*BRIEF ARTICLE*

# ApoE isoforms, treatment of diabetes and the risk of coronary heart disease

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## Abstract

**AIM:** To analyze the risk of coronary heart disease in patients with type 2 diabetes mellitus (T2DM) receiving standard medical treatment.

**METHODS:** We performed a retrospective chart analysis of 269 middle-aged patients (age 45-64 years, mean age, 53.9 ± 5.5 years) with T2DM and without atherosclerotic cardiovascular events who underwent typing to determine their apolipoprotein E (apoE) isoforms. The apoE isoforms were determined using isoelectric focusing, followed by immunoblotting. We retrospectively evaluated the charts of the 269 patients, recorded between their first visit to the hospital (the study's start point, between 1987 and 1992) and the occurrence of an atherosclerotic cardiovascular event (the study's endpoint) or January 2004, whichever came first. The age-adjusted mean values and the prevalences of co-variates were calculated to compare the laboratory data among the apoE phenotypes. To investigate the association of risk factors with the incidence of coronary heart disease during the follow-up period, monovariate and multivariate Cox regression models were used.

**RESULTS:** At enrollment, the mean serum low density lipoprotein (LDL) cholesterol levels were lowest (2.92 ± 0.89 mmol/L) among the subjects with apoE2 (apoE2/2 or apoE2/3) and highest (3.52 ± 0.77 mmol/L) among the subjects with apoE4 (apoE3/4 or apoE4/4). No significant differences in mean age or the percentage of smokers were observed among the three groups. Furthermore, no significant differences were observed in the systolic and diastolic blood pressures, body mass index, HbA1c level or serum triglyceride levels among the three groups. There were 47 cases of coronary heart disease over 3285 person-years of follow-up. An age-adjusted multivariate Cox proportional model identified diabetic retinopathy (hazard ratio, 2.38, 95% CI: 1.28-4.43, $P$ = 0.006), a high systolic blood pressure (hazard ratio, 1.04, 95% CI: 1.02-1.06, $P$ < 0.001) and high HbA1c values (hazard ratio, 1.19, 95% CI: 1.02-1.38, $P$ = 0.0029), but not the LDL cholesterol value at enrollment (hazard ratio, 1.01, 95% CI: 0.97-1.05, $P$ = 0.77) nor the specific apoE isoform, as significant predictors of coronary heart disease.

**CONCLUSION:** Under standard medical treatment of diabetes, including the control of LDL cholesterol levels, the apoE4 isoform was not associated with coronary heart disease among T2DM patients.

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## INTRODUCTION

Among patients with diabetes mellitus, a high prevalence of coronary heart disease is observed at a relatively young age[1]. Thus, risk factors for atherosclerosis must be defined and avoided in patients with diabetes mellitus. The low density lipoprotein (LDL) cholesterol level, which is a major risk factor for atherosclerosis, can be lowered by lifestyle modifications and by the use of statins, reducing the risk of cardiovascular events among diabetic patients[2]. Concomitantly with LDL cholesterol-lowering therapy, the effect of other cardiovascular risk factors was enhanced. Abnormality of lipids such as high triglyceride levels[3] and low HDL cholesterol levels[4] emerged as residual cardiovascular risks for diabetic patients.

The apolipoprotein E gene (*apoE*) is located on chromosome 19q13.2 and commonly exhibits polymorphisms, designated as *epsilon2 (e2)*, *epsilon3 (e3)* and *epsilon4 (e4)*; the apoE protein is mainly produced by the liver and participates in the transportation of cholesterol and fatty acids throughout the body. The structural and functional differences among the three apoE isoforms[5-7] reportedly affect serum lipid levels[8-11] and influence the risks of atherosclerosis[12], coronary heart disease[13] and late-onset Alzheimer disease[14], in addition to being associated with longevity[15].

Does the risk of coronary heart disease increase in the presence of specific apoE isoform even after the application of current standard medical treatment strategies, including the control of LDL cholesterol levels in patients with type 2 diabetes mellitus (T2DM)? To answer this question, we examined the effect of the apoE isoform as well as other possible cardiovascular risk factors on the frequencies of atherosclerotic coronary heart disease in patients with T2DM.

