Association between anti-apolipoprotein A-1 antibodies and cardiovascular disease in the general population

Results from the CoLaus study

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Summary

We aimed to determine the association between autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG) and prevalent cardiovascular (CV) disease (CVD) as well as markers of CV risk in the general population. Cross-sectional data were obtained from 6649 subjects (age 52.6 ± 10.7 years, 47.4 % male) of the population-based CoLaus study. CVD was defined as myocardial infarction, angina pectoris, percutaneous revascularisation or bypass grafting for ischaemic heart disease, stroke or transient ischaemic attack, and was assessed according to standardised medical records. Anti-apoA-1 IgG and biological markers were measured by ELISA and conventional automated techniques, respectively. Prevalence of high anti-apoA-1 IgG levels in the general population was 19.9 %. Presence of anti-apoA-1 IgG was significantly associated with CVD [odds ratio 1.34, 95 % confidence interval (1.05–1.70), p=0.018], independently of established CV risk factors (CVRFs) including age, sex, hypertension, smoking, diabetes, low and high-density lipoprotein cholesterol levels. The n=455 (6.8 %) study participants with a history of CVD (secondary prevention subgroup) presented higher median anti-ApoA-1 IgG values compared with subjects without CVD (p=0.029). Among patients in the secondary prevention subgroup, those with positive anti-apoA-1 IgG levels had lower HDL (p=0.002) and magnesium (p=0.001) levels, but increased uric acid and high-sensitivity C-reactive protein levels (p=0.022, and p<0.001, respectively) compared to patients with negative anti-apoA-1 IgG levels. In conclusion, anti-apoA-1 IgG levels are independently associated with CVD in the general population and also related to CV biomarkers in secondary prevention. These findings indicate that anti-apoA-1 IgG may represent a novel CVRF and need further study in prospective cohorts.

Keywords

Anti-apolipoprotein A-1 antibodies, cardiovascular disease, high-density lipoprotein cholesterol, biomarker, population-based

Introduction

Despite considerable advances having been made in its prevention, diagnosis and treatment, cardiovascular disease (CVD) remains the leading cause of death in the western world. Major discoveries in the pathophysiology of CVD over the last 30 years have shifted the long-standing paradigm that held CVD primarily as a lipid-related metabolism disorder, to the current view of an immune-mediated inflammatory disease, where humoral autoimmunity may play an important role (1, 2).

Different lines of evidence indicate that autoantibodies may represent a novel, independent cardiovascular risk factor (CVRF), (3–5) not only in their potential role as biomarkers for risk stratification but also as mediators of CVD, amenable to targeted therapeutic strategies (6).

Among autoantibodies possibly related to CVD, those directed against apolipoprotein A-1 (anti-apoA-1 IgG), the major protein fraction of high-density lipoproteins (HDL), are of particular interest. During the last decade, numerous translational studies have examined the mechanisms underpinning the role of anti-ApoA-1
IgG in inflammation (7, 8) and atherogenesis (9–12). In addition, preliminary clinical studies have shown encouraging results regarding the association and prognostic value of anti-apoA-1 IgG for CVD in subjects with autoimmune diseases (9–11, 13, 14), subjects at high CV risk (15–17), or in secondary prevention (18–23).

Nevertheless, the prevalence of anti-apoA-1 IgG and their association with CVD or markers of CV risk in the general population have not yet been examined. Therefore, the purpose of our current study was manifold: first, to investigate the prevalence and association of anti-ApoA-1 IgG with CVD in the general population. The second objective was to study the possible association of anti-ApoA-1 IgG with both established and emergent CV markers, in the general and secondary prevention populations. The third objective was to investigate the possible connection between anti-apoA-1 IgG and serum magnesium concentrations, as anti-ApoA-1 IgG have shown to be associated with a higher basal heart rate after myocardial infarction (MI) (23) through activation of L-type calcium channels (24, 25), tightly regulated by intracellular magnesium concentrations (26).

