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Original Article

Mendelian randomization analysis of cholesteryl ester transfer protein and subclinical atherosclerosis: A population-based study

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BACKGROUND: Several trials to prevent cardiovascular disease by inhibiting cholesteryl ester transfer protein (CETP) have failed, except Randomized EValuation of the Effects of Anacetrapib through Lipid-modification. Thus far, it is unclear to what extent CETP is causally related to measures of atherosclerosis.

OBJECTIVE: The aim of the article was to study the causal relationship between genetically determined CETP concentration and carotid intima-media thickness (cIMT) in a population-based cohort study.

METHODS: In the Netherlands Epidemiology of Obesity study, participants were genotyped, and cIMT was measured by ultrasonography. We examined the relation between a weighted genetic risk score for CETP concentration, based on 3 single-nucleotide polymorphisms that have previously been shown to largely determine CETP concentration and cIMT using Mendelian randomization in the total population and in strata by sex, Framingham 10-year risk, (pre)diabetes, high-density lipoprotein cholesterol, triglycerides, and statin use.

RESULTS: We analyzed 5655 participants (56% women) with a mean age of 56 (range 44–66) years, body mass index of 26 (range 17–61) kg/m^2, and serum CETP of 2.47 (range 0.68–5.33) μg/mL. There was no evidence for a causal relation between genetically determined CETP and cIMT in the total population, but associations were differently directed in men (16 μm per μg/mL increase in genetically

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determined CETP; 95% confidence interval: −8, 39) and women (−8 μm; −25, 9). Genetically determined CETP appeared to be associated with cIMT in normoglycemic men (26 μm; −1, 52) and in (pre)diabetic women (48 μm; −2, 98).

CONCLUSION: In this population-based study, there was no causal relation between genetically determined CETP concentration and cIMT in the total population although we observed directionally differing effects in men and women. Stratified results suggested associations in individuals with different cardiometabolic risk factor profiles, which require replication.

Introduction

Recent studies to improve cardiovascular risk prevention have focused on cholesteryl ester transfer protein (CETP) inhibitors since they increase high-density lipoprotein cholesterol (HDL-C) and decrease non-HDL-c concentrations.[1–3]

CETP facilitates the migration of cholesteryl esters from HDL to low-density lipoprotein (LDL) and very-low-density lipoproteins. A high CETP concentration is therefore hypothesized to contribute to an atherogenic lipoprotein profile by increasing (V)LDL cholesterol (LDL-C) and decreasing HDL-C.[4] Several observational studies have suggested that lower concentrations of CETP are associated with reduced cardiovascular disease (CVD) risk.[5,6] Most recent efforts to lower CETP concentration pharmacologically with the purpose of reducing CVD risk have been unsuccessful, except for the Randomized EValuation of the Effects of Anacetrapib through Lipid-modification trial, in which CETP inhibition with anacetrapib successfully lowered the risk of major coronary events in high cardiovascular risk patients.[7,8]The effect of genetically determined CETP has been subject to considerable discussion in recent literature, but, in general, a detrimental effect of high CETP, if any, appears to be restricted to men[2,4,8–18] Close inspection of previous studies on the association of CETP with CVD risk suggested that in addition to sex, other factors potentially modulate the effects of CETP on CVD risk, including HDL-C or triglyceride (TG) concentrations, insulin resistance, or the use of statins or fibrates.[1,9,19–21] This suggests that CETP inhibition could be effective in specific subgroups of the population. To provide more insights in the role of CETP on cardiovascular risk, we aimed to study the causal effect of genetically determined higher CETP concentration on atherosclerosis in the general low-risk population, as well as specific subgroups, using a genetic risk score (GRS) for CETP concentration as determinant.

Methods

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study of 6671 men and women aged between 45 and 65 years. The study design and population are described in detail elsewhere.[22] All inhabitants with a self-reported body mass index (BMI) of $\geq$27 kg/m^2 and living in the greater area of Leiden, the Netherlands, were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from 1 adjacent municipality (Leiderdorp, The Netherlands) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. Participants visited the NEO study center for extensive baseline measurements, including blood sampling and carotid intima-media thickness (cIMT). Research nurses recorded current medication use by means of a medication inventory. Before the study visit, participants completed questionnaires at home with respect to demographic, lifestyle, and clinical information.

The Medical Ethical Committee of the Leiden University Medical Center approved the protocol. All participants gave their written informed consent.

For the present analyses, we excluded participants from non-European ancestry or with poor genotyping quality (n = 927): when the sample call rate was <98%, there was a sex mismatch, heterozygosity rate was not within ±3 standard deviation (SD) of mean heterozygosity rate, participants differed based on the first 2 principal components (±3.5 SD), samples were duplicates, or concordance with another DNA sample was >0.25 (related individuals). Furthermore, we excluded participants with missing CETP (n = 31) and cIMT measurement (n = 58).

Blood sampling

During the visit to the NEO study center, venous blood samples were obtained from the antecubital vein after a >10-hour overnight fast. Fasting serum total cholesterol and TG concentrations were measured with enzymatic colorimetric assays (Roche Modular P800 Analyzer; Roche Diagnostics, Mannheim, Germany) and fasting serum HDL-C concentrations with third generation homogenous HDL-C methods (Roche Modular P800 Analyzer). LDL-C concentrations were calculated using the Friedewald equation.[23] Furthermore, aliquots of plasma and serum were stored at −80°C after centrifugation. DNA was extracted, and genotyping was performed by the Centre National de Génotypage (Evry Cedex, France), using the Illumina