## MATERIALS AND METHODS

The present study was a retrospective cohort follow-up investigation. A total of 560 subjects with T2DM visited the Marunouchi Hospital, which is attached to the Institute for Adult Disease, Asahi Life Foundation (located in the center of Tokyo), between 1987 and 1992 and under-

went an evaluation of the apoE protein phenotype[16]. Of the 560 subjects, we selected 352 middle-aged subjects (age 45-64 years) as this age group was often analyzed in previous epidemiological studies dealing with the risk of coronary heart disease, such as the Honolulu Heart Program[17] and Cohort Component of the Atherosclerosis Risk in Communities study[18]. We excluded patients aged over 65 years in the present analysis because about one-third of the patients in this age group had already experienced cardiovascular events at their first visit to the hospital. We also excluded patients aged under 44 years in the present analysis because of the small number of patients included in this age group. Of the 352 subjects, an additional 44 subjects were excluded from the present analyses because of the poor quality of their medical records, leaving 308 subjects. We finally excluded another 39 subjects with coronary heart disease or stroke at the time of their first visit to the hospital, leaving 269 subjects for the present analysis. Since their first visit to the Marunouchi Hospital, the patients were encouraged to reduce and maintain their body mass index (BMI) at a value under 22 kg/m$^2$, to walk 10 000 steps a day and to consume a low-fat (less than 30% of the daily caloric intake), low-energy (25-27 kcal/ideal body weight/day) diet. An intake ratio of saturated fatty acids: monosaturated fatty acids: poly-unsaturated fatty acids of 3:4:3 was recommended. If lifestyle modifications were not sufficient to lower the LDL cholesterol level, statins were prescribed. The mean ± SD deviation of the total cholesterol level, LDL cholesterol level, HDL level and triglyceride level of the participants in 1995 were 5.06 ± 0.48 mmol/L, 3.08 ± 0.44 mmol/L, 1.27 ± 0.20 mmol/L and 1.55 ± 0.66 mmol/L, respectively. We did not attempt to estimate the patients' adherence to the prescribed pharmaceutical treatment.

Instead of genotyping, the apoE phenotype was determined using isoelectric focusing of plasma proteins followed by immunoblotting[16]. The three isoforms differ in their isoelectrical points by a single charged amino acid; apoE2 is the most acidic isoform and apoE4 is the most basic. We classified the 269 patients into three groups according to the presence/absence of apoE2 or apoE4 (18 patients with apoE2, 207 patients with apoE3 only and 44 patients with apoE4); the three groups were represented genotypically by the e2 allele (apoE2/2 or apoE2/3), e3 homozygosity and the e4 allele (apoE3/4 or apoE4/4), respectively. The allele frequencies for the 269 patients were 3.5% for e2, 84.7% for e3 and 11.8% for e4. At the time of study enrollment, the patients were questioned as to their history of smoking, coronary heart disease and cerebrovascular accidents.

We retrospectively evaluated the charts of the 269 patients, recorded between their first visit to the hospital (the study's start point) and the occurrence of an atherosclerotic cardiovascular event (the study's endpoint) or January 2004, whichever came first. We obtained the patients' medical records and examined their BMI, systolic and diastolic blood pressure, serum total cholesterol levels (determined enzymatically[19]), triglyceride levels

**Table 1 Mean values or prevalence of factors at baseline for apoE2, apoE3 homozygosity and apoE4 among the 269 patients *n* (%)**

|  | ApoE2 | ApoE3 homozygosity | ApoE4 |
|---|---|---|---|
| No. of patients (men/women) | 18 (14/4) | 207 (156/51) | 44 (29/15) |
| Age at enrollment | 53.4 ± 5.8 | 53.8 ± 5.7 | 54.2 ± 4.8 |
| Observation period (yr) | 11.9 ± 5.2 | 12.6 ± 5.3 | 12.2 ± 5.4 |
| Smoking status |  |  |  |
|   Never smokers | 6 | 66 | 17 |
|   Past smokers | 5 | 63 | 11 |
|   Smokers | 7 | 78 | 16 |
| Body mass index (kg/m$^2$) | 22.4 ± 3.1 | 22.3 ± 2.6 | 22.9 ± 3.3 |
| HbA1c (NGSP equivalent value) (%) | 8.7 ± 1.5 | 8.7 ± 1.4 | 8.6 ± 1.1 |
| Medication for hyperglycemia before enrollment |  |  |  |
|   None | 7 (38.9) | 80 (38.6) | 22 (50.0) |
|   Oral hypoglycemic agents | 9 (50.0) | 104 (50.2) | 19 (43.2) |
|   Insulin | 2 (11.1) | 23 (11.1) | 3 (6.8) |
| Total cholesterol (mmol/L) | 4.83 ± 1.00 | 5.51 ± 1.21 | 5.45 ± 0.93 |
| HDL cholesterol (mmol/L) | 1.14 ± 0.22 | 1.17 ± 0.32 | 1.12 ± 0.36 |
| LDL cholesterol (mmol/L) | 2.92 ± 0.89 | 3.55 ± 1.10 | 3.52 ± 0.76 |
| Triglyceride (mmol/L) | 1.69 ± 0.97 | 1.79 ± 1.32 | 1.94 ± 1.82 |
| Medications for hyperlipidemia | 0 (0.0) | 1 (0.01) | 2 (5.0) |
| Systolic blood pressure (kPa) | 17.3 ± 1.9 | 17.3 ± 2.5 | 17.8 ± 2.5 |
| Diastolic blood pressure (kPa) | 10.6 ± 1.3 | 10.5 ± 1.5 | 10.5 ± 1.5 |
| Medications for hypertension | 3 (16.7) | 21 (10.1) | 8 (18.2) |
| Proteinuria | 4 (22.2) | 50 (24.2) | 10 (22.7) |
| Diabetic retinopathy | 7 (38.9) | 61 (29.6) | 11 (25.0) |