**Materials and methods**

**Study population and design**

We obtained cross-sectional clinical and biological data from the CoLaus study, a population-based observational study investigating cardiovascular disease (CVD) and risk factors in a random sample of 6733 subjects, aged between 35 and 75 and living in the city of Lausanne, Switzerland. Recruitment began in June 2003 and ended in May 2006. All participants of the CoLaus study were eligible for participation in the current study and were included in the analysis. The study was approved by the Institutional Ethics Committee of the University of Lausanne and informed consent was obtained from all participants. A detailed description of the study design and sampling procedures has been reported elsewhere (27).

All participants attended the outpatient clinic of the University Hospital of Lausanne in the morning, after an overnight fast. Data were collected from each participant by trained field interviewers in a single visit lasting about 60 minutes (min).

Blood pressure and heart rate were measured three consecutive times using an automated sphygmomanometer (Omron® HEM-907, Matsusaka, Japan) and the average of the last two measurements was used. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg and/or presence of anti-hypertensive treatment. Diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/l and/or oral or insulin anti-diabetic treatment. Body weight was measured in kilograms to the nearest 0.1 kg using a Seca® scale (Hamburg, Germany) and height was measured to the nearest 5 cm using a Seca® height gauge (Hamburg, Germany). Body mass index (BMI) was defined as weight/height². Metabolic syndrome was identified according to the NCEP-ATP III criteria (28) in subjects presenting with at least three of the following components: 1) SBP>130 mm Hg or DBP>85 mm Hg or treatment; 2) waist size>88 cm if female or >102 cm if male; 3) HDL-C<1.30 mmol/l for women or <1.03 mmol/l for men; 4) TG>1.7 mmol/l or lipid-lowering drug treatment; and 5) glucose>5.6 mmol/l or antidiabetic drug treatment. Aggregate CV risk was assessed using the Systematic COronary Risk Evaluation (SCORE) algorithm. Autoimmune disease was defined as history of rheumatoid arthritis or systemic lupus erythematosus, independently of treatment status.

Prevalent CVD was defined by the presence of myocardial infarction (MI); angina pectoris; percutaneous revascularisation or bypass grafting for ischaemic heart disease; stroke or transient ischaemic attack and assessed according to standardised medical records (27).

A venous blood sample (50 ml) and a spot urine sample were collected from each participant under fasting conditions. The analytical procedures and clinical assays used for determining serum and urine biological markers are available in Suppl. Table 1 (available online at www.thrombosis-online.com). The urinary fractional excretion of magnesium was calculated according to the following formula (29):

\[
FeMg = \frac{UMg \times Scr}{0.7 \times SMg \times UCr},
\]

where \(UMg\) = urinary magnesium (mmol/l), \(Scr\) = serum creatinine (µmol/l), \(SMg\) = serum magnesium (mmol/l) and \(UCr\) = urinary creatinine (µmol/l). Glomerular filtration rate (GFR) was estimated by the simplified Modification of Diet in Renal Disease (MDRD) prediction equation:

\[
GFR (ml/min/1.73 m^2) = 186 \times (Scr)^{-1.154} \times (Age)^{0.203} \times (0.742 \text{ if female}),
\]

where \(Scr\) = plasma creatinine concentration in mg/dl, and \(age\) = years.

