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Authors: Antiochos P, Marques-Vidal P, Virzi J, Pagano S, Satta N, Bastardot F, Hartley O, Montecucco F, Mach F, Waeber G, Vollenweider P, Vuilleumier N

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Panagiotis Antiochos [1], Pedro Marques-Vidal [1], Julien Virzi [2, 3], Sabrina Pagano [2, 3], Nathalie Satta [2, 3], François Bastardot [1], Oliver Hartley [4], Fabrizio Montecucco [2, 5], François Mach [5], Gerard Waeber [1], Peter Vollenweider [1]*, Nicolas Vuilleumier [2, 3]*

* These authors contributed equally

Authors' affiliations:

[1] Department of Internal Medicine, University Hospital of Lausanne, 46 rue du Bugnon, 1011 Lausanne, Switzerland

[2] Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 4 rue Gabrielle-Perret-Gentil 1205 Geneva, Switzerland

[3] Department of Human Protein Sciences, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, 1206 Geneva, Switzerland

[4] Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, 1206 Geneva, Switzerland

[5] Division of Cardiology, Foundation for Medical Researches, Department of Medical Specialties, University of Geneva, 64 avenue de la Roseraie, 1211 Geneva, Switzerland

CORRESPONDING AUTHOR:

Dr. Panagiotis Antiochos

CoLaus Study, Bâtiment des Instituts

19, Rue du Bugnon

CH-1005 Lausanne

Switzerland

Tel.: +41 79 556 03 11

Fax: +41 21 314 80 37

Email: panagiotis.antiochos@chuv.ch

ABSTRACT

Objective: 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.

Methods: 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 revascularization or bypass grafting for ischemic heart disease stroke or transient ischemic attack, and was assessed according to standardized medical records. Anti-apoA-1 IgG and biological markers were measured by ELISA and conventional automated techniques, respectively.

Results: Prevalence of high anti-apoA-1 IgG 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.

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 connexion 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 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.

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 antidiabetic 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 mm using a Seca® height gauge (Hamburg, Germany). Body mass index (BMI) was defined as weight/height^2. Metabolic syndrome was identified according to the NCEP-ATP III criteria (28) in subjects presenting with at least 3 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; angina pectoris; percutaneous revascularization or bypass grafting for ischemic heart disease; stroke or transient ischemic attack and assessed according to standardized 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 **Supplemental Table I.** The urinary fractional excretion of magnesium was calculated according to the following formula (29):

$$FeMg = (UMg \times SCr) / (0.7 \times SMg \times UCr),$$

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^{TM}, Denmark) were coated with purified, human-derived delipidated apolipoprotein A-1 (20 µg/ml; 50 µl/well) for 1h at 37°C. After being washed, all wells were blocked for 1h 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), diluted 1:1000 in a PBS/BSA 2% solution, was added and incubated for 1h at 37°C. After washing six more times, phosphatase substrate p-nitrophanylphosphate disodium (Sigma-Aldrich) dissolved in a diethanolamine buffer (pH 9.8) was added and incubated for 20 min at 37°C (Molecular Devices^{TM} 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 detection was assessed using conventional saturation tests by Western blot analysis. (20, 21)

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 x 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, Texas, USA). 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 summarized 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 **Supplemental Figure I**.

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 odds ratio, 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 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 summarizes 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).

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 **(Supplemental Table II)**. 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) **(Supplemental Table III)**.

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 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, and remain so 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 (30) reported that patients with high anti-apoA-1 IgG levels entirely lacked mature HDL particles, suggesting decreased ability of pre-beta HDL to become lipidated. 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 immunization 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 paraoxonase 1 (PON1) 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 endotoxin-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. (31, 32)

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, (33) this descriptive association could provide a model to support our previous observation that showed elevated anti-apoA-1 IgG levels after myocardial infarction 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 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.

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 characterized sample of the general population.

There are several limitations to this study. First, owing to the 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, 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 stabilization of PON1, (34) 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.

Thirdly, 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. (35, 36) 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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LEGENDS TO FIGURES AND TABLES

Figure 1: Study flowchart and endpoints.

