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Association of *APOE* polymorphisms with diabetes and cardiometabolic risk factors and the role of *APOE* genotypes in response to anti-diabetic therapy: results from the AIDHS/SDS on a South Asian population

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Abstract

Background and Objectives—Apolipoprotein E (*APOE*) gene polymorphisms have been examined extensively in multiple global populations particularly due to their crucial role in lipid metabolism and cardiovascular disease. However, the overall contribution of *APOE* polymorphisms in type 2 diabetes (T2D) and coronary artery disease (CAD) in South Asians is still under-investigated. The objectives of this investigation were: 1) to evaluate the distribution of *APOE* polymorphisms in a large diabetic case–control sample from South Asia, 2) to examine the impact of *APOE* polymorphisms on quantitative risk factors of T2D and CAD, and 3) to explore the contribution of *APOE* genotypes in the response to anti-diabetic therapy.

Subjects and Methods—A total of 3564 individuals (1956 T2D cases and 1608 controls) used in this study were part of the Asian Indian Diabetic Heart Study/Sikh Diabetes Study (AIDHS/SDS). We assessed the association of *APOE* polymorphisms with T2D, CAD and cardiometabolic traits using logistic and linear regression analysis.

Results and Conclusions—No significant differences in the distribution of *APOE* genotypes were observed between T2D and CAD cases and controls. The *APOE4* genotype carriers had moderately higher diastolic blood pressure (BP) ($p = 0.022$), and lower HDL-cholesterol ($p = 0.026$) compared to *E4* non-carriers. Overall, the *APOE* genotype was not a significant predictor of cardiometabolic disease in this population. Further stratification of data from diabetic patients by *APOE* genotypes and anti-hyperglycemic agents revealed a significant (~23%) decrease in 2-

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hour glucose (p = 0.004) and ~7% decrease in systolic BP (p < 0.001) among *APOE4* carriers compared to non-carriers on metformin and sulphonylurea (SU) combination therapy, and no such differences were seen in patients on other agents. Our preliminary findings point to the need for evaluating population-specific genetic variation and its interactions with therapeutic effects.

Keywords

Apolipoprotein E polymorphism; Type 2 diabetes; Coronary artery disease; Anti-diabetic drugs; Treatment response

1. Introduction

The global epidemic of type 2 diabetes (T2D) has dramatically increased worldwide and is influenced by complex interactions between genes and environment (Ridderstrale & Groop, 2009; Sanghera & Blackett, 2012). According to the latest statistics released by the International Diabetes Federation, the number of people living with diabetes is expected to rise from 382 million in 2013 to 592 million by 2035 (Guariguata et al., 2014). Despite advances in treatment, T2D continues to contribute to the development of cardiovascular disease, stroke, peripheral vascular disease, renal failure, blindness, and amputation and greatly impacts quality of life. The six most commonly used antidiabetic agents are insulin, sulfonylureas (SUs), meglitinides, metformin, thiazolidinediones (TZDs) and α-glucosidase inhibitors. Other less commonly used anti-diabetic agents include amylin analogues, incretin hormone mimetics, and dipeptidyl peptidase 4 (DPP4) inhibitors. Metformin is the most-widely accepted and established first line therapy. In addition to providing effective control of hyperglycemia, it effects improvements in endothelial dysfunction, oxidative stress, insulin resistance, lipid profiles, and fat redistribution (Rojas & Gomes, 2013). Metformin ameliorates hyperglycemia by decreasing hepatic glucose output, and improving both gastrointestinal glucose absorption and insulin sensitivity. Metformin and SU is a commonly used combination that can attain a reduction in HbA1c of 0.8 to 1.5 percentage points (Hanefeld et al., 2004). However, despite overall improvements in glycemic control, the efficacy of the most widely used anti-hyperglycemic agents is variable, and 35–40% of T2D patients do not achieve acceptable control of fasting glucose levels or HbA1c (Hoerger, Segel, Gregg, & Saaddine, 2008). Inter-individual variability in response to drug efficacy and toxicity is heritable (Alving, Carson, Flanagan, & Ickes, 1956). However, the mechanisms by which genetic factors affect response of anti-diabetic agents are poorly understood.