**Assessment of autoantibodies against apolipoproteinA-1**

Anti-apoA-1 IgG were measured as previously described (20, 21), using the CoLaus study (2003–2006) serum aliquots that had been previously frozen and stored at −80°C. Maxisorp plates (Nunc™, Roskilde, Denmark) were coated with purified, human-derived delipidated apolipoprotein A-1 (20 µg/ml; 50 µl/well) for 1 hour (h) at 37°C. After being washed, all wells were blocked for 1 h with 2% bovine serum albumin (BSA) in a phosphate buffer solution (PBS) at 37°C. Patient samples were also added to a non-coated well to assess individual non-specific binding. After six washing cycles, a 50 µl/well of signal antibody (alkaline phosphatase-conjugated anti-human IgG; Sigma-Aldrich, St Louis, MO, USA), diluted 1:1000 in a PBS/BSA 2% solution, was added and incubated for 1 h at 37°C. After washing six more times, phosphatase substrate p-nitrophenylphosphate disodium (Sigma-Aldrich) dissolved in a diethanolamine buffer (pH 9.8) was added and incubated for 20 min at 37°C (Molecular Devices™ Versa Max). Optical density (OD) was determined at 405 nm, and each sample was tested in duplicate. Corresponding non-specific binding was subtracted from mean OD for each sample. The specificity of
Elevated levels of anti-apoA-1 IgG were set at an OD cut-off of OD 0.64, corresponding to the 97.5th percentile of a reference population of 140 healthy blood donors. In order to limit the impact of inter-assay variation, we calculated an index consisting in the ratio between sample net absorbance and the positive control net absorbance × 100. The index value corresponding to the 97.5th percentile of the normal distribution was 37. Accordingly, to be considered as positive (presenting elevated anti-apoA-1 IgG levels), samples had to display both an absorbance value >0.64 OD and an index value ≥37.

Sample size and power calculation

Based on previous published studies on healthy blood donors (21) we assumed an expected prevalence of anti-apoA-1 IgG in healthy subjects devoid of CVD of 5–10%. Taking into account the CVD rate in CoLaus (455 events or 6.8%) and a two-sided alpha of 5%, our study had 80% power to detect an odds ratio (OR) of anti-apoA-1 IgG for CVD at OR 1.51.

Statistical analysis

Univariate analysis of continuous variables was performed using the Student’s t-test or the non-parametric Mann-Whitney test to account for non-parametric distributions, and results were expressed as mean ± standard deviation (SD) or as median (interquartile range), as appropriate. Analysis of discrete variables was performed using Chi-square test and results were expressed as number of participants and (percentage).

Multivariate analysis was performed using logistic regression adjusting for age, sex, hypertension, diabetes, smoking, low-density (LDL) and high-density lipoprotein cholesterol (HDL). Results were expressed as OR and 95% confidence interval (CI). All analyses were performed using STATA 13.0 (Stata Corp, College Station, TX, USA).

All conducted analyses were predefined at the moment of study conception and disclosed when applying for funding. Contrary to confirmatory studies where correcting for multiple testing is required, for exploratory analyses correction for multiple testing increases the type II error for non-null associations (30), leading to reduced statistical power and precluding the identification of potentially interesting associations (30–32). Thus, for testing pre-planned associations of anti-apoA-1 IgG with specific and independent CV features in the current study, a two-tailed p < 0.05 was considered statistically significant.

Results

Association between anti-apoA-1 IgG and CVD, independently of established CVRF

The flowchart and objectives of the study are summarised in Figure 1. N = 6194 (93.2%) study participants were devoid of baseline CVD (primary prevention subgroup), while n = 455 (6.8%) were in secondary prevention. The distribution of raw optical density (OD) values for anti-apoA-1 IgG in the sample is illustrated in Suppl. Figure 1 (available online at www.thrombosis-online.com).

As described in Table 1, elevated levels of anti-apoA-1 IgG were present in 1323 out of 6649 (19.9%) study subjects. In the general population, the prevalence of clinical CVRF did not differ between subjects with presence vs absence of anti-apoA-1 IgG. The same observation was true both in the primary and the secondary prevention subgroups. Furthermore, we did not find any significant difference in CV drugs rates depending on presence vs absence of anti-apoA-1 IgG. Among study subjects, n = 154 had a history of autoimmune disease (either rheumatoid arthritis or systemic lupus erythematosus). We observed no association between history of autoimmune disease and elevated levels of anti-apoA-1 IgG in the population (Table 1).

Prevalence of CVD in the general population was significantly associated with presence vs absence of anti-ApoA-1 IgG (8.3% vs 6.5%, respectively, p = 0.018). Indeed, patients with history of CVD had higher median OD values for anti-ApoA-1 IgG than subjects without (OD: 0.412, interquartile range (IQR): 0.273–0.661 vs 0.395, IQR: 0.253–0.589, p = 0.029).

