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AUTOANTIBODIES TO C-TERMINAL APOA-1 AS A BIOMARKER OF CARDIOVASCULAR DISEASE

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UNIL | Université de Lausanne
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préparée sous la direction du Professeur Peter Vollenweider
(avec la co-direction du Professeur Pedro Marques-Vidal)

et présentée à la Faculté de biologie et de médecine de
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DOCTEUR EN MEDECINE

par

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Médecin diplômé de la Grèce
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Autoantibodies to C-terminal apoA-1 as a biomarker of cardiovascular disease

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de la Faculté de Biologie et de Médecine

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Vice-Directeur de l'Ecole doctorale

Résumé

Ce travail de thèse s'intéresse aux anticorps dirigés contre l'apolipoprotéine A-1 (anti-apoA-1 IgG), la principale fraction protéique des particules de HDL cholestérol.

Jusqu'à présent, des travaux scientifiques fondamentaux ont établi le rôle des anti-apoA-1 IgG en tant que molécules pro-inflammatoires et pro-athérogènes, associées à une mortalité accrue dans des modèles murins. Par ailleurs, des travaux cliniques préliminaires ont démontré la valeur pronostique indépendante des anti-apoA-1 IgG dans la maladie cardiovasculaire (CV) dans des populations des patients atteints de maladies auto-immunes, des patients à risque CV élevé ou post infarctus du myocarde.

En utilisant des données de la "Cohorte Lausannoise" (étude CoLaus), une cohorte populationnelle de plus de 6500 habitants de la ville de Lausanne, ce travail de recherche s'est décliné en 3 objectifs : 1) mieux comprendre la prévalence des anti-apoA-1 IgG dans la population générale, 2) examiner l'association entre anti-apoA-1 IgG et la prévalence et facteurs de risque de la maladie CV 3) examiner les déterminants génétiques des anti-apoA-1 IgG et établir leur valeur pronostique pour la mortalité globale et les évènement coronariens dans la population générale.

Concernant les deux premiers objectifs, nos résultats publiés dans la revue *« Thrombosis and Haemostasis »* révèlent, pour la première fois, que des taux élevés d'anti-apoA-1 IgG sont présents dans environ 20% des individus dans la population générale. Ils sont, par ailleurs, associés à une prévalence accrue de maladies CV dans la population générale, indépendamment des facteurs de risque CV traditionnels. Par ailleurs, dans le sous-groupe des sujets atteints de maladie CV dans le passé (prévention secondaire), nous mettons en évidence une association des anti-apoA-1 IgG avec un profil pro-inflammatoire, témoigné par un taux de HDL cholestérol diminué, mais aussi par des valeurs de protéine C-réactive de haute sensibilité et d'acide urique élevées.

En partant de ces observations, nous avons, par la suite, investigué une prédisposition génétique à la présence des anti-apoA-1 IgG. Nos résultats publiés dans le journal *« Frontiers in Immunology »* démontrent que des taux élevés d' anti-apoA-1 IgG sont associés à des polymorphismes du gène FCRL3, gène impliqué dans la susceptibilité aux maladies auto-immunes chez l'être humain. Dans le même manuscrit, nous montrons que la présence des anti-apoA-1 IgG est associée de manière significative et indépendante à la mortalité toutes causes confondues dans la population générale.

Finalement, concernant le lien entre anti-apoA-1 IgG et maladie coronarienne, nous avons récemment démontré que les anti-apoA-1 IgG seraient davantage des prédicteurs indépendants de nouveaux événements coronariens et que cette association serait modulée par un polymorphisme fonctionnel du gène du récepteur CD14. Ses observations sont publiés dans le journal *« Arteriosclerosis, Thrombosis and Vascular Biology (ATVB) »* sous le titre « Impact of CD14 polymorphisms on anti-apolipoprotein A1 IgG related coronary artery disease prediction in the general population ».

D'un point de vue de santé publique, ces résultats innovants, obtenus dans une étude populationnelle à grande echelle, sont prometteurs concernant le rôle des anti-apoA-1 IgG non seulement comme un nouveau facteur de risque CV indépendant, mais également en tant que cible spécifique potentielle de thérapies immuno-modulatrices avec des implications cliniques futures.