Apolipoprotein E (apoE protein encoded by the *APOE* gene) is a serum glycoprotein containing 299 amino acid residues. Variation in apoE is known to have a significant impact on various inflammatory and metabolic diseases, in addition to its well-known regulatory role in lipoprotein metabolism and lipid transport within tissues by enhancing lipoprotein uptake of apoE-bearing receptors (E-specific remnant receptor and low–density lipoprotein [LDL] receptor) (Mahley, 1988; Mahley & Innerarity, 1983). Three common APOE alleles (*E*2*, *E*3*, *E*4*) code the three major apoE isoforms (apoE2, apoE3, apoE4) in plasma. These isoforms differ in amino acid residues at two sites: 112 and 158. The predominant isoform, apoE3, contains cysteine at 112 and arginine at 158; apoE2 has cysteine at both

positions and is associated with higher ApoE plasma concentrations, and apoE4 has arginine at both sites and is associated with lower concentrations compared to apoE3 (Siest et al., 1995). ApoE4 isoform (*E*4* allele) is a well-known marker associated with increased risk of coronary artery disease (CAD) (Bennet et al., 2007; Chaudhary et al., 2012; Song, Stampfer, & Liu, 2004; Stengard, Weiss, & Sing, 1998; Zhang et al., 2014) and late-onset of Alzheimer's disease (Farrer et al., 1997; Kamboh, Sanghera, Ferrell, & DeKosky, 1995; Strittmatter et al., 1993). A large body of data, including several initial findings from our own group, suggests that *E*4* carriers have a propensity for higher levels of total plasma cholesterol along with increased risk of heart disease and T2D when compared with people having the commonest *E*3*, while *E*2* carriers are protective (Bennet et al., 2007; Burman et al., 2009; Sanghera et al., 1996). However, no study has comprehensively examined the contribution of *APOE* gene polymorphisms in T2D, lipid metabolism, and cardiovascular disease risk in South Asians. The objectives of this investigation were: 1) to evaluate the distribution of *APOE* polymorphisms in a large diabetic case–control cohort, 2) to examine the impact of *APOE* polymorphisms on quantitative risk factors of T2D and CAD, and 3) to explore the role of *APOE* genotypes in the response to anti-diabetic therapy.

2. Subjects and methods

2.1. Study subjects

This study involves 3564 participants from the Asian Indian Diabetic Heart Study (AIDHS)/ Sikh Diabetes Study (SDS) (Sanghera et al., 2008; Saxena et al., 2013). Diabetic subjects (n = 1956; male/female = 1078/878) were identified based upon their medical records for symptoms and use of diabetic medications; and were defined as diabetics based on fasting glucose levels following the American Diabetes Association guidelines as described previously (Guidlines, 2004; Sanghera et al., 2010). Non-diabetic control participants (n = 1608; male/female 888/720) were selected based on a fasting glycemia < 100.8 mg/dl (5.6 mmol/l) or 2-hour glucose < 141.0 mg/dl (7.8 mmol/l). Subjects with impaired fasting glucose (IFG) defined as a fasting blood glucose level ≥100 mg/dl (5.6 mmol/l) but ≤126 mg/dl (7.0 mmol/l), or impaired glucose tolerance (IGT) defined as having 2-hour glucose (based on 2-hour oral glucose tolerance test) > 140 mg/dl (7.8 mmol/l) but <200 mg/dl (11.1 mmol/l) were excluded from the this study. Individuals with type 1 diabetes, or with rare forms of T2D such as maturity onset diabetes of the young, or secondary diabetes (e.g., due to hemochromatosis or pancreatitis) were also excluded. Body mass index (BMI) was calculated as (weight [kg]/height [meter^2]). A tape measure was used to measure the waist and hip circumferences at the abdomen and at the hip, respectively. The World Health Organization's (WHO) new guidelines for the BMI thresholds for Asians were followed (Panel, 2004). Blood pressure was measured twice after a five-minute seated rest period with the participant's feet flat on the floor. Serum lipids [low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG)] were measured using standard enzymatic methods (Roche, Basel, Switzerland) as described previously (Sanghera et al., 2010). CAD was considered if there was use of nitrate medication (nitroglycerine), electrocardiographic evidence of angina pain, coronary angiographic evidence of severe (greater than 50%) stenosis, or echocardiographic evidence of myocardial infarction. Diagnosis was based on date of coronary artery bypass graft

(CABG) or angioplasty, and medication usage obtained from patient records as described previously (Saxena et al., 2014).

All blood samples were obtained at the baseline visit. Among diabetics, 61% were taking hypoglycemic agents. Of these, 12% were treated with insulin, 30% were on metformin and metformin-SU combination therapy, 15% were treated with ayurvedic, or 'desi' medicines, 4% were taking TZDs, DPP4 inhibitors, or others medications. The remaining 39% were maintaining glycemic control with diet and exercise or were new cases without any medication history. The vast majority of T2D cases were chronic cases; the average age-of-onset of T2D was 47.2 ± 11.5 years, and average duration of T2D was 7.5 years. Being chronically diabetic, the vast majority of patients were consistently on a specific medication (mono or combination therapy). The cases with duration of anti-hyperglycemic treatment >8 weeks were analyzed for the association of *APOE* genotypes with treatment response. Those not on any medication but controlling hyperglycemia by diet and exercise and the newly detected cases were analyzed separately. All participants in this study were from India and provided written informed consent following procedures approved by institutional review boards (IRBs). All AIDHS/SDS protocols and consent documents were reviewed and approved by the University of Oklahoma Health Sciences Center (OUHSC)'s IRB as well as the Human Subject Protection (Ethics) committees at the participating hospitals and institutes in India.