ApoE: Apolipoprotein E gene; HDL: High density lipoprotein; LDL: Low density lipoprotein; NGSP: National Glycohemoglobin Standardization Program.

(determined enzymatically[20]), HDL-cholesterol level (determined using the deposition method[21]) and glycosylated hemoglobin (HbA1c) level (determined using high-performance liquid chromatography[22]). Prior to 1996, we determined the HbA1c level using HLC-723GHb Ⅱ (Tosoh, Tokyo). Thus, the HbA1c values measured using HLC-723GHb Ⅱ were calibrated to the values obtained by the previous Japanese standard substance and measurement methods [HbA1c (JDS)]. The value for HbA1c (%) was estimated as an National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated using the formula HbA1c (%) = HbA1c (JDS) (%) + 0.4%, considering the relational expression of HbA1c (JDS)(%) and HbA1c (NGSP)[23]. The LDL-cholesterol level was calculated according to the Friedewald equation[24]. The diagnosis of coronary heart disease (coronary insufficiency and myocardial infarction) was made according to criteria defined by the Framingham Heart Study[25]. The diagnosis of myocardial infarction was determined by specified electrocardiographic changes accompanied by an elevation of serum enzymes. Coronary insufficiency was defined as prolonged ischemic chest pain accompanied by transient ischemic abnormalities on electrocardiography. Coronary angiography was performed in all patients and a diagnosis of coronary heart disease was confirmed by narrowed or blocked coronary arteries. This research program conformed to the ethical recommendations for epidemiological studies, as declared by the Ministry of Health, Labour and Welfare in Japan, and was approved by the National Center for Global Health and Medicine Research Ethics Committee.

The age-adjusted mean values and the prevalences of

covariates were calculated to compare the laboratory data among the apoE phenotypes. To investigate the association of risk factors with the incidence of coronary heart disease during the follow-up period, monovariate and multivariate Cox regression models were used to estimate the influence of candidate risk factors on coronary heart disease. The age at which patients entered the study was the study's start point. The end point was defined as the occurrence of a cardiovascular event, death of the participants, dropout or the study's endpoint. *P* values less than 0.05 were considered statistically significant. The data analysis was performed using R[26].

## RESULTS

The baseline characteristics of the 269 patients at the time of their first visit to our hospital are summarized in Table 1. No significant differences in mean age or the percentage of smokers were observed among the three groups. Furthermore, no significant differences were observed in the systolic and diastolic blood pressures, BMI, HbA1c level or serum triglyceride levels among the three groups (Table 1). Patients with apoE4 exhibited higher serum LDL cholesterol values than those with apoE2 but a significant difference was not observed (*P* = 0.053, Table 1). Patients with apoE2 tended to exhibit diabetic retinopathy but a significant difference was not observed.

