Atherosclerosis 240 (2015) 98–104

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

APOA5 variants predispose hyperlipidemic patients to atherogenic dyslipidemia and subclinical atherosclerosis

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ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form
24 February 2015
Accepted 3 March 2015
Available online 9 March 2015

Keywords:
APOA5
Polymorphisms
NMR
IMT
Atherogenic dyslipidemia

ABSTRACT

Background: Triglycerides (TG) are the initiators of the metabolic changes leading to the atherogenic dyslipidemia, which is a major inducer of atherosclerosis as a result of quantitative and qualitative changes in lipoprotein subclass distributions. We hypothesized that variation at the of *APOA5* gene locus, encoding apoAV, a key regulator of TG levels, significantly affect lipoprotein subclass distributions toward a more atherogenic pattern in both hyperTG patients and dyslipemic patients.
Methods: We recruited four hundred and twenty-two subjects attending a Lipid Clinic, prior to lipid-lowering treatment. We genotyped two *APOA5* variants, rs662799 (-1131T>C) and rs3135506 (S19W). Circulating lipoproteins were determined by nuclear magnetic resonance (NMR). Intima-media thickness (IMT) was evaluated using B-mode ultrasound.
Results: Carriers of the rare alleles of rs662799 and rs3135506 compared to common allele homozygotes, had a significantly proatherogenic profile of the VLDL and LDL subclasses, resulting in increased concentrations of the proatherogenic subclasses, large VLDLs (+133%, $p < 0.001$) and small LDLs (+34%, $p = 0.014$). Significant changes in smaller HDL (+71%, $p = 0.032$), as well as an 18% decrease in large HDL ($p = 0.046$), were also been observed. This atherogenic NMR subclass distribution was significantly associated with increased carotid IMT.
The observed effects were significantly stronger in patients with a BMI $\geq$ 25 kg/m^2 and in male and female patients with a waist circumference $\geq$90 cm or $\geq$85 cm, respectively.
Conclusion: In a dyslipemic population, genetic variants of *APOA5* modulate lipoprotein subclass distributions, inducing an atherogenic profile associated with IMT defined subclinical atherosclerosis.

1. Introduction

Plasma lipid levels play a pivotal role in the pathogenesis of atherosclerosis and are a major predictor of coronary artery disease (CAD) [1]. Hence, LDL cholesterol is the focus of most strategies directed at reducing cardiovascular risk. Triglycerides (TG) are also important in this regard, not only because they are a risk factor for atherosclerosis [2] but because they are also the initiators of the metabolic changes leading to an atherogenic lipoprotein profile, the so-called atherogenic dyslipidemia. The rationale behind the present investigation is that atherogenic changes induced by TG occur at concentrations as low as 1.7 mmol/L; the level at which small dense LDL become predominant [3]. Thus small dense LDL are clinically relevant not only for hypertriglyceridemic patients but also for those with other dyslipidemias.

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http://dx.doi.org/10.1016/j.atherosclerosis.2015.03.008
0021-9150/

The condition that best reflects the pernicious role of TG is atherogenic dyslipidemia, a metabolic disturbance characterized by hypertriglyceridemia and low HDLc that entails an increased cardiovascular disease risk [4–6]. It is a feature of obesity, type 2 diabetes mellitus, and metabolic syndrome, conditions with a high prevalence globally [7,8]. It has been suggested that high plasma TG levels modulate the size and number of certain lipoprotein subclasses, triggering an imbalance that promotes increase in circulating proatherogenic small dense LDL particles and cholesterol-rich remnant particles, as well as a decrease in anti-atherogenic HDL particles.

To gain insight into this scenario two additional issues must be addressed. The first involves the factors that predispose an individual to increased TG; the second is our capacity to detect TG-induced lipoprotein changes via traditional lipid and lipoprotein measurements.

Regarding the first issue, the apolipoprotein A5 (*APOA5*) gene, encoding apoAV, is one of the major genetic determinants of circulating TG levels. In animal models, an inverse relationship between hepatic *APOA5* expression and plasma TG levels has been described [9]. In humans, both single-gene and genome-wide association studies in different populations, have confirmed that *APOA5* is one of the strongest genes influencing TG concentrations [10–13]. It has also been reported that *APOA5* variants are associated with other lipid parameters, LDL and HDL, suggesting that they are not limited to determining TG levels, but also play an important role in the overall regulation of lipid metabolism.

To date, several mechanisms of apoAV action have been proposed, including plasma TG removal, either by stimulating TG hydrolysis and stabilizing the lipoprotein lipase (LPL) active dimer, or facilitating the attachment between TG-rich lipoproteins and glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) [14,15], as well as accelerating hepatic uptake of TG-rich lipoproteins and their remnants by heparan sulfate proteoglycan (HSPG) and LDL receptor (LDLR) family members. Intracellular effects of apoAV on VLDL production and secretion by the liver may also explain the effect of apoAV on TG levels, but the evidence supporting this notion is weaker [16].

