthy control. According to Nowak et al, ApoA5 expression is down-regulated by insulin resistance and plasma ApoA5 levels were decreased by following insulin infusion (21). In addition, lower levels of ApoA5 are associated with C allele at *APOA5*-1131 in healthy people and CAD patients (10), which is in the opposite direction with TG levels. In our study, ApoA5 levels were lower in C carriers of healthy control and CC homozygote of MA people. On the other hand, no significant genotype-association was observed in DM patients. It is assumed that hyperinsulinemia or insulin resistance commonly shown in DM patients might down-regulate ApoA5 expression, regardless of *APOA5*-1131T>C genotype. However, no genotype-association was found with fasting glucose levels in all three subject groups. The similar results was observed in the report of Zhai et al (13) which showed no significance in profiles of insulin resistance according to genotypes in both healthy control and type 2 DM. Neither significant genotype association was observed with fasting glucose levels and insulin resistance in our previous study for healthy control and CAD patients (10). Perhaps, measurement of glucose or insulin levels during postprandial states, oral glucose tolerance test or hyperglycemic clamp rather than fasting state will be more relevant to examine the

association with *APOA5*-1131T>C.

Fasting glucose levels were positively correlated with plasma TG levels in whole or in each genotype of total population. On the other hand, in DM patients, positive correlation was observed only in C carriers, particularly in CC genotype (r=0.395, p=0.021), but not in TT genotype (r=0.139, p=0.102). Additionally, DM patients show the dramatically significant associations of TG level with *APOA5*-1131T>C only in those with higher fasting glucose, whereas healthy control and MA people show the significant association regardless of fasting glucose levels. Taken together, these results may suggest that C carriers particularly CC genotype with higher glucose levels is more susceptible to hypertriglyceridemia and DM patients with CC genotype are more easily exposed to the risk of cardiovascular complication.

The present investigation has several limitations. Study subjects were Korean women, thus the results may not be applicable to men or other ethnic samples whose clinical and biochemical characteristics may differ from those in our population. This case-control study is not designed for assessing the time sequential associations because

the exposure and outcomes are collected at one point in time. Postprandial glucose test or hyperglycemic clamping test may be needed to investigate the direct contribution of *APOA5*-1131C allele on the regulation of glucose and insulin status. In addition, other SNPs in *APOA5*gene also need to be considered for the risk of DM. Despite these limitations, our new findings support the contribution of *APOA5*-1131C allele on the increased susceptibility of diabetes mellitus in Korean women, with association of plasma TG concentration.

# References

1. Pennacchio LA, Olivier M, Hubacek JA. Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science. 2001;294:169–73.

2. Jang Y, Kim JY, Kim OY, Lee JE, Cho H, Ordovas JM, Lee JH. The _1131T3C polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriacylglycerolemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men. Am J Clin Nutr. 2004;80:832-40.

3. Kim JY, Kim OY, Koh SJ, Jang Y, Yun SS, Ordovas JM, Lee JH. Comparison of Low-Fat Meal and High-Fat Meal on Postprandial Lipemic Response in Non-Obese Men according to the _1131T>C Polymorphism of the Apolipoprotein A5 (APOA5) Gene (Randomized Cross-Over Design). J Am Coll Nutr. 2006;25:340-7.

4. Bi N, Yan SK, Li GP, Yin ZN, Chen BS. A single nucleotide polymorphism -1131TNC in the apolipoprotein A5 gene is associated with an increased risk of coronary artery disease and alters triglyceride metabolism in Chinese. Mol Genet Metab. 2004;83:280–6.

5. Nabika T, Nasreen S, Kobayashi S, Masuda J. The genetic effect of the apoprotein AV gene on the serum triglyceride level in Japanese. Atherosclerosis. 2002;165:201–4.

6. Endo K, Yanagi H, Araki J, Hirano C, Yamakawa-Kobayashi K, Tomura S. Association found between the promoter region polymorphism in the apolipoprotein A-V gene and the serum triglyceride level in Japanese schoolchildren. Hum Genet. 2002;111:570–2.

7. Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. Hum Mol Genet. 2002;11:3031–8.

