Atherosclerosis 224 (2012) 123–128

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

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

Plasma levels of antibodies against oxidized LDL are inherited but not associated with HDL-cholesterol level in families with early coronary heart disease

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ARTICLE INFO

Article history:
Received 2 February 2012
Received in revised form
7 June 2012
Accepted 12 June 2012
Available online 28 June 2012

Keywords:
Immunology
Lipoproteins
Immunoglobulin isotypes
Coronary artery disease
Genetics
Pedigree
Bivariate analysis
Genetic cross-correlation
Environmental cross-correlation

ABSTRACT

Objective: Oxidized low-density lipoproteins (oxLDL) and antibodies against them (anti-oxLDLs) are thought to play a central role in atherosclerosis. One proposed antiatherosclerotic mechanism for HDL is to prevent oxidation of LDL. This study examined whether plasma HDL-cholesterol (HDL-C) is related to plasma anti-oxLDL levels.

Methods: We collected families based on probands with low HDL-C and premature coronary heart disease (CHD). Antibody levels were determined in samples from 405 subjects. Immunoglobulin G, M and A levels against two in vitro models of oxLDL, malondialdehyde-acetaldehyde-modified LDL (MAA-LDL) and copper oxidized LDL (CuOx-LDL), were measured by ELISA. We carried out heritability estimation of antibody traits and bivariate analyses between HDL-C, LDL-C and antibody traits.

Results: All the antibody levels were significantly inherited ($p < 0.001$), heritability estimates ranging from 0.28 to 0.65. HDL-C exhibited no environmental or genetic cross-correlations with antibody levels. Significant environmental correlations were detected between LDL-C and both IgG levels ($\rho_E = 0.40$, $p = 0.046$ and $\rho_E = 0.39$, $p < 0.001$). There were no differences in antibody levels between subjects with normal and low HDL-C, or between CHD-affected and non-affected subjects.

Conclusion: In this study, low HDL-C level displayed no significant associations with the anti-oxLDL levels measured. The heritability of the anti-oxLDL levels was a novel and interesting finding.

1. Introduction

Modified lipoproteins, including oxidized low-density lipoproteins (oxLDL), and humoural immunity against these compounds are thought to play a central role in the initiation and propagation of atherosclerosis [1]. Plasma levels of antibodies against oxLDL (anti-oxLDL) have been associated with atherosclerosis, but these associations have been found to vary in different studies e.g. depending on the antibody type and the model of atherosclerosis being studied. Plasma levels of IgG-type anti-oxLDL (IgG-oxLDL) have been associated positively [2], inversely [3] or not at all with atherosclerosis [4,5]. High plasma levels of IgM-oxLDL have been considered to protect against atherosclerosis in most studies [2,3]. The role of IgA-oxLDL in atherosclerosis has not been studied so far.

Low plasma HDL-cholesterol (HDL-C) level is an independent risk factor for atherosclerosis [6] and it is the most common dyslipidemia associated with premature and familial coronary heart disease (CHD) [7]. The major antiatherogenic mechanism of HDL-particles is thought to be reverse cholesterol transport, but HDL-particles are also anti-oxidative and anti-inflammatory [8–10]. Few studies have focused on the relationship between plasma levels of HDL-C and anti-oxLDL, although it could be speculated that high HDL-C levels could confer protection against oxidation of LDL and therefore be associated with a reduced oxLDL burden and lower anti-oxLDL levels. Plasma HDL-C has been shown to be inversely related to oxLDL-levels [11]. Low plasma HDL-C levels have been linked with higher total anti-oxLDL [12]. An inverse association between HDL-C and total anti-oxLDL binding to malondialdehyde-modified LDL (MDA-LDL) has been reported [13]. The level of IgM-oxLDL has been found to be positively correlated with HDL-C [14], whereas the level of IgG-MDA-LDL correlated inversely with HDL-C [4].