Translated into OR, elevated levels of anti-ApoA-1 IgG were associated with a 1.3-fold increased risk for prevalent CVD; an association which remained unchanged after adjustment for established CVRFs, including age, sex, hypertension, diabetes, smoking, LDL and HDL cholesterol. Alternatively, there was a 15% risk
Table 1: Clinical characteristics of the sample according to anti-apolipoprotein A-1 IgG status: A) general population, B) primary prevention and C) secondary prevention subgroups. Continuous data are expressed as mean ± standard deviation or median (interquartile range) according to the variable distribution. Categorical data are expressed as number of participants and (percentage). Statistical analysis by chi-square for categorical variables and Mann-Whitney U test for continuous variables. Anti-apolipoprotein A-1 IgG, Autoantibodies against Apolipoprotein A-1; BP; blood pressure; CVD, cardiovascular disease; SCORE, Systematic Coronary Risk Evaluation. The primary and secondary prevention subgroups are issued from the general study population.

<table>
<thead>
<tr>
<th>Anti-apolipoprotein A-1 IgG</th>
<th>General population (n=6649)</th>
<th>Primary prevention subgroup (n=6194)</th>
<th>Secondary prevention subgroup (n=455)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>P-value</td>
</tr>
<tr>
<td>Sample size, n (%)</td>
<td>5326 (80.1)</td>
<td>1323 (19.9)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>52.7 ± 10.8</td>
<td>52.3 ± 10.7</td>
<td>0.201</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>2527 (47.5)</td>
<td>626 (47.3)</td>
<td>0.933</td>
</tr>
<tr>
<td>CVD, n (%)</td>
<td>345 (6.5)</td>
<td>110 (8.3)</td>
<td>0.018</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>1851 (34.8)</td>
<td>456 (34.5)</td>
<td>0.844</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>351 (6.6)</td>
<td>81 (6.1)</td>
<td>0.537</td>
</tr>
<tr>
<td>Metabolic syndrome, n (%)</td>
<td>1196 (22.5)</td>
<td>309 (23.4)</td>
<td>0.484</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>1422 (26.7)</td>
<td>358 (27.1)</td>
<td>0.791</td>
</tr>
<tr>
<td>Autoimmune disease, n (%)</td>
<td>116 (2.2)</td>
<td>38 (2.9)</td>
<td>0.133</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68.0 ± 9.7</td>
<td>68.1 ± 10.3</td>
<td>0.656</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>128 ± 18</td>
<td>128 ± 18</td>
<td>0.471</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.8 ± 4.5</td>
<td>25.8 ± 4.7</td>
<td>0.853</td>
</tr>
<tr>
<td>CV risk (SCORE)</td>
<td>0.6 (0.2–2.2)</td>
<td>0.6 (0.2–2.1)</td>
<td>0.165</td>
</tr>
<tr>
<td>CV drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>855 (16.0)</td>
<td>191 (14.4)</td>
<td>0.148</td>
</tr>
<tr>
<td>Statins</td>
<td>581 (10.9)</td>
<td>123 (9.30)</td>
<td>0.088</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>284 (5.3)</td>
<td>81 (6.1)</td>
<td>0.259</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>161 (3.0)</td>
<td>43 (3.2)</td>
<td>0.668</td>
</tr>
<tr>
<td>IEC/ARB</td>
<td>408 (7.7)</td>
<td>88 (6.7)</td>
<td>0.211</td>
</tr>
<tr>
<td>Diuretics</td>
<td>120 (2.2)</td>
<td>22 (1.7)</td>
<td>0.184</td>
</tr>
</tbody>
</table>

Table 2: Odds ratio of anti-apolipoprotein A-1 IgG for cardiovascular disease in unadjusted and adjusted models. Results are expressed as odds ratio (95% confidence interval). Statistical analysis was performed by multivariate logistic regression. The adjusted model was adjusted for age, sex, hypertension, diabetes, smoking, HDL cholesterol and LDL cholesterol. Anti-apolipoprotein A-1 IgG, autoantibodies against apolipoprotein A-1; OD, optical density. * Subjects positive for anti-apolipoprotein A-1 (OD≥0.64, n=1323) were divided into three groups of equal size and increasing anti-apolipoprotein A-1 titres: 1st group (0.64≤OD<0.77), 2nd group (0.77≤OD<0.98) and 3rd group (OD≥0.98).