8. Evans D, Buchwald A, Beil FU. The single nucleotide polymorphism -1131T>C in the apolipoprotein A5 (APOA5) gene is associated with elevated triglycerides in patients with hyperlipidemia. J Mol Med. 2003;81:645–54.

9. Ribalta J, Figuera L, Fernandez-Ballart J, Vilella E, Castro Cabezas M, Masana L, Joven J. Newly identified apolipoprotein AV gene predisposes to high plasma triglycerides in familial combined hyperlipidemia. Clin Chem. 2002;48:1597–600.

10. Y Jang Y, Paik JK, Hyun YJ, Chae JS, Kim JY, Choi JR, Lee SH, Shin DJ, Ordovas JM, Lee JH. The apolipoprotein A5 -1131T>C promoter polymorphism in Koreans: Association with plasma APOA5 and serum triglyceride concentrations, LDL particle size and coronary artery disease. Clinica Chimica Acta. 2009;402:83–87.

11. Lehto S, Ronnemaa T, Haffner SM, Pyorala K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with type 2 diabetes. Diabetes. 1997;48:1354–9.

12. Teno S, Uto Y, Nagashima H, Endoh Y, Iwamoto Y, Omori Y, Takizawa T. Association of postprandial hypertriglyceridemia and carotid intima-media thickness in patients with type 2 diabetes. Diabetes Care. 2000;23:1401-6.

13. Zhai GH, Wen P, Guo LF, Chen L Yi Chuan. Association of apolipoprotein A5 gene -1131T/C polymorphism with lipid metabolism and insulin resistance in patients with type II diabetes mellitus. 2007;29:541-6.

14. Yan SK, Cheng XQ, Song YH, Xiao XH, Bi N, Chen BS. Apolipoprotein A5 gene polymorphism –1131T>C: association with plasma lipids and type 2 diabetes mellitus

with coronary heart disease in Chinese. Clin Chem Lab Med. 2005;43:607–612.

15. Cardona F, Guardiola M, Queipo-Ortuño MI, Murri M, Ribalta J, Tinahones FJ/ The -1131T>C SNP of the APOA5 gene modulates response to fenofibrate treatment in patients with the metabolic syndrome: A postprandial study. Atherosclerosis. 2009 Epub print

16. Merkel M, Loeffler B, Kluger M, et al. Apolipoprotein AV accelerates plasmahydrolysis of triglyceride-rich lipoproteins by interaction with proteoglycanbound lipoprotein lipase. J Biol Chem 2005;280:21553–60.

17. Schaap FG, Rensen PC, Voshol PJ, et al. ApoAV reduces plasma triglycerides by inhibiting very low density lipoprotein-triglyceride (VLDL-TG) production and stimulating lipoprotein lipase-mediated VLDL-TG hydrolysis. J Biol Chem 2004;279:27941–7.

18. Hirano T, Hayashi T, Adachi M, et al. Marked decrease of apolipoprotein AV in both diabetic and nondiabetic patients with end-stage renal disease. Metabolism 2007;56:462–3.

19. Pruneta-Deloche V, Ponsin G, Groisne L, et al. Postprandial increase of plasma

apoAV concentrations in Type 2 diabetic patients. Atherosclerosis 2005;181:403–5.

20. Hyun YJ, Jang Y, Chae JS, Kim JY, Paik JK, Kim SY, Yang JY, Ordovas JM, Ko YG, Lee JH. Association of apolipoprotein A5 concentration with serum insulin and triglyceride levels and coronary artery disease in Korean men. Atherosclerosis 2009, EuPub.

21. Nowak M, Helleboid-Chapman A, Jakel H, et al. Insulin-mediated downregulation of apolipoprotein A5 gene expression through the phosphatidylinositol 3-kinase pathway: role of upstream stimulatory factor. Mol Cell Biol 2005;25:1537-48.