Anti-apoA-1 IgG, Autoantibodies against Apolipoprotein A-1; CVD, Cardiovascular Disease; CV, Cardiovascular

Table 1: 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-apoA-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 2: 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-apoA-1 IgG, Autoantibodies against Apolipoprotein A-1; OD, optical density

* Subjects with positive anti-ApoA-1 (n=1323) were divided in tertiles of increasing titers: 1st tertile ($0.64 \le OD < 0.77$), 2nd tertile ($0.77 \le OD < 0.98$) and 3rd tertile ($OD > 0.98$).

Table 3: 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, Cardio Vascular Disease; hs-CRP, high-sensitivity C-Reactive Protein. The primary and secondary prevention subgroups are issued from the general study population.

Table 1: Clinical characteristics of the sample according to anti-apoA-1 IgG status: a) general population, b) primary prevention and c) secondary prevention subgroups.

	General population (n=6649)			Primary prevention subgroup (n=6194)			Secondary prevention subgroup (n=455)		
Anti-apoA-1 IgG	**Negative**	**Positive**	**p-value**	**Negative**	**Positive**	**p-value**	**Negative**	**Positive**	**p-value**
Sample size, n (%)	5326 (80.1)	1323 (19.9)		4981 (80.4)	1213 (19.6)		345 (75.8)	110 (24.2)	
Age, years	52.7 ± 10.8	52.3 ± 10.7	0.201	52.1 ± 10.6	51.6 ± 10.6	0.130	61.6 ± 9.2	60.0 ± 9.4	0.099
Male sex, n (%)	2527 (47.5)	626 (47.3)	0.933	2336 (46.9)	556 (45.8)	0.506	191 (55.4)	70 (63.6)	0.127
CVD, n (%)	345 (6.5)	110 (8.3)	0.018	.0 (0.0)	0 (0.0).	.	345 (100.0)	110 (0.0)	.
Hypertension, n (%)	1851 (34.8)	456 (34.5)	0.844	1619 (32.5)	382 (31.5)	0.499	232 (67.3)	74 (67.3)	0.996
Diabetes, n (%)	351 (6.6)	81 (6.1)	0.537	294 (5.9)	61 (5.0)	0.240	57 (16.5)	20 (18.2)	0.686
Metabolic syndrome, n (%)	1196 (22.5)	309 (23.4)	0.484	956 (19.6)	257 (19.5)	0.907	58 (21.5)	52 (28.1)	0.105
Current smoking, n (%)	1422 (26.7)	358 (27.1)	0.791	1347 (27.0)	326 (26.9)	0.906	75 (21.7)	32 (29.1)	0.113
Autoimmune disease	116 (2.2)	38 (2.9)	0.133	105 (2.1)	32 (2.6)	0.260	11 (3.2)	6 (5.5)	0.275
Heart rate, bpm	68.0 ± 9.7	68.1 ± 10.3	0.656	68.1 ± 9.6	68.2 ± 10.3	0.920	66.5 ± 10.6	68.0 ± 9.6	0.170
Systolic BP, mmHg	128 ± 18	128 ± 18	0.471	128 ± 18	127 ± 18	0.402	136 ± 120	136 ± 20	0.972

Body mass index, kg/m^2	25.8 ± 4.5	25.8 ± 4.7	0.853	25.7 ± 4.4	25.6 ± 4.6	0.813	27.6 ± 5.0	28.1 ± 5.2	0.396
CV risk (SCORE)	0.6 (0.2 – 2.2)	0.6 (0.2 – 2.1)	0.165	0.6 (0.2 – 1.9)	0.5 (0.2 – 1.8)	0.059	3.1 (0.9 – 6.6)	2.8 (0.9 – 5.8)	0.546
CV drugs									
Aspirine	855 (16.0)	191 (14.4)	0.148	677 (13.6)	140 (11.5)	0.058	178 (51.6)	51 (46.4)	0.339
Statins	581 (10.9)	123 (9.30)	0.088	432 (8.7)	81 (6.7)	0.024	149 (43.2)	42 (38.2)	0.354
Beta blockers	284 (5.3)	81 (6.1)	0.259	198 (4.0)	48 (4.0)	0.977	86 (24.9)	33 (30.0)	0.292
Calcium channel blockers	161 (3.0)	43 (3.2)	0.668	113 (2.3)	30 (2.5)	0.670	48 (13.9)	13 (11.8)	0.574
IEC/ARB	408 (7.7)	88 (6.7)	0.211	339 (6.8)	70 (5.8)	0.193	69 (20.0)	18 (16.4)	0.398
Diuretics	120 (2.2)	22 (1.7)	0.184	98 (2.0)	17 (1.4)	0.190	22 (6.4)	5 (4.6)	0.479

Table 2: Odds ratio of anti-apoA-1 IgG for cardiovascular disease in unadjusted and adjusted models.