Regarding lipid measurements, it has been known for some time that lipid constituents of major lipoprotein classes (such as cholesterol and TG), may be further characterized into subclasses using various techniques, among them nuclear magnetic resonance (NMR), a method that helps quantify lipoprotein subclasses [17]. The profile of these subclasses represents an important prognostic factor in both the manifestation and the progression of CAD.

Therefore, the objective of this study was to analyze the effects of *APOA5* variants on lipid profiles, lipoprotein subclass sizes and numbers, and carotid atherosclerosis, determined by measures of intima media thickness (IMT) in hyperlipidemic subjects recruited from a lipid clinic.

2. Subjects and methods

2.1. Study subjects

Subjects were recruited at 3 Lipid Clinics (Hospital Universitari Sant Joan de Reus, Hospital Clínic of Barcelona and Hospital Universitario Miguel Servet of Zaragoza) in Spain [18]. The study began in 2005, when clinical, analytical and sonographic methodologies were standardized among centers. All patients ≥17 years of age with a clinical diagnosis of familial hyperlipidemia were included and provided informed consent to participate in a protocol approved by the ethical review boards of each of the participating institutions. Within 2–6 weeks of their first visit, all participants had venipuncture to collect fasting blood samples and underwent a carotid ultrasound according to a predefined protocol. We studied 422 untreated subjects.

Isolated primary hypercholesterolemia was diagnosed in subjects with off-treatment LDLc levels above the age- and sex-specific 95th percentile of a Spanish reference population [19], as well as TG levels below 5.17 mmol/L. The diagnosis of primary hypertriglyceridemia was based on the presence of either combined hyperlipidemia or isolated hypertriglyceridemia in untreated patients whose serum cholesterol and TG concentrations were above the sex- and age-specific 90th percentiles for the Spanish population. The criterion for combined hyperlipidemia was having serum total apolipoprotein B levels ≥120 mg/dL, and for isolated hypertriglyceridemia this was the presence of high TG alone. Secondary causes of hyperlipidemia were excluded in all subjects. A control group consisting of healthy, unrelated male and female volunteers, aged 18–75 years, who underwent a medical examination at the Hospital Miguel Servet of Zaragoza was also studied. Exclusion criteria for control subjects included a personal or parental history of CAD or dyslipidemia, an existing acute illness, or the use of drugs capable of influencing either glucose or lipid metabolism.

Eighteen participants did not report data on BMI and 10 participants did not report data on waist circumference so they were not included into the analysis.

2.2. Laboratory measurements

Fasting blood for biochemical profiles was drawn after patients were off hypolipidemic drug treatment for at least 4 weeks. Cholesterol and TG levels were determined by standard enzymatic methods. HDLc was measured by a precipitation technique. LDLc was estimated with the Friedewald equation, except in samples with triglycerides ≥3.5 mmol/L, when it was measured in the d = 1.063 g/mL fraction separated by density gradient ultracentrifugation. Apolipoprotein (apo) B and lipoprotein(a) levels were determined by immunoturbidimetry (Unimate 3, Roche, Basel, Switzerland).

2.3. Nuclear magnetic resonance lipoprotein profile measurements

Both lipoprotein subclass particle concentrations and the average sizes of lipoprotein particles were measured by proton NMR spectroscopy (LipoScience, Inc., Raleigh, North Carolina), as previously described [20]. The particle concentrations for lipoprotein subclasses of different size were obtained directly using the measured amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Weighted-average lipoprotein particle sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal. The concentrations of the following subclasses were measured: small LDL (diameter 18.0–21.2 nm), large LDL (21.2–23.0 nm), intermediate-density lipoprotein (IDL) (23.0–27.0 nm), large high-density lipoprotein (HDL) (8.8–13.0 nm), medium HDL (8.2–8.8 nm), small HDL (7.3–8.2 nm), large very low-density lipoprotein (VLDL) (>60 nm), medium VLDL (35.0–60.0 nm), and small VLDL (27.0–35.0 nm). VLDL and LDL particle concentrations are expressed in nmol/L, and HDL, in μmol/L.

2.4. Carotid intima media thickness (IMT) measurements

For carotid sonography, we used at each center an Acuson Sequoia instrument (Siemens Medical Solutions, Erlangen, Germany) equipped with a linear array ultrasound transducer (L7, 5–12 MHz). Scanning and image analysis procedures were standardized as previously described [21]. In summary, scans were