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Apolipoprotein E levels and apolipoprotein E genotypes in incident cardiovascular disease risk in subjects of the Prevention of Renal and Vascular End-stage disease study

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BACKGROUND: Apolipoprotein E (apoE) is a component of all major lipoprotein classes with multiple functions including clearance of circulating triglyceride-rich lipoprotein particles and hepatic production of triglyceride-rich lipoprotein, thus affording several avenues for apoE involvement in atherosclerosis development. ApoE has 3 isoforms (E2, E3, and E4) based on a common genetic polymorphism. Numerous studies have been performed assessing cardiovascular disease (CVD) risk relative to the 6 resulting genotypes; however, surprisingly, few studies have been performed assessing risk attributable to apoE plasma levels either alone or in addition also taking into account apoE genotypes.

OBJECTIVE: To examine the role of apoE levels together with apoE genotypes on incident CVD risk in a large population-based cohort and also to afford preliminary characterization of atherogenic apoE-containing lipoprotein particles.

METHODS: Cox multivariable proportional hazards modeling was performed on a cohort of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study as a function of apoE levels and apoE genotypes adjusted for age, gender, and past history of CVD. Further modeling was performed with single addition of clinical and biomarker parameters to elucidate the nature of apoE-associated risk.

RESULTS: High apoE levels were demonstrated to be associated with CVD risk (hazard ratio per apoE standard deviation, 1.20; 95% confidence interval, 1.11–1.31; $P < .0001$) both overall and within the high-frequency apoE genotype groups (ε2ε3, ε3ε3, and ε3ε4). Only on addition of apoB-containing lipoprotein parameters to models, did apoE levels lose association with risk.

CONCLUSIONS: ApoE levels positively associate with incident CVD risk with apoE-associated risk likely residing in apoB-containing lipoproteins.

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Introduction

Apolipoprotein E (apoE) is a 34 kDa protein comprised of 299 amino acid residues.[1] Its encoding gene, *APOE*, is located on chromosome 19 in close proximity to *APOC1*, *APOC2*, and *APOC4*.[2,3] ApoE is a component of all major lipoprotein classes, and as such, it has multiple functions in metabolism and inflammation-related processes including clearance of circulating atherogenic triglyceride-rich lipoproteins (TRLs); hepatic secretion of very low–density lipoprotein (VLDL); stimulation of cholesterol efflux; inhibition of platelet aggregation; inhibition of T-lymphocyte, smooth muscle cell, and endothelial cell proliferation; and inhibition of inflammation and oxidative stress.[4–8] The apoE gene demonstrates 3 well-known common alleles, ε2, ε3 (wild type), and ε4, which give rise to 3 protein isoforms: apoE2 (Cys112, Cys158), apoE3 (Cys112, Arg158), and apoE4 (Arg112, Arg158), respectively. The apoE isoforms are known to demonstrate differential functionality relating to clearance of TRL from the circulation based primarily on differences in affinities of the isoforms for various receptors mediating TRL uptake and on differences in affinities of the isoforms for the various lipoprotein classes.[7,9–11] The differences in affinities derive from structural differences in apoE isoforms. The apoE N-terminal domain contains the lipoprotein receptor–binding site[12–15]; whereas the C-terminal domain exhibits the principal lipoprotein-binding region.[13,14] The most notable change involving lipoprotein receptor binding is the decreased affinity of apoE2 arising from structural alterations close to the receptor-binding site brought about by the ε2 allele.[16,17] With regard to lipoprotein preferences, apoE2 and apoE3 favor association with small phospholipid-rich high-density lipoprotein (HDL), whereas apoE4 favors association with large, triglyceride-rich VLDL.[16,18,19]

In view of functional differences in apoE isoforms relating to lipoprotein metabolism, there has been a great deal of work oriented toward assessing potential associations of apoE isoforms with cardiovascular disease (CVD) risk.[20–24] In this regard, recent meta-analyses have generally concluded associations of the ε4 allele with higher risk and the ε2 allele with lower risk.[20–24] Specifically, results have demonstrated: (1) a 42% higher coronary risk for carriers of the ε4 allele in comparison to ε3ε3 individuals and no significant risk association for the ε2 allele[20]; (2) slightly higher risk for ε4 carriers compared with ε3ε3 individuals and 20% lower coronary risk for ε2 carriers[21]; (3) the ε4 allele to be a risk factor for development of myocardial infarction (MI) and the ε2 allele to be a protective factor for development of MI[22]; (4) a tentative association of increased risk with the ε4 allele[23]; and (5) suggestion of increased risk for the ε4 allele and no association with risk for the ε2 allele.[24] However, also should be noted are studies not supportive of such results.[5,25]

In contrast to the extensive work on apoE isoforms and CVD risk, there have been remarkably few studies dealing with associations of apoE levels with risk and even fewer studies assessing potential roles for interactions of apoE levels with apoE isoforms in the establishment of risk.[26–30] Thus, the current work was conducted in a large cohort of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study to assess the potential association of apoE levels with incident CVD risk, to assess potential associations of interaction of apoE levels with apoE genotypes in risk, and to preliminarily characterize apoE-containing atherogenic lipoprotein particles.

Methods

Subjects for the current work were participants of PREVEND, a large prospective general population-based study begun in 1997 to investigate CVD and renal disease with focus on albuminuria.[31] Briefly, a questionnaire was sent to all inhabitants (28–75 years old, N = 85,421) of the city of Groningen, the Netherlands, requesting demographic and CVD morbidity data and to supply an early morning urine specimen. Response rate was 47.8%. Included in the study were all subjects with urine albumin levels ≥10 mg/L and a group of randomly selected subjects with urine albumin level <10 mg/L. Exclusions included subjects with insulin-using diabetes mellitus and pregnant women after which resulted the PREVEND cohort of 8592 study subjects. CVD outcomes were followed and included cardiovascular mortality and any of the following at hospitalization: fatal and nonfatal MI, ischemic heart disease, percutaneous transluminal coronary angioplasty, and coronary artery bypass grafting. The municipal register was the source of mortality data with cause of death obtained by linkage of death certificate number to primary cause of death (Dutch Central Bureau of Statistics). Hospital morbidity data were from PRISMANT (Dutch national registry of hospital discharge diagnoses). Follow-up time was from initial urine collection in 1997 to date of either first CVD event or study termination (31 December 2008) if no CVD event. For the present study, there were additional exclusions as follows: lack of apoE level or apoE genotyping and lack of metabolic syndrome (MetS) status. This resulted in a study group of 5485 subjects with mean follow-up time of 9.4 years. The PREVEND study has been approved by the Medical Ethics Committee of the University of Groningen. Informed written consent was obtained from all participants.

Serum and plasma were prepared from venous blood samples with collection after overnight fast and after 15 minutes of rest with laboratory analyses performed after storage overnight at −20°C. Lipids, lipoproteins, high-sensitivity C-reactive protein (CRP), and glucose were determined by standard analytical methods as described previously.[32,33] Low-density lipoprotein cholesterol (LDL-C) was determined from the Friedewald equation. ApoE genotyping was performed as described previously.[34,35] Clinical variables were defined as follows. Past cardiac history included: before entry into PREVEND