	Unadjusted model OR (95% CI)	**p-value**	**Adjusted model OR (95% CI)**	**p-value**
Positive anti-ApoA-1 IgG (OD≥0.64) *vs* negative (OD<0.64)	1.31 (1.05 – 1.64)	0.018	1.34 (1.05 – 1.70)	0.018
1SD change in OD levels	1.15 (1.05 – 1.25)	0.003	1.18 (1.07 – 1.30)	0.001
Anti-ApoA-1 IgG levels *				
Negative (OD<0.64)	1 (ref.)		1 (ref.)	
1st tertile (0.64≤OD<0.77)	1.02 (0.69 – 1.50)	0.936	1.15 (0.76 – 1.74)	0.507
2nd tertile (0.77≤OD<0.98)	1.24 (0.87 – 1.79)	0.236	1.14 (0.76 – 1.70)	0.529
3rd tertile (OD≥0.98)	1.68 (1.22 – 2.33)	0.002	1.71 (1.21 – 2.42)	0.002
P-value for linear trend	0.002		0.008	

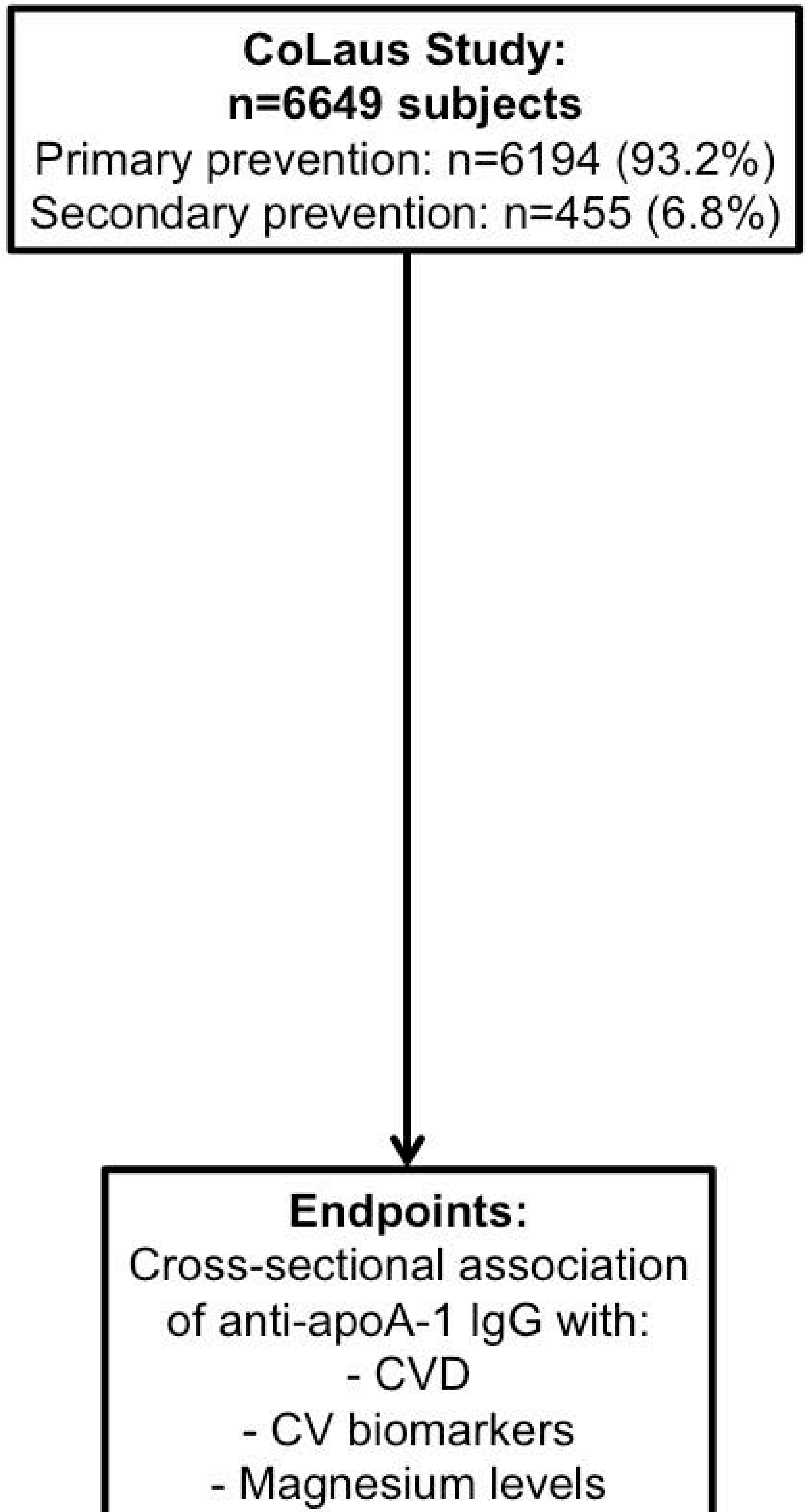
Table 3: Biological characteristics of the sample according to anti-apoA-1 IgG status: a) general population, b) primary prevention and c) secondary prevention subgroups.