In addition to HDL-C, anti-oxLDL levels could be associated with other well-known risk factors of atherosclerosis such as a high low-density lipoprotein cholesterol (LDL-C) level [4]. Statins, drugs which cause an extensive reduction of plasma LDL-C levels, have been claimed to affect anti-oxLDL levels. Atorvastatin, fluvastatin

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http://dx.doi.org/10.1016/j.atherosclerosis.2012.06.056

and pravastin have been shown to decrease the levels of autoantibodies [15–17].

Little is known about the genetic regulation of plasma anti-oxLDL levels, and the heritabilities of anti-oxLDL levels have not been studied earlier. IgG- and IgM-oxLDL have been reported to be associated with a single nucleotide polymorphism (SNP) in the lipoprotein lipase gene (LPL) and IgM-oxLDL with an SNP in the opiate-like receptor 1 gene (OLR1) [18]. In diabetic subjects, anti-oxLDL levels have been associated with polymorphisms of peroxisome proliferator-activated receptor gamma2 gene (PPARγ2) [19].

The purpose of this study was to investigate whether anti-oxLDL levels are linked to the phenotype of low plasma HDL-C levels predisposing to early CHD. Two in vitro models of oxLDL, namely malondialdehyde-acetaldehyde-modified LDL (MAA-LDL) and copper oxidized LDL (CuOx-LDL), were studied in Finnish families with low HDL-C levels and early onset CHD. The heritabilities of anti-oxLDL levels were studied and in addition, bivariate analysis between anti-oxLDL levels and HDL-C or LDL-C was carried out to examine whether they share a common genetic or environmental background.

2. Materials and methods

2.1. Subjects

Probands with premature CHD (i.e. acute myocardial infarction, coronary artery bypass graft operation or percutaneous transluminal coronary angioplasty before the age of 55 years), low HDL-C levels (<1.1 mmol/l) and normal to moderately elevated levels of triglycerides (<3.5 mmol/l) and total cholesterol (<7.0 mmol/l), no diabetes and an entry about family history of CHD in the hospital records were selected from the Oulu University Hospital as described earlier [20]. All the relatives of the proband (independent of their CHD status and HDL-C levels) who were willing to participate were examined and extended pedigrees were recruited to ascertain the genetic background. There were 39 pedigrees with three generations on average (minimum 2 and maximum 5) and with an average pedigrees size of 19 subjects (minimum 5 and maximum 89). A total number of 405 samples (39 families) were available for antibody measurements. From CHD-patients, lipid measurements were taken before or at least three months after myocardial infarction or coronary bypass operation. Information about smoking (recoded as current smoker or not, as pack-years of smoking), average alcohol consumption (grams of alcohol/week), medication and past medical history of the subjects was obtained by using a questionnaire. The study material was collected at 1990s before the era of intensive statin treatment and therefore only forty-eight study subjects were being treated with a statin; simvastatin at a daily dose of 10 mg was used by 50% of statin users and the most effective statin in use was atorvastatin at a daily dose of only 10 mg. Aspirin was used by 106 subjects, beta blocker by 125 subjects, an ACE inhibitor by 44 subjects, an angiotensin II receptor blocker by 5 subjects, a calcium channel blocker by 47 subjects, nitrates by 35 subjects, a hormone replacement therapy by 13 subjects and vitamin supplements by 41 subjects.Informed consent was obtained from all the study subjects. The ethical committee of the Oulu University Hospital approved this study.