2.2. APOE genotyping

Genomic DNA was extracted from buffy coats or whole blood using QIAamp kits (Qiagen, Chatworth, CA). DNA amplification for *APOE* used a forward primer (E1), 5'-GCGGACATGGAGGACGTG-3' and a reverse primer (E2), 5'-GGCCTGGTACACTGCCAG-3' immediately flanking codon 112 and codon 158, the substitution sites determining the apoE isoforms as described by Kamboh et al., 1995. PCR amplification was carried out in a 50 μl reaction volume containing 200 μM of each dNTP, 0.2 μM of each primer and 1 U of Flexi *Taq* DNA polymerase. The cycling conditions were 95 °C for 10 minutes followed by 36 cycles of 95 °C for 30 seconds, 65 °C for 30 seconds and 72 °C for 45 seconds with a final extension of 72 °C for 5 minutes. The PCR amplicon (10 μl) was digested with *Hha-I* restriction enzyme at 65 °C overnight according to manufacturer's instructions. Restriction fragments were resolved on 8% polyacrylamide gel and stained with ethidium bromide. *APOE* genotypes were determined as described by Kamboh et al. (1995) and Sanghera et al. (1996). The entire genotyping was carried out on the samples blinded for phenotypes or treatment groups.

2.3. Statistical analysis

APOE allele frequencies were calculated by allele counting. Departure from Hardy-Weinberg expected frequencies in controls was tested using Pearson's chi-square test. Variables with skewed distributions were normalized by log-transformation before statistical comparisons (e.g. fasting glucose, 2-hour glucose, TG, LDL-C, and HDL-C), and p-values reported are from analyses of the transformed data. Transformed variables were retransformed into the original measurement scale and reported as geometric means. Multiple-linear regression analyses were used to examine the impact of *APOE* genotypes on

quantitative traits adjusted for covariates. Covariates considered included T2D, antihypertensive medications, lipid-lowering medications (statins), alcohol, age, BMI, and gender. The Punjabi Sikh population studied here is a non-smoking population. Significant covariates for each dependent trait were identified by Spearman's correlation and step-wise multiple-linear regression, and an overall 5% level of significance was used to select appropriate subsets of significant covariates. For analysis, *APOE* genotypes were grouped as *APOE2* carriers (*E2/2, E2/3*) and *APOE4* carriers (*E3/4, E4/4*) with *E3/3* as the reference group; E2/4 individuals were included among *APOE4* carriers for comparisons of *E4* carriers to non-*E4* carriers. Interaction terms were included to determine whether the effects of anti-hyperglycemic medications were modified by *APOE* genotypes, particularly by *APOE4* carrier and *APOE4* non-carrier genotype on plasma glucose levels and other cardiometabolic traits. All statistical analyses were performed using SPSS for windows statistical package (version 18.0) (SPSS Inc., Chicago, USA).

3. Results

3.1. Distribution and association of APOE polymorphism with T2D and CAD

Clinical and physical characteristics of the study subjects stratified by T2D status are presented in Table 1. *APOE* genotype and allele frequencies in the study population are presented in Table 2 stratified by T2D and CAD status. Genotype data of a total of 3564 study subjects showed all six possible *APOE* genotypes (*E2/2, E3/2, E3/3, E3/4, E2/4,* and *E4/4*) based on the three common alleles (*E*2, E*3, E*4*). As expected, the *E3/3* genotype was the most common (81.2% cases and 80.7% controls); *E2/2* was the least common with just three individuals. *APOE* genotype frequencies in control subjects did not deviate significantly from Hardy-Weinberg expectations ($p = 0.137$). As shown in Table 2a, the frequencies of *APOE* genotypes and *E*2, E*3* and *E*4* alleles did not vary between T2D patients and controls. Similarly, no significant difference in the distribution of *APOE* genotypes was observed among CAD group (Table 2b). Our study has 80% power at $\alpha = 0.05$ to detect maximum protective OR of 0.64 for the *E2* and *E4* genotypes compared to the reference *E3* genotype in T2D. The general estimates of power in our sample to detect the ORs between 1.2 and 1.3 would range between 72% and 80% at $\alpha = 0.05$ to detect the *E4*-associated CAD risk when the frequency of *E4* allele is 0.08.