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 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

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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 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 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):

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

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-nitrophanylphosphate 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 de-

tection 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 × 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 preplanned associations of anti-apoA-1 IgG with specific and interdependent 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 in-

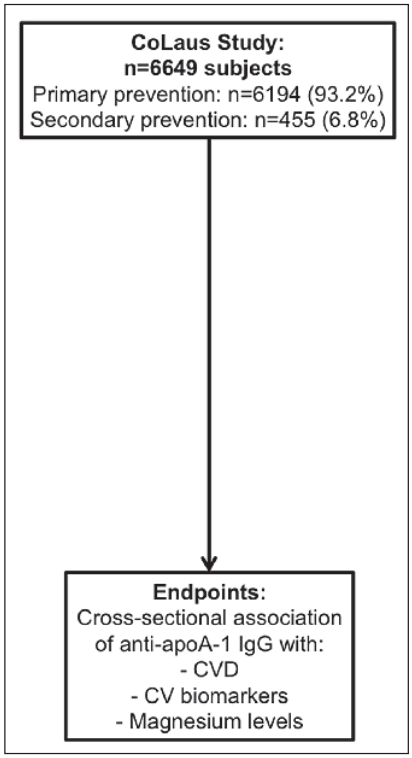

Figure 1: Study flowchart and endpoints. Anti-apoA-1 IgG, autoantibodies against apolipoprotein A-1; CVD, cardiovascular disease; CV, cardiovascular.

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. 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.

	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									
Aspirin	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

	Unadjusted model	P-value	Adjusted model	P-value
	OR (95 % CI)		OR (95 % CI)	
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 for anti-ApoA-1 IgG (OD<0.64)	1 (ref.)		1 (ref.)	
Positive for anti-ApoA-1 IgG (OD≥0.64) *				
1st group (0.64≤OD<0.77)	1.02 (0.69–1.50)	0.936	1.15 (0.76–1.74)	0.507
2nd group (0.77≤OD<0.98)	1.24 (0.87–1.79)	0.236	1.14 (0.76–1.70)	0.529
3rd group (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 2: Odds ratio of anti-apoA-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-apoA-1 IgG, autoantibodies against apolipoprotein A-1; OD, optical density. * Subjects positive for anti-ApoA-1 (OD≥0.64, n=1323) were divided into three groups of equal size and increasing anti-ApoA-1 titres: 1st group (0.64≤OD<0.77), 2nd group (0.77≤OD<0.98) and 3rd group (OD≥0.98).

crease 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.

	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

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 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 (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) (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 anti-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-phosholipid, 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.

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.

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.

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.

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Anti-Apolipoprotein A-1 IgG Predict All-Cause Mortality and Are Associated with Fc Receptor-Like 3 Polymorphisms

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Background: Autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG) have emerged as an independent biomarker for cardiovascular disease and mortality. However, their association with all-cause mortality in the community, as well as their genetic determinants, have not been studied.

Objective: To determine whether anti-apoA-1 IgG: (a) predict all-cause mortality in the general population and (b) are associated with single-nucleotide polymorphisms (SNPs) in a genome-wide association study (GWAS).

Methods: Clinical, biological, and genetic data were obtained from the population-based, prospective CoLaus study, including 5,220 participants (mean age 52.6 years, 47.3% men) followed over a median duration of 5.6 years. The primary study outcome was all-cause mortality.

Results: After multivariate adjustment, anti-apoA-1 IgG positivity independently predicted all-cause mortality: hazard ratio (HR) = 1.54, 95% confidence interval (95% CI): 1.11–2.13, $P = 0.01$. A dose–effect relationship was also observed, each SD of logarithmically transformed anti-apoA-1 IgG being associated with a 15% increase in mortality risk: HR = 1.15, 95% CI: 1.02–1.28, $P = 0.028$. The GWAS yielded nine SNPs belonging to the Fc receptor-like 3 (*FCRL3*) gene, which were significantly associated with anti-apoA-1 IgG levels, with the lead SNP (rs6427397, $P = 1.54 \times 10^{-9}$) explaining 0.67% of anti-apoA-1 IgG level variation.