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted model</th>
<th>P-value</th>
<th>Adjusted model</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive anti-ApoA-1 IgG (OD≥0.64) vs negative (OD&lt;0.64)</td>
<td>1.31 (1.05–1.64)</td>
<td>0.018</td>
<td>1.34 (1.05–1.70)</td>
<td>0.018</td>
</tr>
<tr>
<td>1SD change in OD levels</td>
<td>1.15 (1.05–1.25)</td>
<td>0.003</td>
<td>1.18 (1.07–1.30)</td>
<td>0.001</td>
</tr>
<tr>
<td>Anti-Apo-1 IgG levels *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative for anti-ApoA-1 IgG (OD&lt;0.64)</td>
<td>1 (ref.)</td>
<td></td>
<td>1 (ref.)</td>
<td></td>
</tr>
<tr>
<td>Positive for anti-ApoA-1 IgG (OD≥0.64)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st group (0.64≤OD&lt;0.77)</td>
<td>1.02 (0.69–1.50)</td>
<td>0.936</td>
<td>1.15 (0.76–1.74)</td>
<td>0.507</td>
</tr>
<tr>
<td>2nd group (0.77≤OD&lt;0.98)</td>
<td>1.24 (0.87–1.79)</td>
<td>0.236</td>
<td>1.14 (0.76–1.70)</td>
<td>0.529</td>
</tr>
<tr>
<td>3rd group (OD≥0.98)</td>
<td>1.68 (1.22–2.33)</td>
<td>0.002</td>
<td>1.71 (1.21–2.42)</td>
<td>0.002</td>
</tr>
<tr>
<td>P-value for linear trend</td>
<td>0.002</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
increase for prevalent CVD per standard deviation increase in anti-apoA-1 IgG values, which was also independent of established CVRFs (Table 2). Finally, the association between presence of anti-apoA-1 IgG and CVD in the multivariate model remained unchanged after further adjusting for history of autoimmune disease as well as after excluding subjects with history of autoimmune disease (n=154) from the analysis.

Association between anti-apoA-1 IgG levels and biological markers of CV risk

Table 3 summarises variations in biological markers of CV risk, according to presence or absence of anti-apoA-1 IgG.

Subjects with elevated anti-ApoA-1 IgG levels tended to have lower serum levels of total cholesterol, HDL and magnesium than patients with low anti-apoA-1 IgG levels. No trends or significant differences were observed for other lipid parameters, renal function, hs-CRP or uric acid between these two groups. Inverse and significant Spearman correlations were retrieved for anti-apoA-1 IgG and total cholesterol as well as anti-apoA-1 IgG and magnesium levels both in the general population (r=-0.05, p<0.001; r=-0.06, p<0.001, respectively) and in the primary prevention subgroup (r=-0.05, p<0.001; r=-0.05, p<0.001, respectively).

In the secondary prevention subgroup, patients with elevated anti-apoA-1 IgG levels presented significantly lower HDL and magnesium values, but higher hs-CRP and uric acid values than patients with low anti-apoA-1 IgG levels (Table 3). Additionally, inverse significant Spearman correlations were observed between anti-apoA-1 IgG and HDL (r=-0.10, p=0.03) or anti-apoA-1 IgG and magnesium levels (r=-0.19; p<0.001), while significant positive correlations were found between anti-apoA-1 IgG and hs-CRP levels (r=0.11, p=0.02). The correlation for anti-apoA-1 IgG and uric acid levels was also positive (r=0.09; p=0.05). No other significant correlations were observed between anti-apoA-1 IgG levels and biological parameters (data not shown). Notably, the negative association between anti-apoA-1 IgG and HDL levels remained robust after adjusting for statin treatment. Furthermore, no overall difference in the fractional excretion of magnesium in urine was found between subjects with presence vs absence of anti-apoA-1 IgG, either in the general population or in the primary or secondary prevention subgroups (data not shown).