The 269 patients were followed for an average of 12.2 years and the total person-years in the study was 3285. Among the 269 patients who had no history of cardiovascular events at the start point, 47 patients (14.3 per 1000 person-years) developed coronary heart disease

**Table 2 Factors associated with coronary heart disease in the present cohort[1]**

| Factors | Hazard ratio (95% CI) | *P* value |
|---|---|---|
| Age | 1.03 (0.98-1.09) | 0.22 |
| Male | 1.44 (0.77-2.69) | 0.26 |
| Present smokers | 0.62 (0.35-1.1) | 0.071 |
| Height (m) | 0.006 (0.00-1.56) | 0.11 |
| BMI (compared with $22 \leqslant$ BMI < 25) (kg/m$^2$) | | |
| BMI < 18.5 | 0.80 (0.18-3.56) | 0.78 |
| $18.5 \leqslant$ BMI < 22 | 1.94 (0.88-4.28) | 0.10 |
| BMI $\geqslant$ 25 | 1.58 (0.80-3.14) | 0.19 |
| HbA1c | 1.18 (1.04-1.34) | 0.013 |
| Systolic blood pressure | 1.02 (1.01-1.04) | 0.002 |
| Diastolic blood pressure | 1.01 (0.99-1.04) | 0.52 |
| Total cholesterol | 1.00 (0.99-1.01) | 0.38 |
| HDL-cholesterol | 0.97 (0.94-1.00) | 0.027 |
| LDL-cholesterol | 1.00 (0.99-1.00) | 0.26 |
| Triglyceride | 1.00 (0.99-1.00) | 0.32 |
| Diabetic retinopathy | 2.77 (1.57-4.93) | 0.0005 |
| Proteinuria | 1.22 (0.63-2.36) | 0.55 |
| ApoE phenotype (compared with apoE3 homozygosity) | | |
| ApoE2 | 0.76 (0.27-2.13) | 0.60 |
| ApoE4 | 0.72 (0.21-2.43) | 0.60 |

[1]Univariate analysis. BMI: Body mass index; ApoE: Apolipoprotein E gene; HDL: High density lipoprotein; LDL: Low density lipoprotein.

**Table 3 Factors associated with coronary heart disease in the present cohort[1]**

| Factors | Hazard ratio (95% CI) | *P* value |
|---|---|---|
| Male | 0.76 (0.33-1.79) | 0.54 |
| BMI at enrollment (compared with 22 < BMI < 25) (kg/m$^2$) | | |
| BMI $\leqslant$ 18.5 | 0.87 (0.18-4.36) | 0.87 |
| 18.5 < BMI $\leqslant$ 22 | 1.86 (0.80-4.36) | 0.15 |
| 25 < BMI $\leqslant$ 30 | 1.60 (0.75-3.41) | 0.23 |
| HbA1c | 1.19 (1.02-1.38) | 0.029 |
| Systolic blood pressure | 1.04 (1.02-1.06) | 0.0005 |
| Diastolic blood pressure | 0.96 (0.92-1.01) | 0.06 |
| Total cholesterol | 0.99 (0.95-1.04) | 0.79 |
| HDL-cholesterol | 0.97 (0.92-1.02) | 0.21 |
| LDL-cholesterol | 1.01 (0.97-1.05) | 0.77 |
| Triglyceride | 1.00 (0.99-1.01) | 0.86 |
| Diabetic retinopathy | 2.38 (1.28-4.43) | 0.006 |
| Proteinuria | 0.71 (0.34-1.48) | 0.36 |
| ApoE phenotype (compared with apoE3 homozygosity) | | |
| ApoE2 | 0.93 (0.31-2.80) | 0.89 |
| ApoE4 | 0.69 (0.18-1.79) | 0.58 |

[1]Multivariate Cox proportional hazard analysis adjusted with age and smoking status at enrollment. ApoE: Apolipoprotein E gene; HDL: High density lipoprotein; LDL: Low density lipoprotein.

during the observation period. Four patients with apoE2, 36 with apoE3 homozygosity and 7 with apoE4 experienced atherosclerotic coronary heart disease. Twenty patients (3 with apoE2, 15 with apoE3 homozygosity and 2 with apoE4) died during the study period without experiencing any coronary heart disease and 140 continued to visit the hospital without having experienced coronary heart disease. Sixty-two patients (3 with apoE2, 51 with apoE3 homozygosity and 8 with apoE4) had stopped visiting the hospital before January 2004 without having experienced coronary heart disease.

Using a Cox proportional hazards regression analysis, we examined the predictors of coronary heart disease for the patients who developed coronary heart disease during the follow-up period compared with those who did not. A univariate analysis identified a high systolic blood pressure, the existence of diabetic retinopathy, a high HbA1c value and a low HDL cholesterol value as influencing the occurrence of coronary heart disease (Table 2). None of the specific apoE isoforms reached a significant correlation with the occurrence of coronary heart disease. An age-adjusted multivariate Cox proportional hazard analysis also identified high systolic blood pressure, the existence of diabetic retinopathy at enrollment and high HbA1c value, but none of the specific apoE isoforms, as independent risk factors for the occurrence of coronary heart disease in this cohort (Table 3).