	General population (n=6649)			Primary prevention subgroup (n=6194)			Secondary prevention subgroup (n=455)		
Anti-apoA-1 IgG	**Negative**	**Positive**	**p-value**	**Negative**	**Positive**	**p-value**	**Negative**	**Positive**	**p-value**
Sample size, n (%)	**5326 (80.1)**	**1323 (19.9)**		**4981 (80.4)**	**1213 (19.6)**		**345 (75.8)**	**110 (24.2)**	
Lipids (mmol/L)									
Total cholesterol	5.59 ± 1.04	5.53 ± 1.05	0.067	5.60 ± 1.03	5.55 ± 1.03	0.064	5.32 ± 1.04	5.35 ± 1.20	0.854
LDL cholesterol	3.34 ± 0.92	3.30 ± 0.92	0.168	3.35 ± 0.91	3.31 ± 0.91	0.117	3.13 ± 0.92	3.20 ± 1.01	0.449
HDL cholesterol	1.63 ± 0.43	1.62 ± 0.46	0.052	1.64 ± 0.43	1.63 ± 0.46	0.351	1.53 ± 0.42	1.40 ± 0.36	0.002
Triglycerides	1.10 (0.8–1.6)	1.10 (0.8–1.6)	0.082	1.1 (0.8–1.6)	1.1 (0.8–1.5)	0.150	1.3 (0.9–1.8)	1.4 (1 – 2.1)	0.083
Renal function									
eGFR, mL/min/1.73 m^2	77.8 (69–88)	78.3 (68–89)	0.312	77.9 (69–88)	78.4 (69–89)	0.287	76.2 (66–86)	75.7 (66–91)	0.877
Serum ions (mmol/L)									

Magnesium	0.85 ± 0.07	0.84 ± 0.07	<0.001	0.85 ± 0.07	0.84 ± 0.07	<0.001	0.84 ± 0.07	0.81 ± 0.07	0.001
Calcium	2.29 ± 0.09	2.28 ± 0.09	0.103	2.28 ± 0.09	2.28 ± 0.09	0.120	2.31 ± 0.09	2.30 ± 0.10	0.413
Surrogate markers of CVD									
Uric acid, µmol/L	306 (251–365)	302 (249–365)	0.689	304 (249–363)	299 (247–358)	0.173	335 (274–394)	351 (299–415)	0.022
Homocysteine, µmol/L	9.5 (7.9–11.7)	9.5 (7.8–11.6)	0.596	9.5 (7.9–11.5)	9.4 (7.7–11.4)	0.198	10.2 (8.6–13.0)	11.3 (9.0–14.4)	0.078
hs-CRP, mg/L	1.3 (0.6–2.7)	1.2 (0.6–2.8)	0.588	1.3 (0.6–2.7)	1.1 (0.6–2.5)	0.082	1.6 (0.7–3.0)	2.1 (1.2–5.0)	<0.001