2.2. Lipid and lipoprotein measurements

Blood samples were obtained after an overnight fast, and plasma was separated by centrifugation at 800 × *g* for 10 min and kept at +4 °C until further analysis. From plasma, VLDL-fraction (d < 1.006 g/ml) was separated by ultracentrifugation in a Kontron TFT 45.6 rotor at 105,000 × *g* for 18 h. Thereafter, the other apoB-containing lipoproteins were precipitated by adding 25 μl of 2.8% (weight/volume) heparin and 25 μl of 2 M $MgCl_2$ into 1 ml of VLDL-free fraction. After precipitation and centrifugation at 1000 × *g* and +4 °C for 30 min, the HDL-C concentration of the supernatant was measured. The plasma LDL-C concentration was calculated by subtracting the cholesterol concentration of the HDL fraction from that of the VLDL-free fraction. The plasma concentrations of cholesterol and triglycerides and the lipoprotein fractions were measured by enzymatic colorimetric methods (Boehringer Mannheim GmbH, Germany) using a Kone Specific Analyser (Kone Instruments, Espoo, Finland).

2.3. Antibody measurements

The experimental models of oxidized LDL used in this study were CuOx-LDL and MAA-LDL. MAA-LDL and CuOx-LDL were generated and tested as previously described [21]. LDL for antigen preparation was isolated from three healthy male subjects using no medication. The antigens were from a single batch for each antibody type (IgG-MAA-LDL, IgG-CuOx-LDL etc.). ELISA method was used to measure the levels of antibodies binding to modified LDL from 405 subjects as previously described [3,22]. Briefly, plates were coated with 5 ug/ml of CuOxLDL and MAA-LDL, in PBS-EDTA (PBS with 0.27 mmol/l EDTA), for overnight at +4 °C in white microfluor plates (Thermo electron Corp., Milford, MA, USA) and the PBS-washed plates blocked with PBS–EDTA containing 0.5% gelatin - protein from cold water fish skin (PBS-EDTA-FG) for 1 h at room temperature (RT). To determine the total IgG, IgM and IgA against CuOx-LDL and MAA-LDL, the plasma samples were added on PBS-washed plates and incubated for 1 h at RT. The secondary antibody (alkaline phosphatase-labelled goat anti-human (ALP-anti-human) IgG, IgM or IgA (Sigma–Aldrich, Saint Louis, Missouri, MO, USA)) diluted into TBS-FG-buffer, was added on washed plates, and incubated for 1 h at RT. As the substrate for the ALP-anti-human, a volume of 25 uL of 30% water solution of LumiPhos 530 (Lumigen, Inc., Southfield, MI, USA) was used. Luminescence was measured after 60 min dark incubation by Victor^2 Luminometer (Wallac, Perkin–Elmer, Boston, MA, USA). The plates were washed three times with PBS–EDTA with an automated plate washer after every incubation before Lumiphos. For IgG determinations plasma samples were diluted into PBS-EDTA-FG as follows: 1:2000 (MAA-LDL and CuOx-LDL), 1:4000 (MAA-LDL high samples) or 1:8000 (CuOx-LDL high samples). For IgM determinations, 1:1500 (MAA-LDL), 1:4000 (MAA-LDL high samples), 1 : 2500 (CuOx-LDL) or 1:7000/10,000/500/5000 (CuOx-LDL high and low samples) dilutions, and for IgA determinations, 1:4000 (MAA-LDL) or 1:3000 (CuOx-LDL) dilutions were used.

For each plasma sample, triplicate determinations were performed and an average relative luminescence unit (RLU) value was calculated. Each plate contained a triplicate standard of purified immunoglobulin, a zero-sample of pure PBS-EDTA-FG, and two triplicate control samples ('high' and 'low'), which were diluted to cover as wide a range of the standard as possible. Linear equation for the standard curve was determined before the calculation of relative plasma antibody concentrations of the samples. The relative concentration was divided by 1000 and it is expressed as relative units (RU). To detect variation in an assay and between all the assays of an antibody type, intra-assay and inter-assay coefficients of variation (CV) were calculated, respectively, using control samples. The inter-assay coefficients of variation (CVs) were 15% or below in all assay series. The intra-assay CVs were 15% or below, except in the following cases: IgG-MAA-LDL high (control) 23.1% and low (control) 17.5% (in one