3.2. Impact of APOE polymorphism as quantitative risk factors for cardiovascular and metabolic traits

We examined the impact of *APOE* polymorphism on quantitative traits associated with obesity, metabolic and cardiovascular disease on the entire sample as well as in the data stratified by T2D. The *APOE4* genotype was not associated with obesity (BMI), mean plasma fasting glucose, or 2-hour glucose levels in healthy controls. The *APOE4* genotype carriers had higher diastolic BP compared to *APOE2* ($p = 0.030$) and *APOE3* ($p = 0.020$) genotype carriers in healthy controls. A marginally significant step-wise increase in the mean levels of diastolic BP ($p = 0.022$) and step-wise decrease in mean levels of HDL-C ($p = 0.026$) was observed in the order of *APOE2* to *APOE4* carriers after adjusting for covariates of age, gender, and BMI (Fig. 1). We extended our analysis to determine the relationship between *APOE* polymorphism and cardiovascular parameters in T2D patients

not taking any medication. The *APOE4* genotype carriers had significantly higher 2-hour glucose ($p = 0.012$) and slightly elevated fasting glucose levels ($p = 0.088$) compared to *APOE3* and *APOE2* genotype carriers (Fig. 1). However, no such difference was observed in healthy non-T2D controls.

3.3. APOE polymorphism and response to T2D medications

We next explored the effect of *APOE* polymorphism in response to anti-diabetic treatments. We carried out this analysis in data stratified by *APOE4* carriers vs. *APOE4* non-carriers by comparing the mean fasting blood glucose and 2-hour glucose among patients who were on various anti-hyperglycemic treatments.

As shown in Fig. 2, patients on metformin and metformin-SU combination therapy showed a significant decrease in the mean levels of systolic BP ($p < 0.001$) and 2-hour glucose ($p = 0.004$) in *APOE4* carriers compared to *APOE4* non-carriers. Interestingly, the *APOE4* carriers who were not on any medication but were controlling hyperglycemia by diet and exercise had significantly higher mean levels of 2-hour glucose (218.3 ± 73.0) compared to *APOE4* non-carriers (183.0 ± 65.6) ($p = 0.015$) (Fig. 2). No significant changes were observed on HDL-C or LDL-C among *APOE4* carriers on metformin and metformin-SU combination therapy. We further investigated the impact of *APOE* polymorphism on the response to anti-hyperglycemic medications in data stratified by gender. Again, the correlation between *APOE* polymorphism and response to T2D medications was only seen in 2-hour glucose and systolic blood pressure. As shown in Supplementary Table 1, the *APOE4* carriers had significant lower systolic blood pressure in response to metformin and metformin-SU combination therapy in both male ($p = 0.003$) and female ($p = 0.009$) patients compared to *APOE4* non-carriers. Although, mean levels of 2-hour glucose were also lower among *APOE4* carriers on metformin and metformin-SU combination therapy, the difference was statistically significant only in male patients ($p = 0.009$) and not in female patients ($p = 0.236$).

To confirm if the effects of medications (such as metformin and metformin-SU combination therapy) were modified by *APOE* genotypes, we included both no-medication and all the medication groups in the model and analyzed *APOE* *medication (yes/no) interactions with all cardiometabolic traits. As shown in Table 3, our data revealed significant interaction of *APOE**medication in 2-hour glucose ($p = 0.001$). Also, the mean 2-hour glucose levels were higher among the *APOE4* carriers from the no-medication group and those on Dianil (composed of lyophilized *Momordica Charantia*) and herbal medications compared to non-*APOE4* carriers, while these effects were in opposite direction in other medication groups (including insulin, metfromin-SU and TZD/others) (Fig. 2). The effect of *APOE* * medication interaction was most significant in metformin-SU therapy ($p < 0.0001$), and no significant evidence of interaction was observed in other medication groups (Table 3). A marginally significant interaction in *APOE** metformin-SU was observed in diastolic BP ($p = 0.01$) however, it did not remain significant after Bonferroni correction ($p = 0.0063$). Taken together, metformin and metformin-SU combination therapy shows a significant improvement on the cardiometabolic response among the T2D patients carrying *E*4* allele.

4. Discussion

APOE gene polymorphisms have been examined extensively in multiple global populations particularly due to their crucial role in lipid metabolism and cardiovascular disease (Bennet et al., 2007). However, most of the genetic association studies, particularly in South Asian populations, have been dominated by reports comprising small sample size from heterogeneous populations with relatively less characterized disease phenotypes, and the overall contribution of *APOE* polymorphisms in T2D and cardiovascular disease in South Asians is still understudied. Here, we, for the first time have performed a large comprehensive evaluation to assess the impact of genetic variation in the *APOE* for affecting cardiometabolic risk factors associated with T2D and CAD. We also explored the association of *APOE* genotypes with response to anti-diabetic therapy and their effects on cardiometabolic outcomes.