Extra Table

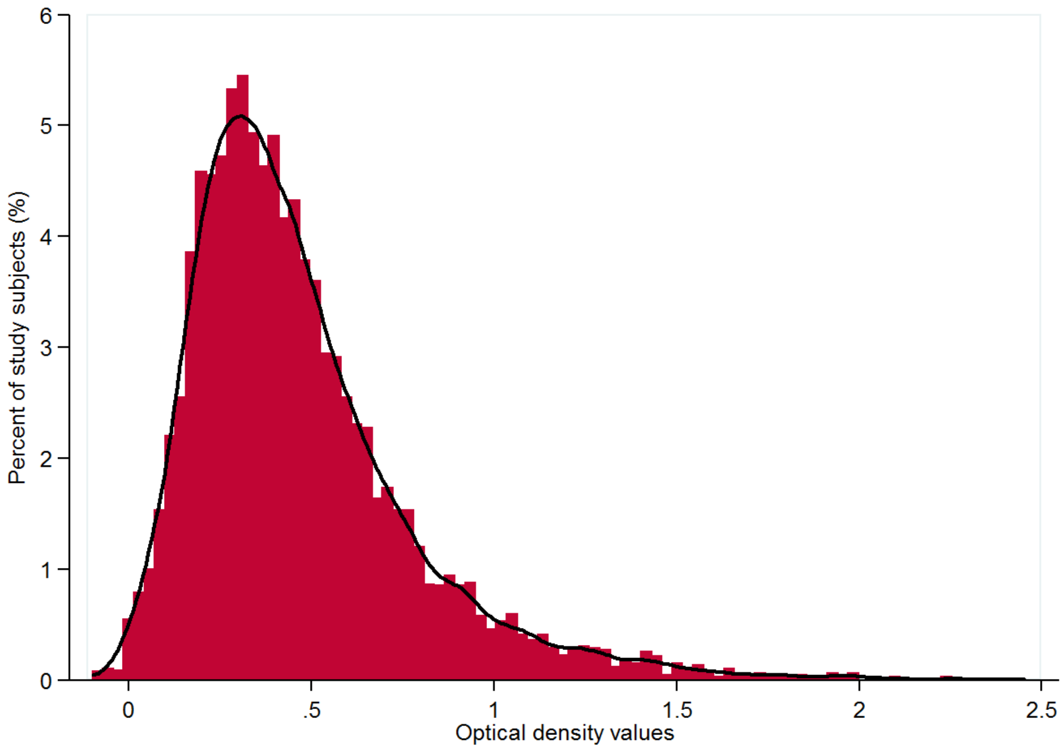
1. What is known on this topic
• Numerous translational studies have established the role of autoantibodies against apolipoprotein A-1 in inflammation and atherogenesis. • Small-scale clinical studies have shown promising results regarding the association and prognostic value of autoantibodies against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


CoLaus Study:
n=6649 subjects
Primary prevention: n=6194 (93.2%)
Secondary prevention: n=455 (6.8%)
Endpoints:
Cross-sectional association of anti-apoA-1 IgG with:
- CVD
- CV biomarkers
- Magnesium levels

SUPPLEMENTAL MATERIAL

Association between anti-apolipoprotein A-1 antibodies and cardiovascular disease in the general population: results from the CoLaus study

Panagiotis Antiochos, Pedro Marques-Vidal, Julien Virzi, Sabrina Pagano, Nathalie Satta, François Bastardot, Oliver Hartley, Fabrizio Montecucco, François Mach, Gerard Waeber, Peter Vollenweider, Nicolas Vuilleumier



Supplemental Figure I: Distribution of anti-apoA-1 IgG levels (in optical density) in the CoLaus study population

Supplemental Table I: Clinical assays used to evaluate biological markers in the CoLaus study.

Markers	Type of assay	Maximum inter and intra-batch CVs	Manufacturer
Lipids (mmol/L)			
Total Cholesterol	CHOD-PAP method	1.6% – 1.7%	Cobas 8000, Roche Diagnostics, CH
LDL cholesterol	Friedewald formula, only if triglycerides <4.6 mmol/l		
HDL cholesterol	CHOD-PAP + PEG + cyclodextrin method	3.6% – 0.9%	Cobas 8000, Roche Diagnostics, CH
Triglycerides	GPO-PAP method	2.9% – 1.5%	Cobas 8000, Roche Diagnostics, CH
Renal function			
Creatinine, µmol/L	Jaffe kinetic compensated method	2.9% – 0.7%	Cobas 8000, Roche Diagnostics, CH
Serum ions (mmol/L)			
Magnesium	Colorimetric Assay (Xylidyl Blue-I method)	2.9% – 0.8%	Cobas 8000, Roche Diagnostics, CH
Calcium	Colorimetric Assay (O-cresolphtalein method)	2.1% – 1.5%	Cobas 8000, Roche Diagnostics, CH
Surrogate markers of CVD			
Uric acid, µmol/L	Uricase-PAP method	1.0% – 0.5%	Cobas 8000, Roche Diagnostics, CH
Homocysteine, µmol/L	High pressure liquid chromatography following ammonium 7-fluorobenzo-2-oxa-1, 3-diazole -4-sulphonate (SBD-F) derivatisation	3.1% – 2.9%	Agilent 1100 apparatus
hs-CRP, mg/L	Latex-enhanced turbidimetric immunoassay method	4.6% – 1.3%	Cobas 8000, Roche Diagnostics, CH