# 국문요약

## 한국 여성에서 *APOA5*-1131C 대립유전자형의 당뇨병 위험증가에 대한 기여도: 혈중 중성지방 농도와 관련하여

**배경 및 연구 목적:** *APOA5*-1131SNP의 C 대립유전자는 혈중 중성지방 농도 증가와 관련이 있으며 한국여성에서 심혈관계 질환(cardiovascular disease: CVD)의 위험도 증가에 기여하는 것으로 알려져 있다. 고중성지방혈증은 CVD에 대한 전통적인 위험 요인일 뿐만 아니라, 당뇨병(diabetes mellitus: DM)에서 흔히 나타나는 지질대사이상 현상중 하나이며, 제2형 당뇨병(type 2 DM)에서 보이는 죽상경화증의 독립적인 위험인자로 인지되고 있다. 본 연구는 한국 여성에서 *APOA5*-1131T>C와 당뇨병의 상대적 위험도 간의 연관성을 분석하고, 혈중 지질 지표들과 어떠한 관련성이 있는지 조사할 목적으로 시행되었다.

**연구 방법**: 총 3450명의 한국 여성들[정상 대조군 2033명, DM군 304 명,

대사 이상군 (metabolic abnormality; MA) 1113명]을 대상으로 하였다. *APOA5*-1131T>C와 DM 위험도와의 연관성은 교란 변수들을 보정한 로지스틱 회귀 분석을 통해 측정하였다. 각 집단들 사이에서의 일반적인 특징 및 생화학적 지표들의 차이에 대해서는 공분산분석(ANCOVA)을 시행하였다.

**결과:** DM 집단에서 *APOA5*-1131C 대립유전자 빈도가 DM이 아닌 집단에 비해 더 높게 나타났다. C 대립유전자를 지닌 대상자들(C carriers)은 TT 유전자형 집단에 비해 당뇨병의 위험도가 높았고[$OR_0$ 1.39 (95% CI, 1.09-1.77), P=0.008], 연령, BMI, 폐경 여부, 흡연 및 음주 여부에 대해서 보정한 다음에도 통계적 유의성이 유지 되었다[$OR_1$ 1.61 (95% CI, 1.23-2.10), P<0.001]. 특히 CC 유전자형 집단은 TT 집단에 비해서 당뇨병의 위험도가 매우 높았다[$OR_0$ 1.93 (95% CI, 1.27-2.92), P=0.002, $OR_1$ 2.35 (95% CI, 1.46-3.77), P<0.001]. 대사 증후군에 대해 보정한 다음에도 C carriers 에서의 당뇨병 위험도 증가는 여전이 통계적 유의성이 유지되었으며[$OR_2$ 1.98 (95% CI, 1.09-3.59, p=0.024]. CC 유전자형 집단에서도 보정후에 당뇨병 위험도가 높게 유지되었으나, 경계역 수준에서

유의한 정도로 나타났다[$OR_2$ 2.51 (95% CI, 0.90-6.96, p=0.078].

지질 지표들과 관련하여, C carriers (특히 CC 유전자형)는 다른 집단들에 비해 중성지방이 높고, HDL 콜레스테롤은 낮았다. 정상 대조군과 MA 집단에서 아포지단백 A5 농도는 유전자형과 유의한 연관성을 보였으나, DM 집단에서는 연관성이 없었다. 공복 혈당농도와 중성지방 농도는 전체 대상자 및 이들을 유전형에 따라 나눈 집단 모두에서 양의 상관관계를 보인 반면, DM 집단에서는 TT 집단에서는 유의한 상관성이 없었고(r=0.139, p=0.102), C carriers 에서만 양의 상관관계를 보였으며(r=0.165, p=0.035), 이러한 상관관계는 CC 유전자형 집단에서 더 크게 나타났다(r=0.395, p=0.021). 또한 DM 환자집단에서 공복 혈당농도가 특히 높은 경우 CC 유전자형에서의 TG 농도가 TT 유전자형에 비해 현저히 높게 나타났다.

**결론:** 본 연구에서 나타난 새로운 결과들을 통해, *APOA5*-1131C 대립유전자가 한국 여성의 혈중 중성지방과 연관하여 당뇨병 이환의 위험도를 높이는 데에 기여할 가능성이 있다는 것을 유추해 볼 수 있었다.

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핵심 단어: APOA5-1131C 대립유전자, 당뇨병, 중성지방 한국 여성