Conclusion: Anti-apoA-1 IgG levels (a) independently predict all-cause mortality in the general population and (b) are linked to *FCRL3*, a susceptibility gene for numerous autoimmune diseases. Our findings indicate that preclinical autoimmunity to anti-apoA-1 IgG may represent a novel mortality risk factor.

Keywords: autoimmunity, autoantibodies, apolipoprotein A-1, mortality, genome-wide association study, Fc receptor-like 3

INTRODUCTION

Autoimmune diseases (ADs) represent a major health issue affecting up to 10% of the population (1–4). Multiple studies have established the relationship between a chronic inflammatory state due to clinical or quiescent autoimmunity and poor outcomes in various clinical settings (5, 6). Marking sustained B-cell activation, presence of autoantibodies specifically represents a signature of humoral autoimmunity, which can drive disease pathogenesis even in the absence of overt clinical AD (7–9).

More than a decade ago, early clinical studies have supported the role of autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG), the principal component of high-density lipoproteins (HDLs), as mediators of chronic low-grade inflammation and predictors of unfavorable outcomes in patients with ADs (10–17). Subsequently, *in vitro* and animal studies demonstrated the ability of these autoantibodies to elicit a pro-inflammatory and pro-atherogenic response through interaction with the TLR2/TLR4/CD14 complex, followed by NF-κB and MAPK downstream activation as the main molecular pathway (18–24).

Recently, due to these pro-inflammatory and pro-atherogenic properties, anti-apoA-1 IgG gained interest as independent biomarkers for incident cardiovascular (CV) disease (CVD) and mortality, as well as potential therapeutic targets for immune-modulating interventions, in high CV risk populations (16, 19, 20, 22, 25–28). Moreover, similar to what has been reported with other autoantibodies (7), our group recently demonstrated that elevated anti-apoA-1 IgG levels were present in up to 20% of individuals in the general population and associated with prevalent CVD independently of traditional CV risk factors (25). Nevertheless, the association of anti-apoA-1 IgG with all-cause mortality in the general population has not yet been studied.

From another point of view, while rare autoimmunity syndromes can result from monogenic mutations, common human ADs are complex disorders arising from the interaction between polygenic and environmental risk factors, disrupting mechanisms of immune tolerance. In recent years, genome-wide association studies (GWASs) have provided insight into the subtle immune dysregulation caused by common genetic variants that predispose to clinical autoimmunity (29, 30) and autoantibody production in particular (31–34).

Although the risk attributable to most of the identified individual nucleotide variants is modest, modern GWAS have the potential to provide an unbiased view of biological pathways that drive autoimmunity (1–4). However, despite the associations of anti-apoA-1 IgG with adverse outcomes in different patient populations, no study has so far investigated common genetic variants potentially related to their serum values.

Thus, the goal of the present study was twofold. We aimed to determine whether anti-apoA-1 IgG: (a) predict all-cause mortality in the general population in a prospective study and (b) are associated with single-nucleotide polymorphisms (SNPs) in a GWAS.

METHODS

Study Population and Design

Clinical and biological data were obtained from the CoLaus study, a population-based prospective cohort of 6,733 participants recruited between 2003 and 2006 in the city of Lausanne, Switzerland. Of the initial baseline sample of 6,733 participants, 5,220 (mean age 52.6 ± 10.7 years, 47.3% men) had complete clinical and biological data over a median follow-up (FU) time of 5.6 years and were included in the prospective analysis. A detailed description of the study design, variables, and sampling procedures has been reported elsewhere (35).

All participants attended the outpatient clinic of the University Hospital of Lausanne. Clinical data and fasting venous blood samples were collected from each participant by trained field interviewers during a single visit lasting about 60 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. Body weight and height were measured with participants standing without shoes in light indoor clothes. Body weight was measured in kilograms to the nearest 100 g using a Seca® scale, and height was measured to the nearest 5 mm using a Seca® height gauge. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Hypertension was defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg and/or the 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. History of CVD was defined by the presence of myocardial infarction, angina pectoris, percutaneous revascularization or bypass grafting for ischemic heart disease, and stroke or transient ischemic attack and assessed according to standardized medical records (35). History of ADs was obtained *via* questionnaire. Estimated glomerular filtration rate (eGFR) was calculated by the simplified "Modification of Diet in Renal Disease" prediction equation. Absolute risk for CVD was computed using the Systematic Coronary Risk Evaluation algorithm (36).