Urine			
Creatinine, µmol/L	Jaffe kinetic compensated method	2.2% – 2.1%	Cobas 8000, Roche Diagnostics, CH
Magnesium, mmol/L	Colorimetric Assay (Xylidyl Blue-I method)	2.1% – 1.4%	Cobas 8000, Roche Diagnostics, CH

CV, coefficient of variation; CHOD, cholesterol oxidase; PAP, phenol aminophenazone; PEG, polyethylene glycol; GPO, glycerol-3-phosphate oxidase; LDL, low density lipoprotein; HDL, high density lipoprotein; hs-CRP, high-sensitivity C-reactive protein

Supplemental Table II: Clinical characteristics of subjects with established coronary heart disease according to anti-apoA-1 IgG status.

	Subjects with established CHD (n=235)		
Anti-apoA-1 IgG	**Negative**	**Positive**	**p-value**
Sample size, n (%)	**183 (77.9)**	**52 (22.1)**	
Age, years	63.1 ± 8.8	61.0 ± 8.6	0.084
Male sex, n (%)	127 (69.4)	41 (78.9)	0.183
Hypertension, n (%)	137 (74.9)	38 (73.1)	0.794
Diabetes, n (%)	34 (18.6)	13 (25.0)	0.307
Metabolic syndrome, n (%)	78 (42.6)	31 (59.6)	0.030
Current smoking, n (%)	44 (24.0)	14 (26.9)	0.671
Heart rate, bpm	64.2 ± 10.8	66.9 ± 9.36	0.042
Systolic BP, mmHg	136.7 ± 17.8	137.1 ± 22.3	0.797
Body mass index, kg/m^2	28.0 ± 4.8	29.2 ± 4.4	0.067

Data are expressed as mean ± standard deviation or number of participants and (percentage). Statistical analysis performed by chi-square for categorical variables and Mann-Whitney U test for continuous variables. Anti-apoA-1 IgG, Autoantibodies against Apolipoprotein A-1; BP, blood pressure

Supplemental Table III: Biological characteristics of subjects with established coronary heart disease according to anti-apoA-1 IgG status.

	Subjects with established CHD (n=235)		
Anti-apoA-1 IgG	**Negative**	**Positive**	**p-value**
Sample size, n (%)	**183 (77.9)**	**52 (22.1)**	
Lipids (mmol/L)			
Total cholesterol	5.06 ± 1.00	5.21 ± 1.05	0.159
LDL cholesterol	2.94 ± 0.88	3.09 ± 0.95	0.154
HDL cholesterol	1.43 ± 0.37	1.30 ± 0.29	0.015
Triglycerides	1.3 (1.0 – 1.9)	1.7 (1.1 – 2.3)	0.010
Serum ions (mmol/L)			
Magnesium	0.85 ± 0.07	0.81 ± 0.07	0.004
Calcium	2.31 ± 0.09	2.31 ± 0.11	0.531
Renal function			
eGFR, ml/min/1.73 m^2	75.7 (65.4 – 86.2)	75.7 (67.9 – 91.9)	0.593
Surrogate markers of CVD			
Uric acid, µmol/L	355 (299 – 417)	369 (331 – 420)	0.294
Homocysteine, µmol/L	11 (8.8 – 14.1)	12.6 (9.4 – 14.4)	0.127

hs- CRP, mg/L	1.6 (0.9 – 3)	2.3 (1.3 – 5.5)	0.005

Data are expressed as mean ± standard deviation or median (inter quartile 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