Table 3: Biological characteristics of the sample according to anti-apoA-1 IgG status: A) general population, B) primary prevention and C) secondary prevention subgroups. Data are expressed as mean ± standard deviation or median (interquartile range) according to the variable distribution. Statistical analysis was performed by Mann-Whitney test. Anti-apoA-1 IgG, autoantibodies against apolipoprotein A-1; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate (MDRD equation); CVD, cardiovascular disease; hs-CRP, high-sensitivity C-reactive protein. The primary and secondary prevention subgroups are issued from the general study population.

<table>
<thead>
<tr>
<th>Anti-apoA-1 IgG</th>
<th>General population (n=6649)</th>
<th>Primary prevention subgroup (n=6194)</th>
<th>Secondary prevention subgroup (n=455)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size, n (%)</td>
<td>5326 (80.1)</td>
<td>4981 (80.4)</td>
<td>345 (75.8)</td>
</tr>
<tr>
<td>Lipids (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.59 ± 1.04</td>
<td>5.60 ± 1.03</td>
<td>5.32 ± 1.04</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.34 ± 0.92</td>
<td>3.35 ± 0.91</td>
<td>3.13 ± 0.92</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.63 ± 0.43</td>
<td>1.64 ± 0.43</td>
<td>1.63 ± 0.46</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.10 (0.8–1.6)</td>
<td>1.1 (0.8–1.6)</td>
<td>1.1 (0.9–1.8)</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>77.8 (69–88)</td>
<td>77.9 (69–88)</td>
<td>76.2 (66–86)</td>
</tr>
<tr>
<td>Serum ions (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.85 ± 0.07</td>
<td>0.84 ± 0.07</td>
<td>0.84 ± 0.07</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.29 ± 0.09</td>
<td>2.28 ± 0.09</td>
<td>2.31 ± 0.09</td>
</tr>
<tr>
<td>Surrogate markers of CVD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid, µmol/l</td>
<td>0.588</td>
<td>1.1 (0.6–2.7)</td>
<td>1.6 (0.7–3.0)</td>
</tr>
<tr>
<td>Homocysteine, µmol/l</td>
<td>9.5 (7.9–11.7)</td>
<td>9.4 (7.7–11.4)</td>
<td>11.3 (9.0–14.4)</td>
</tr>
<tr>
<td>hs-CRP, mg/l</td>
<td>1.3 (0.6–2.7)</td>
<td>1.1 (0.6–2.5)</td>
<td>2.1 (1.2–5.0)</td>
</tr>
</tbody>
</table>

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In a subgroup analysis performed specifically on coronary heart disease (CHD) patients (n=235), subjects with elevated anti-apoA-1 IgG levels were more likely to present with metabolic syndrome and a higher basal heart rate when compared to patients with low anti-apoA-1 IgG levels (Suppl. Table 2, available online at www.thrombosis-online.com). Elevated levels of anti-apoA-1 IgG were also associated with significantly lower HDL and magnesium levels (p=0.015, p=0.004, respectively), and increased triglyceride (p=0.01) and hs-CRP levels (p=0.005) (Suppl. Table 3, available online at www.thrombosis-online.com).

Discussion

The main finding of the present large-scale study is the novel association between anti-apoA-1 IgG and CVD in a population-based sample, which is independent of established CVRF. Our results validate initial reports, which suggested that presence of these autoantibodies could be associated with prevalent CVD (14, 18), as well as CV complications in rheumatoid arthritis patients (13) and in high CV risk populations (19, 22, 23).

Furthermore, in this study we confirm that the presence of elevated anti-apoA-1 IgG levels in secondary prevention patients is associated with a pro-inflammatory systemic profile (7–9, 13, 14, 22), as reflected by lower HDL concentrations, but higher hs-CRP and uric acid levels. While different studies previously reported anti-apoA-1 IgG presence being associated with a loss of anti-atherogenic properties of HDL due to decreased paraoxonase (PON) activity, in specific settings (9–12), this study is the first to indicate that elevated anti-apoA-1 IgG levels are inversely associated with HDL levels in subjects with established CVD, independently of statin treatment.