Venous blood samples were drawn after an overnight fast, and assays were performed on fresh plasma samples within 2 h of blood collection for standard lipid profile and on unthawed serum aliquots for anti-apoA-1 IgG determination, (see below) that were immediately processed and stored at −80°C. Standard lipid profile was performed by the CHUV Clinical Laboratory using a Modular P apparatus (Roche Diagnostics, Switzerland). The following analytical procedures (with maximum inter- and intra-batch CVs) were used: total cholesterol by the "CHOD-PAP" method (1.6–1.7%); HDL cholesterol by the "CHOD-PAP/PEG/Cyclodextrin" method (3.6–0.9%); triglycerides by the "GPO-PAP" method (2.9–1.5%); glucose by glucose dehydrogenase (2.1–1.0%); and serum creatinine by the Jaffe kinetic compensated method (2.9–0.7%).

Determination of Anti-apoA-1 IgG Levels

Autoantibodies against apolipoprotein A-1 were measured as previously described (19, 22, 37), using the CoLaus study

(2003–2006) frozen serum aliquots, stored at −80°C. Maxisorp plates (Nunc™, Denmark) were coated with purified, human-derived delipidated apolipoprotein A-1 (20 μg/ml; 50 μl/well) for 1 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. Participants' 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 1:1,000 in a PBS/BSA 2% solution, was added and incubated for 1 h at 37°C. After washing six more times, phosphatase substrate *p*-nitrophenyl phosphate 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 detection was assessed using conventional saturation tests by Western blot analysis.

As previously described (19, 22, 37), 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 further 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%.

Genome-Wide Association Study

Genotyping was performed using the Affymetrix GeneChip® Human Mapping 500K array set and genotypes were called using BRLMM. SNPs with a call rate <70% and individuals with call rate <90% were excluded from further analysis. Participants found to be of non-European ancestry by principal component analysis of the genotype data were also excluded, leaving 5,402 participants eligible for GWAS. Imputation was performed using IMPUTE version 0.2.0 and CEU haplotypes from HapMap release 21. The dataset used for imputation consisted in 390,631 genotyped SNPs with a call rate >0.9, Hardy–Weinberg *P*-value $>10^{-7}$, and MAF >1%.

Before performing the GWAS, anti-apoA-1 IgG levels were adjusted for age, sex, and ancestry principal components. The residuals were then inverse normal quantile transformed and regressed onto genetic allele dosages. To fine map the genome-wide significant association at the *FCRL2/3* locus, we re-imputed the 400-kb window around the top HapMap-associated SNP using haplotypes from the HRC reference panel.

Study Endpoints

The primary study endpoint was overall mortality, but specific causes of death were also considered. All deaths and related causes were adjudicated by an independent panel of internal medicine physicians, blinded to all study variables.

Statistics

Statistical analyses were conducted using Stata v14.1 (Stata Corp., TX, USA) and MatLab v8.3 (MathWorks, MA, USA). Bivariate analysis of continuous variables was performed using Student's or Mann–Whitney test as appropriate, while analysis of categorical variables was performed using chi-square test. The association of anti-apoA-1 IgG levels with all-cause mortality was assessed by the log-rank test and by Cox proportional hazards regression, adjusting for age, gender, hypertension, diabetes, smoking, BMI, eGFR, HDL and low-density lipoprotein (LDL) cholesterol, baseline CVD, and AD. Anti-apoA-1 IgG concentrations were natural log transformed to account for skewed distributions, and results were expressed as hazard ratios (HRs) and 95% confidence intervals (95% CIs). Considering a two-sided alpha of 0.05, our study had 80% power to detect a relative risk for all-cause mortality of 1.45 in participants positive for anti-apoA-1 IgG. All tests were two tailed, and *P* values <0.05 were considered as statistically significant.