## DISCUSSION

The long-term follow-up period used in this study enabled a statistical power sufficient to confirm previously identified risk factors (diabetic retinopathy[27,28] and high systolic blood pressure[29]) as risk factors for atherosclerosis among patients with T2DM.

Pleiotropic effects of the apoE4 isoform with regard to the risk of atherosclerosis have been reported. People carrying the apoE4 isoform exhibit a lower blood pressure during youth[30], a lower frequency of hyperhomocysteinemia[31], lower values of remnant lipoproteins[32], lower serum levels of C-reactive protein[33,34] and, most importantly, higher serum LDL cholesterol values[8].

High serum LDL cholesterol values are well-known risk factors for atherosclerotic cardiovascular disease[35] and the polymorphism of ApoE is one genetic factor that affects serum lipid levels[11]. Also in the present study, the serum LDL cholesterol values at the time of first admission were higher (but not statistically significant) among patients with the apoE4 than among those with the apoE2 isoforms. These results were similar to those previously reported for patients with type 2 diabetes[16,36,37], as well as in the general population[9]. In the present study, the level of LDL cholesterol in patients with apoE4 was well controlled after first admission, mostly with lifestyle modifications and with additional medication for hyperlipidemia. The present standard medical treatment strategies for diabetes might be sufficient to lower the LDL cholesterol value so that the LDL cholesterol value[4] as well as the e4 allele might not behave as risk factors for atherosclerotic coronary heart disease. Ward *et al*[10] reported that after adjustments for the baseline levels of risk factors, including LDL cholesterol, the e4 allele failed to reach statistical significance as a risk factor for atherosclerosis: our findings proved that the result of a prospective population study by Ward *et al*[10]

was also applied to the cohort of type 2 diabetic men. To date, lifestyle interventions are known to interfere with various genetic risk alleles. The *e4* allele as a high-risk allele acting against longevity[15] might be overcome by consuming low-fat meals[38]. The percent intake of saturated fat modified the association between the apoE polymorphism and the CHD risk[39]. Lifestyle interventions also interfered with the progression to diabetes in patients with a diabetes-prone risk allele (TT genotype at rs7903146 of TCF7L2)[40]. Our findings appear to be similar to those of the above-mentioned previous reports.

The results regarding the contribution of the *e2* allele to atherosclerosis of diabetic patients are controversial[41,42]. Diabetic patients with apoE2 are prone to develop elevated levels of serum triglycerides if they have poor metabolic control[43] and high levels of serum triglycerides may act as a risk factor for coronary heart disease in diabetic patients[4]. In the present cohort, triglyceride levels did not act as a risk factor for coronary heart disease so our present study might demonstrate equivalence between patients with apoE2 and those with apoE3 homozygosity.

In conclusion, under standard medical treatment of diabetes, including the control of LDL cholesterol levels, the apoE4 phenotype was not associated with coronary heart disease among type 2 diabetic patients.

## ACKNOWLEDGMENTS

## COMMENTS

### Background
There are three major types of amino acid variations with apolipoprotein E (apoE), namely apoE2, apoE3 and apoE4, which are genetically determined. The structural differences among the three apoE isoforms reportedly affect serum lipid levels and influence the risks of atherosclerosis and coronary heart disease.

### Research frontiers
Elevated serum low density lipoprotein (LDL) cholesterol is often observed in people who have the apoE4 protein (apoE4 carriers). ApoE4 carriers are reported to have or not to have increased risk for atherosclerotic disease. A recent meta-analysis suggests that the increased risk of atherosclerosis in ApoE4 carriers was not observed after adjustments for the baseline levels of risk factors, including LDL cholesterol.

### Innovations and breakthroughs
It examined whether intervention to lower the LDL cholesterol level might remove the yoke from the risk of coronary heart disease in diabetic patients carrying apoE4.

### Applications
The study results suggest that under standard medical treatment of diabetes, including the control of LDL cholesterol levels, the apoE4 carrier does not suggest an elevated risk for coronary heart disease.

### Terminology
Apolipoprotein E is a protein mainly produced by the liver and participates in the transportation of cholesterol and fatty acids. ApoE4 carriers are reported to be at increased risk for Alzheimer disease.

### Peer review
The study offers an interesting insight into the correlation between various risk factors and the development of coronary heart disease. It adds value to the body of research in the field.

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