The overall patterns of allelic diversity in our Punjabi sample are comparable to earlier published reports in Indian populations (Singh, Singh, & Mastana, 2006). We did not observe any significant difference in the distribution of *APOE* genotypes between T2D patients and non-T2D controls. Although, a large percentage of CAD patients (24%) were carriers of *APOE4* genotype compared to (20%) of *APOE2* and *APOE3*, the association of *APOE4* genotype for predicting CAD risk was not statistically significant (odds ratio 1.14 (0.85–1.52, p = 0.376) comparing CAD patients with healthy (non-T2D) controls (Table 2b) or using non-CAD controls from the entire subset (1.18 (95% 0.93–1.51), p = 0.180). Agreeing with earlier published reports predominantly from Caucasian populations, there was a modestly significant linear increase in diastolic BP and linear decrease in serum levels of HDL-C when *APOE* genotypes were ordered in increasing orders from *APOE2* to *APOE4* genotype carriers in controls, suggesting higher propensity for developing CAD, especially in *APOE4* carriers. These results are also modestly in agreement with a large meta-analysis study of 37,850 patients with coronary events and 82,727 controls, where *APOE4* genotype was shown to be a potential risk factor for cardiometabolic susceptibility (Bennet et al., 2007). Of note, while for the purpose of discussion we have provided statistical significant differences in cardio-metabolic traits among *APOE* genotypes, but after applying Bonferroni correction for multiple testing (0.05/10 = 0.005), no differences in Fig. 1 would remain statistically significant.

In our exploratory analysis of the association of *APOE* polymorphism and anti-hyperglycemic therapy, our data revealed significantly improved cardiometabolic outcomes among *APOE4* carriers in response to metformin and metformin-SU combination therapy. It is of interest to note that the overall means particularly for 2-hour glucose, fasting glucose, and systolic BP did not significantly differ among *APOE* genotypes for patients (Fig. 1). However, in comparison to *APOE4* non-carriers, the mean plasma levels among *APOE4* carriers were reduced by ~23% in 2-hour glucose (p = 0.004), and ~7 % in systolic BP (p < 0.001) among individuals on metformin and metformin-SU combination therapy. This difference was not observed in insulin or other herbal/homoeopathic medications as well as other therapies or in those taking no medication (Fig. 2). Our observed gender-specific correlation between *APOE* polymorphism and response to metformin and metformin-SU combination therapy showing 29% reduction in 2-hour glucose in males (p = 0.009) and

17% reduction in females (p = 0.236) is suggestive of physiological differences and would need further evaluations. Additionally, the improved treatment response in *APOE4* carriers to metformin-SU combination therapy was again confirmed by *APOE** drug-treatment interactions, where a significant evidence of interaction was observed in *APOE** metformin-SU (p < 0.0001 for 2-hour glucose; Bonferroni p = 0.0063).The exact mechanism of this improved response in cardiometabolic outcomes among *APOE4* genotype carriers is currently unknown. However, previous studies on metformin and ApoE using animal models support up-regulation of the expression of ApoE in response to metformin in peripheral nerve injury (Melemedjian, Yassine, Shy, & Price, 2013). ApoE is considered a potential regulator during peripheral nerve injury, and the expression of ApoE in response to metformin has been shown to be enhanced in ApoE-associated nerve regeneration and neuro-protection (Ignatius et al., 1986). *APOE4* with arginine at both sites (codon 112 and 158) is associated with lower ApoE plasma concentrations; perhaps the improved glycemic response among *APOE4* carriers could be due to improved (or restored) apoE concentration with metformin or metformin-SU combination therapy (Siest et al., 1995). Furthermore, beneficial effects of metformin-SU combination therapy have been reported to reduce all-cause mortality (Charbonnel et al., 2005; Isoda et al., 2006; Johnson, Majumdar, Simpson, & Toth, 2002). Metformin has also been implicated in vascular protection such as improvements in inflammatory pathways (Isoda et al., 2006), oxidative stress (Bailey & Turner, 1996), endothelial function (Vitale et al., 2005), insulin resistance (Glueck et al., 2001), and lipid profile (Glueck et al., 2001), beyond its known role in glycemic control. From these findings, it appears that the metformin-SU combination therapy may provide cardioprotection perhaps by up-regulation of ApoE4 and rendering it less atherogenic. However, further investigations would be required to understand the mechanism. It is noteworthy that despite improved cardiometabolic outcomes in patients with metformin and SU combination therapy, mean triglyceride levels still remained higher among *APOE4* carriers compared to *APOE4* non-carriers (data not shown), which perhaps suggest separate/additional intervention for lowering hypertriglyceridemia. Evidently, our results support the use of combination therapy to simultaneously treat multiple components of T2D pathogenesis. Strengths of our study include a carefully collected well-characterized T2D cohort with deeply phenotype traits related to cardiovascular complications. All non-diabetic controls were tested twice for fasting and post-prandial glucose to identify individuals with IGT. We recognize that our findings on the improved treatment response among *APOE4* carriers are provisional and a perspective follow-up of patients at different time points would provide better evaluation of genotype-associated response to treatment and reduction in risk factor levels.