RESULTS

Figure 1A shows the participants' selection procedure, and Table S1 in Supplementary Material summarizes the baseline characteristics of participants, with or without FU data. Overall, subjects lost at FU were more likely to be smokers, hypertensive, obese, and with a less favorable lipid profile than subjects included in the analysis, but did not differ with regards to anti-apoA-1 IgG positivity or serum levels.

During FU, 191 deaths (3.7%) occurred. The three major causes of death were cancer, CVD, and infectious diseases. Participants who died presented with a significantly higher prevalence of anti-apoA-1 IgG positivity (26.7 vs. 19.6%, $P = 0.016$) and higher anti-apoA-1 IgG levels (median [interquartile range]: 0.43 [0.30–0.70] vs. 0.39 [0.25–0.59] AU, $P = 0.007$), than participants alive at FU (**Table 1**). Of note, median anti-apoA-1 IgG values of anti-apoA-1 IgG-positive subjects who died at FU were 0.89 [0.79–1.05] AU.

Kaplan–Meier curves for participants with positive and negative anti-apoA-1 IgG titers are shown in **Figure 1B**; participants positive for anti-apoA-1 IgG had higher mortality rates than participants negative for the marker (4.9 vs. 3.4%, log-rank $P = 0.034$). Conversely, 95.1% of anti-apoA-1 IgG-positive subjects survived, compared to 96.6% of anti-apoA-1 IgG-negative subjects corresponding to an absolute survival difference of 1.5% between the two groups.

Cox regression analysis indicated that anti-apoA-1 IgG positivity was associated with a 1.5-fold increased risk of death, and this hazard rate remained unchanged after adjustment for the aforementioned mortality risk factors, including baseline CVD and AD: HR = 1.54 (1.11–2.13), $P = 0.01$. Similarly, 1 SD increase of the log-transformed anti-apoA-1 IgG levels was associated with a 15% increase in the risk of all-cause mortality: HR = 1.15 (1.02–1.28), $P = 0.028$ (**Table 2**). Sensitivity analyses excluding participants with CVD or AD at baseline led to comparable findings (**Table 2**).