Because anti-apoA-1 IgG target the major protein component of HDL, the association between anti-apoA-1 IgG and lower HDL levels may be related to the clearance of immune anti-apoA-1 IgG and HDL complexes by the reticular-endothelial system. In accordance with this hypothesis, human case reports (33) described that patients with high anti-apoA-1 IgG levels entirely lacked mature HDL particles, suggesting decreased ability of pre-beta HDL to become lipidated, although additional mechanisms could also account for this finding. Along the same line, in a murine model of SLE, anti-apoA-1 IgG could lower HDL levels without affecting hepatic HDL biosynthesis, possibly by accelerating HDL clearance (12). On the other hand, anti-apoA-1 IgG passive immunisation in mice has not shown to impact HDL levels, despite otherwise marked effects on atherogenesis, myocardial necrosis and survival (8, 20), leaving the question open as to whether a causal link between apoA-1 IgG and HDL levels does exist. Lastly, the presence of these antibodies may also contribute to loss of HDL function by impairing ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux or interfering with PON activity, although this assumption requires further experimental validation (9–12).

Association between anti-apoA-1 IgG levels and biological markers of CV risk

The fact that patients with high anti-apoA-1 IgG levels had higher hs-CRP levels than patients without anti-apoA-1 IgG is in line with previous studies showing that elevated anti-apoA-1 IgG levels were associated with higher circulating levels of pro-inflammatory cytokines, including IL-6, TNF- alpha, myeloperoxidase, hs-CRP and matrix-metalloproteinase 9 (7, 13, 17, 20, 22). Because endotxin-free anti-apoA-1 IgG was shown to induce a dose-dependent production of IL-6 and other inflammatory cytokines by human macrophages in a TLR2/CD14 dependent manner (7), a straightforward explanation for this association could be that the anti-apoA-1 IgG-driven production of IL-6 by macrophages directly induces CRP production by hepatocytes, a phenomenon highly dependent on IL-6 stimulus (34, 35).

Another compelling finding of our study was that patients with high anti-apoA-1 IgG levels had significantly lower serum magnesium concentrations compared to patients with low anti-apoA-1 IgG levels. Previous studies showed anti-apoA-1 IgG to elicit in vitro a sustained positive chronotropic response on cardiomyocytes through the activation of L-type calcium channels (24, 25), the activity of which is enhanced by intracellular magnesium deficiency (26). Since magnesium depletion predisposes for cardiac arrhythmias and CHD (36), this descriptive association could provide a model to support our previous observation that showed elevated anti-apoA-1 IgG levels after MI to be associated with a higher basal heart rate, as assessed by Holter monitoring (23). Interestingly, in the present study – apart from the difference in circulating concentrations of magnesium not previously assessed (23) – we found the same association between elevated anti-apoA-1 IgG levels and basal heart rate in the subgroup of CHD patients. It is therefore plausible that the higher basal heart rate observed in CHD patients with elevated anti-apoA-1 IgG levels could be mediated by concomitant lower magnesium concentrations, or that these patients have increased chronotropic susceptibility to relative hypomagnesaemia. Since the fractional excretion of magnesium in the urine did not differ between subjects with high vs. low anti-apoA-1 IgG levels, one can reasonably exclude urinary magnesium wasting as the cause of this finding.

Prevalence of anti-apoA-1 IgG in the general population

Our study raises an important question with regards to the prevalence of elevated anti-apoA-1 IgG levels in the general population. Indeed, while we were able to demonstrate that the prevalence of elevated anti-apoA-1 IgG levels was significantly higher in patients with CVD than in those without (using the same ELISA protocol and the same definition of elevated anti-apoA-1 IgG levels as in our previous studies), the overall prevalence of elevated anti-apoA-1 IgG levels in the CoLaus study approached 20%, whereas previously reported values varied between 0 to 6.5% for healthy blood donors or matched controls (14, 17, 21) and between 11–29% for subjects in secondary prevention (Suppl. Table 4, available online at www.thrombosis-online.com).
available online at www.thrombosis-online.com) (19, 22, 23). The reasons for such a discrepancy are not fully understood, although one could argue that due to stringent selection criteria, healthy blood donors may not be representative of the general population (37). In addition, sample size may play a role since this is the first large-scale study of anti-apoA-1 IgG in the community. Furthermore, as only baseline anti-apoA-1 IgG levels were measured, we could not assess potential variations of anti-ApoA-1 IgG levels over time (38) and especially after cardiovascular events, a point that will need to be determined in future studies.