5. Conclusions

In summary, our study suggests a modest impact of *APOE* genetic variation for increasing cardiometabolic susceptibility in patients with and without T2D. Our results also suggest significantly improved cardiometabolic outcomes among high-risk *APOE4* carriers in response to metformin and metformin-SU combination therapy. These data point to the need for evaluating population-specific genetic variation and its interactions with therapeutic

effects. However, these findings are exploratory and warrant confirmation in other independent datasets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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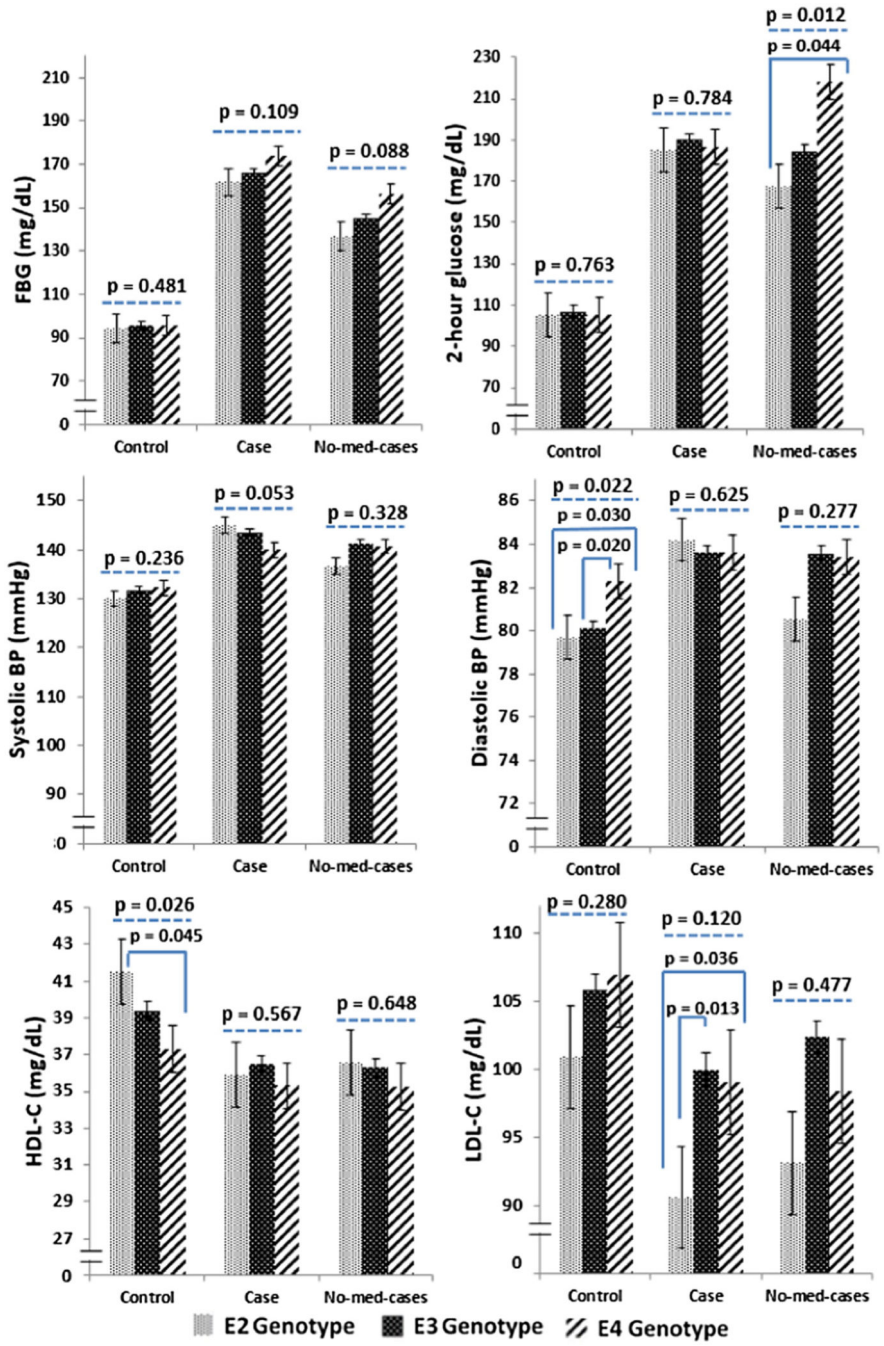

Fig. 1.

Effects of *APOE* genotype on cardiometabolic traits in data stratified by T2D status. Effects of *APOE* genotypes were also separately analyzed in T2D patients who were not taking any anti-hyperglycemic medications. Analysis was controlled for significant covariates — age, gender, BMI and medication status, where appropriate.