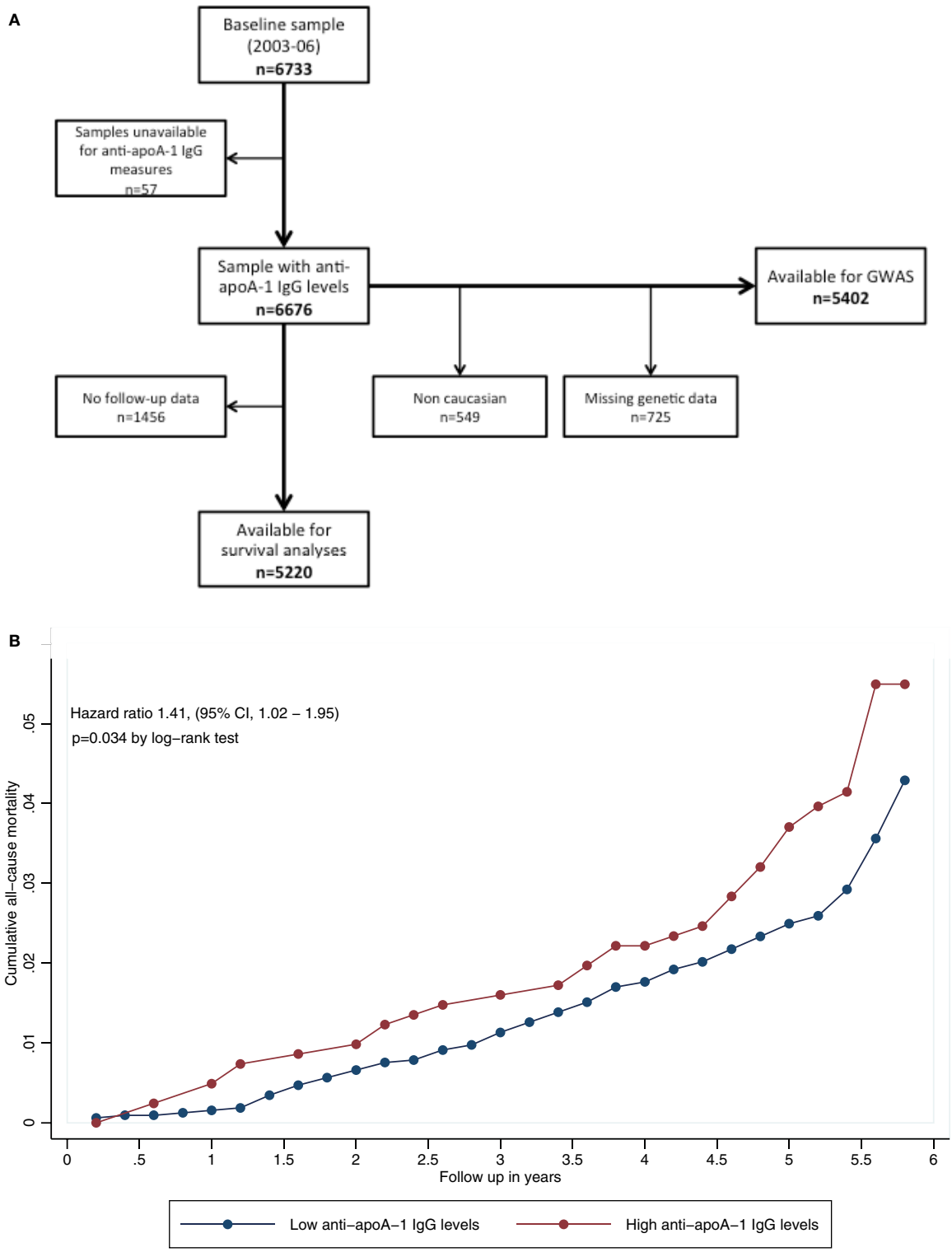

FIGURE 1 | **(A)** Participants' selection procedure. **(B)** Kaplan–Meier curves for all-cause mortality, according to autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG) status. *P*-value according to log-rank test ($P = 0.034$).

TABLE 1 | Baseline characteristics of the sample according to all-cause mortality during follow-up.

	Overall (*n* = 5,220)	Survivors (*n* = 5,029)	Non-survivors (*n* = 191)	*P*-value
Age, years	52.6 ± 10.7	52.2 ± 10.6	62.7 ± 9.5	<0.001
Male sex, *n* (%)	2,461 (47.3)	2,337 (46.6)	124 (64.9)	<0.001
History of CVD, *n* (%)	379 (7.3)	337 (6.7)	42 (22.0)	<0.001
Current smoking, *n* (%)	1,356 (26.1)	1,277 (25.5)	79 (41.4)	<0.001
Diabetes, *n* (%)	326 (6.3)	282 (5.6)	44 (23.0)	<0.001
BMI (kg/m^2)	25.6 ± 4.4	25.6 ± 4.4	26.7 ± 5.5	<0.001
Hypertension, *n* (%)	1,723 (33.1)	1,613 (32.2)	110 (57.6)	<0.001
SBP (mmHg)	127.6 ± 17.7	127.2 ± 17.6	135.3 ± 19.1	<0.001
eGFR (ml/min/1.73 m^2)	78.7 ± 15.7	78.8 ± 15.5	75.4 ± 20.0	0.014
Total cholesterol (mmol/l)	5.56 ± 1.02	5.57 ± 1.01	5.53 ± 1.19	0.625
HDL cholesterol (mmol/l)	1.64 ± 0.44	1.64 ± 0.44	1.54 ± 0.47	0.001
LDL cholesterol (mmol/l)	3.32 ± 0.91	3.32 ± 0.90	3.26 ± 1.02	0.362
Triglycerides (mmol/l)	1.37 ± 1.14	1.36 ± 1.06	1.78 ± 2.33	<0.001
SCORE risk (%)	2.08 ± 3.57	1.91 ± 3.35	6.01 ± 5.80	<0.001
Known AD (RA, SLE)	115 (2.2)	110 (2.2)	5 (2.6)	0.696
Anti-apoA-1 IgG positivity	1,035 (19.9)	984 (19.6)	51 (26.7)	0.016
Anti-apoA-1 IgG levels (AU)	0.39 [0.34]	0.39 [0.33]	0.43 [0.40]	0.007
Incident overall death rate, *n* (%)	191 (3.7)		191 (3.7)	
Cancer-related, *n* (%)	69 (36.1)		69 (36.1)	
CVD-related, *n* (%)	36 (18.9)		36 (18.9)	
Infectious-related, *n* (%)	25 (13.1)		25 (13.1)	
Other causes[a], *n* (%)	51 (26.7)		51 (26.7)	
Undetermined, *n* (%)	10 (5.2)		10 (5.2)	