Strengths and limitations

The strength of our study lies in its unbiased, community-based approach that enabled us to confirm that elevated anti-apoA-1 IgG are independently associated with CVD in a large and extensively characterised sample of the general population.

There are several limitations to this study. First, owing to the observational and cross-sectional nature of the data, a causal relationship between anti-ApoA-1 IgG and CVD cannot be firmly established. This hypothesis is currently being tested in an ongoing longitudinal study aiming at exploring the prognostic value of anti-ApoA-1 IgG in the general population.

Secondly, apart from anti-ApoA-1 IgG titres, we did not have data on other clinically relevant autoantibodies, such as oxidised LDL, anti-phospholipid or anti-nuclear antibodies. Previous work demonstrated that presence of anti-apoA-1 IgG was independent of the existence of other autoantibodies (such as anti-oxLDL, anti-phospholipid, anti-nuclear, anti-heat shock protein antibodies) and provided the strongest prognostic accuracy for CVD, especially after MI (23, 39). However, further studies are required to challenge these preliminary results.

What is known about this topic?

- Numerous translational studies have established the role of autoantibodies against apolipoprotein A-1 in inflammation and atherogenesis.
- Small-scale clinical studies have shown promising results regarding the association and prognostic value of autoantibodies against apolipoprotein A-1 for cardiovascular disease in subjects with autoimmune diseases, subjects at high CV risk or following myocardial infarction.

What does this paper add?

- This is the first study to demonstrate that autoantibodies against apolipoprotein A-1 are significantly associated with prevalent cardiovascular disease in the general population, independently of established cardiovascular risk factors.
- Our results suggest that autoantibodies against apolipoprotein A-1 may prove useful as a novel biomarker as well as a potential target for specific immune modulation strategies for cardiovascular disease.

Moreover, we did not measure apoA-1 levels nor did we assess the possible qualitative changes in HDL, such as PON1 activity, reverse cholesterol efflux capacity or HDL anti-inflammatory properties. As apoA-1 is largely responsible for reverse cholesterol transport and stabilisation of PON1 (40), it is plausible that anti-apoA-1 IgG could decrease apoA-1 levels, rendering the protein less able to promote cholesterol efflux and thereby manifest its anti-atherogenic effects. We assume that both apoA-1 levels and HDL functional properties would be altered in anti-apoA-1 IgG positive patients, as reported earlier (11); however, we did not collect data to challenge this hypothesis.

Lastly, it remains elusive as to whether elevated anti-apoA-1 IgG levels are associated with susceptibility to infections. Previous studies reported that polyclonal human anti-apoA-1 IgG response is focused against epitopes present on the C-terminal part of apoA-1 (41, 42). Since the latter shares structural homologies with TLR2 (7), it is suggested that pathogen molecular mimicry may be the underlying mechanism for the deleterious properties of these autoantibodies. The design of this study did not allow for the exploration of association between the existence of anti-apoA-1 IgG and previous exposure to specific pathogens.

Conclusion

The present study confirms that elevated anti-apoA-1 IgG levels are independently associated with CVD in the general population, while also being associated with CV biomarkers in patients with a history of CVD. Prospective studies are needed to evaluate the prognostic value of anti-apoA-1 IgG as a biomarker for CVD in the general population as well as to investigate the possible therapeutic value of developing immune therapies directed against anti-apoA-1 IgG.

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Conflicts of interest

None declared.

References

Antiochos et al. Anti-apolipoprotein A-1 antibodies and CVD