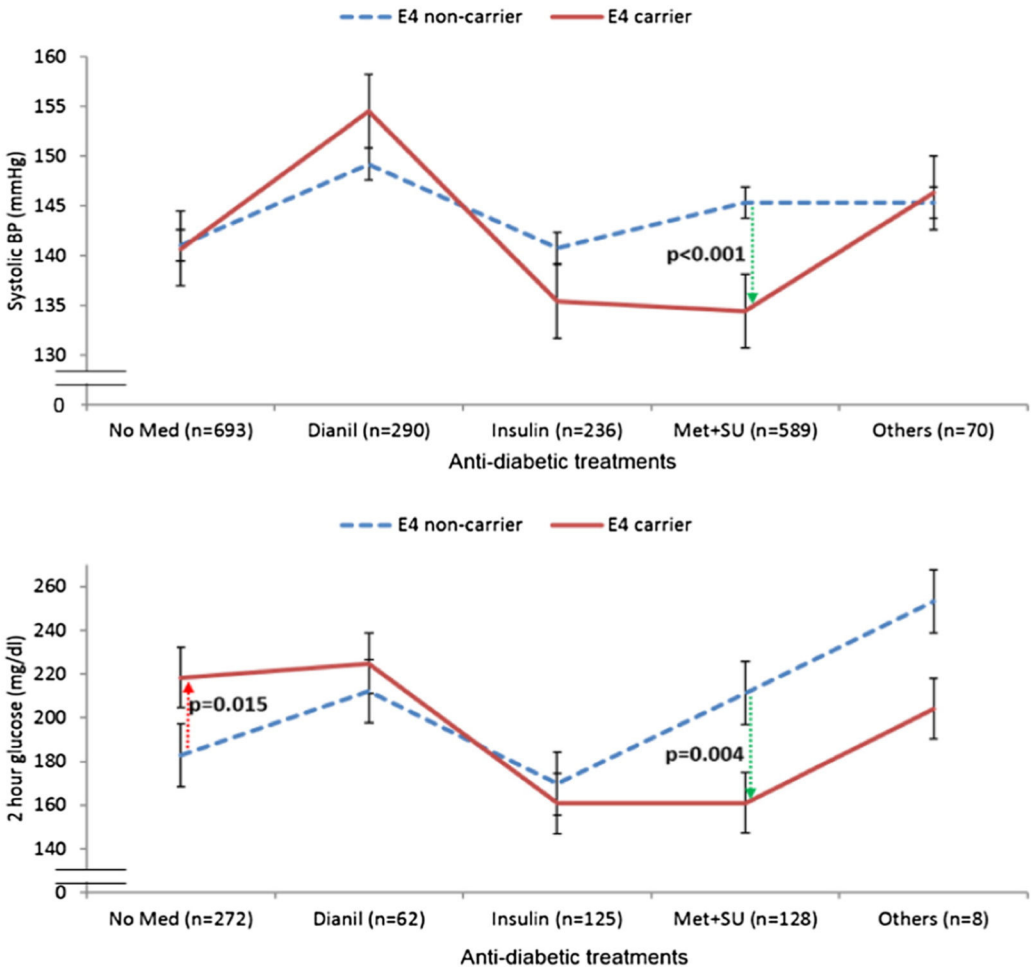

Fig. 2.

Data show the variation in the mean levels of systolic blood pressure and 2-hour glucose in patient data stratified by *APOE4* genotype-carriers and *APOE4* non-carriers treated with different anti-diabetic medications. Met – metformin, SU – sulfonylurea, others include TZD – thiazolidinedione, DPP4 – dipeptidylpeptidase 4 and other oral anti-diabetic medications, BP – blood pressure.

Table 1

Clinical characteristics of study population stratified by type 2 diabetes.

Traits	Control	Case	p value[a]
n (%)	1608 (45.1)	1956 (54.9)	–
Age (years)	50.0 ± 14.5	55.4 ± 11.2	<0.0001
Gender (M/F)	888/720	1078/878	–
BMI (kg/m^2)	25.6 ± 4.8	26.6 ± 4.9	<0.0001
Systolic BP (mmHg)	133.5 ± 22.3	145.2 ± 24.0	<0.0001
Diastolic BP (mmHg)	81.3 ± 12.3	84.5 ± 12.3	<0.0001
FBG (mg/dl)	96.3 ± 11.5	179.0 ± 69.8	<0.0001
2-hour glucose (mg/dl)	107.7 ± 17.7	202.7 ± 77.4	<0.0001
TG (mg/dl)	162.5 ± 99.0	177.1 ± 99.7	<0.0001
HDL-C (mg/dl)	42.2 ± 15.2	38.6 ± 13.7	<0.0001
LDL-C (mg/dl)	112.5 ± 39.7	106.1 ± 37.8	<0.0001

BMI – body mass index, BP – blood pressure, FBG – fasting blood glucose, TG – triglycerides, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, values are mean ± SD.

[a]Statistical probability that traits are different between cases and controls.