P values are derived from the comparison of patients with low vs. high levels of anti-apoA-1 IgG. Variables are expressed as mean ± SD or median [interquartile range] as appropriate or number of participants and percentage. Statistical analysis for continuous variables was performed by Student's t-test or Mann–Whitney test depending on the normality assumption. Chi-squared test was used for categorical variables.

AD, autoimmune disease; CVD, cardiovascular disease; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate according to the MDRD formula; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SCORE, Systematic Coronary Risk Evaluation; AU, arbitrary units of optical density; BMI, body mass index; anti-apoA-1 IgG, autoantibodies against apolipoprotein A-1.

[a]*Other causes include lung disease (n = 12), bleeding and trauma-related complications (n = 11), suicide (n = 9), chronic renal failure (n = 6), non-ischemic heart failure (n = 5), chronic liver failure (n = 5), and dementia (n = 3).*

Genome-wide association study on anti-apoA-1 IgG levels identified a single locus located in chromosome 1 (157.6–157.9 Mb) including 21 SNPs significantly associated with anti-apoA-1 IgG levels (**Figure 2A**; Table S2 in Supplementary Material). The T allele of the lead SNP (rs6427397) was associated with an increase of anti-apoA-1 IgG levels by 0.113 AU and explained 0.67% of anti-apoA-1 IgG variation. Conversely, in logistic regression (yes vs. no anti-apoA-1 IgG positivity) analysis, the T allele of rs6427397 had an odds ratio (OR) of 1.27 (95% CI 1.15–1.40) for anti-apoA-1 IgG positivity in our sample. The 20 remaining SNPs identified in the same region were in strong linkage disequilibrium and associated with anti-apoA-1 levels at *P*-value $<10^{-7}$ (**Figure 2B**). Most of these SNPs were located in or near the Fc receptor-like 2 (*FCRL2*) and 3 (*FCRL3*) genes and to some extend in the regions spanning the *FCRL1* gene (**Figures 2B,C**). SNPs with a previously known biological function or association with a clinical trait were located in the *FCRL3* gene (Table S2 in Supplementary Material). Additionally, the only coding polymorphism among the identified SNPs represents a missense variant (rs7522061) also located in the *FCRL3* gene. We also observed an association between anti-apoA-1 IgG and a missense variant (rs1047989) in the human leukocyte antigen (HLA)-DQA1 gene, which did not, however, achieve stringent genome-wide significance ($P = 7.27 \times 10^{-7}$, data not shown).

DISCUSSION

This is the first study to demonstrate that anti-apoA-1 IgG levels are significantly and independently associated with all-cause mortality in the general population. In our population, after 5 years of FU, 95.1% of anti-apoA-1-positive subjects survived, compared to 96.6% of anti-apoA-1-negative participants. This corresponds to a modest, nevertheless, significant ($P = 0.03$) absolute survival difference of 1.5% between the two groups.