Table 2a

Distribution of *APOE* genotypes and allele frequencies in sample stratified by T2D status.

		Control (n = 1608)	Cases (n = 1956)	Combine (n = 3564)		
	***APOE* genotypes**	**n (%)**	**n (%)**	**n (%)**	**OR (95% CI)**	**p value[a]**
Genotypes	22	1 (0.1)	2 (0.1)	3 (0.1)	Genotype OR E2 Vs E3 0.93 (0.69–1.24)	0.604
	32	113 (7.0)	126 (6.4)	239 (6.7)		
	33	1287 (80.0)	1588 (81.2)	2875 (80.7)	Reference	
	24	11 (0.7)	16 (0.8)	27 (0.8)	Genotype OR E4 Vs E3 0.95 (0.76–1.19)	0.670
	34	190 (11.8)	216 (11.0)	406 (11.4)		
	44	6 (0.4)	8 (0.4)	14 (0.4)		
Allele frequency	*E**2	0.04	0.04	0.04		
	*E**3	0.89	0.90	0.90		
	*E**4	0.07	0.06	0.06		

OR – odds ratio.

[a] *APOE3* genotype was used as reference and adjusted for age, gender and blood pressure medication.

Table 2b

Distribution of *APOE* genotypes and allele frequencies in sample stratified by CAD status.

		Controls[a] (n = 1212)	CAD (n = 723)	Combined (n = 1935)		
	***APOE* genotypes**	**n (%)**	**n (%)**	**n (%)**	**OR (95% CI)**	**p value[b]**
Genotypes	22	1 (0.1)	1 (0.1)	2 (0.1)	Genotype OR E2 Vs E3 0.79 (0.53–1.18)	0.250
	32	83 (6.8)	46 (6.4)	129 (6.7)		
	33	977 (80.6)	572 (79.1)	1549 (80.1)	Reference	
	24	10 (0.8)	4 (0.6)	14 (0.7)	Genotype OR E4 Vs. E3 1.14 (0.85–1.52)	0.376
	34	137 (11.3)	95 (13.1)	232 (12.0)		
	44	4 (0.3)	5 (0.7)	9 (0.5)		
Allele frequency	*E**2	0.04	0.04	0.04		
	*E**3	0.90	0.89	0.90		
	*E**4	0.06	0.08	0.07		

CAD – coronary artery disease, OR – odds ratio.

[a] Non-CAD controls were healthy subjects without T2D or CAD.

[b] *APOE3* genotype was used as reference and adjusted for age and gender.

Table 3

Interaction between *APOE* gene polymorphisms and anti-hyperglycemic medications for affecting cardiometabolic traits.

	(A) *APOE**all medications			(B) *APOE**Dianil			(C) *APOE**Insulin			(D) *APOE**Met + SU Only			(E) *APOE** TZD, DPP4 or others		
Trait	**Mean square**	**F**	**p value**	**Mean square**	**F**	**p value**	**Mean square**	**F**	**p value**	**Mean square**	**F**	**p value**	**Mean square**	**F**	**p value**
BMI (kg/m^2)	0.027	0.807	0.369	0.092	2.507	0.114	0.002	0.055	0.815	0.000	0.006	0.941	0.013	0.337	0.562
Systolic BP (mmHg)	0.066	2.465	0.117	0.036	1.382	0.240	0.033	1.200	0.274	0.166	6.585	**0.010**	2.8×10^{-5}	0.001	0.974
Diastolic BP (mmHg)	0.000	0.019	0.889	0.038	1.823	0.177	0.015	0.735	0.391	0.020	1.001	0.317	0.065	3.158	0.076
FBG (mg/dl)	0.132	1.035	0.309	0.003	0.027	0.870	0.208	1.565	0.211	0.101	0.825	0.364	0.001	0.011	0.915
2-hour glucose (mg/dl)	1.552	11.466	**0.001**	0.033	0.267	0.606	0.384	2.932	0.088	1.879	14.868	**<0.0001**	0.101	0.800	0.372
TG (mg/dl)	0.170	0.644	0.422	0.001	0.003	0.956	0.003	0.010	0.919	0.550	2.051	0.152	0.231	0.798	0.372
HDL-C (mg/dl)	0.000	0.004	0.952	0.095	0.740	0.390	0.119	0.933	0.334	0.166	1.272	0.260	0.188	1.383	0.240
LDL-C (mg/dl)	0.087	0.600	0.439	0.040	0.273	0.601	0.047	0.357	0.550	0.000	0.004	0.173	0.000	0.003	0.960

BMI – body mass index, BP – blood pressure, FBG – fasting blood glucose, TG – triglycerides, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, Met – metformin, SU – sulfonylurea, TZD – thiazolidinedione, DPP4 – dipeptidylpeptidase 4, Bonferroni p = 0.0063.