Our results extend previous findings (19, 22, 25, 26) and suggest that preclinical autoimmunity against apoA-1/HDL—which affects up to one-fifth of the general population—may identify individuals at increased risk of death. Our findings raise the possibility that presence of anti-apoA-1 IgG leads to pathophysiological events affecting not only CV prognosis, but also survival in the long term. Based upon previous studies, such events could be related to a chronic low-grade inflammatory state through sustained activation of the TLR2/TLR4/CD14 complex and production of pro-inflammatory cytokines (14, 18, 21, 24, 38), associations with elevated high-sensitivity C-reactive protein and uric acid levels (23, 25), impairment of HDL function (10–13, 16, 17), interference with basal heart rate regulation (22, 24, 25) or B-cell epitope spreading (39). Other pathophysiological mechanisms, other than those currently ascribed to anti-apoA-1 IgG could also be involved, in the same way that multiple molecular pathways underlie the associations of other autoantibodies with all-cause mortality in the community (7, 9, 40, 41). These challenging points will have to be investigated in future clinical and translational research efforts.

The second notable finding of our study is that anti-apoA-1 IgG levels are related to genetic polymorphisms belonging or regulating the *FCRL3* gene. Indeed, our lead SNP (rs6427397) is an intergenic variant that represents a strong expression quantitative trait loci for the *FCRL3* gene in whole blood (1.2×10^{-12}) (42). Additionally, among the 20 remaining *FCRL* SNPs achieving genome-wide significance, the only coding variant identified (rs7522061) is a missense variant of the *FCRL3* gene.

Fc receptor-like genes are located in the human chromosome regions 1q21–23 and belong to the immunoglobulin genes superfamily. *FCRL3* in particular is known to encode a mature B-cells co-receptor primarily expressed in secondary lymphoid organs and involved in B cell maturation, regulation, and production of autoantibodies (43–45). Previous work suggests that *FCRL3* expression further affects T regulatory cells development and function, with high expression resulting in abnormal immune activation and breakdown of self-tolerance (46). Corroborating

TABLE 2 | Association between anti-apoA-1 IgG and all-cause mortality.

	Events (*n*)	Unadjusted HR HR (95% CI)	*P*-value	Adjusted HR[a] HR (95% CI)	*P*-value
Overall sample (*n* = 5,220)					
– High vs. low levels	191	1.41 (1.02–1.95)	0.035	1.54 (1.11–2.13)	0.010
– For 1 SD increase in log (anti-apoA-1 IgG)	191	1.14 (1.01–1.28)	0.040	1.15 (1.02–1.30)	0.028
Participants without CVD at baseline (*n* = 4,825)					
– High vs. low levels	149	1.42 (0.98–2.04)	0.062	1.62 (1.12–2.34)	0.011
– For 1 SD increase in log (anti-apoA-1 IgG)	149	1.14 (0.99–1.31)	0.062	1.19 (1.03–1.38)	0.019
Participants without AD at baseline (*n* = 5,105)					
– High vs. low levels	186	1.42 (1.02–1.97)	0.036	1.68 (1.21–2.34)	0.002
– For 1 SD increase in log (anti-apoA-1 IgG)	186	1.15 (1.01–1.30)	0.030	1.23 (1.07–1.40)	0.002

Data are expressed as adjusted HRs and 95% CI. Statistical analysis by Cox proportional hazards regression.

AD, autoimmune disease; HR, hazard ratio; CVD, cardiovascular disease; 95% CI, 95% confidence interval; anti-apoA-1 IgG, autoantibodies against apolipoprotein A-1.

[a]Adjusted for age, sex, hypertension, diabetes, smoking, high- and low-density lipoprotein cholesterol, body mass index, estimated glomerular filtration rate baseline CVD and AD.

these *in vivo* findings, large-scale GWAS have identified *FCRL3*-related SNPs as major susceptibility genes for numerous ADs in humans (44, 47, 48).

The observed association of *FRCL3* with anti-apoA-1 IgG values in our sample is in line with previous studies showing that two-thirds of candidate loci for autoimmunity discovered by GWAS represent shared risk factors for multiple ADs (1, 3, 49). Among potential pathophysiological mechanisms, *FCRL3* may predispose to clinical autoimmunity by pleiotropic regulation of the production of other deleterious autoantibodies. *FCRL3* has been associated with the production of cyclic citrullinated peptide autoantibodies in rheumatoid arthritis (RA) (47) and antibodies to thyroid peroxidase in patients suffering from Graves' disease (33). In a study of genetic determinants of autoantibody production in over 8,000 type 1 diabetes cases, Plagnol et al. (31) identified the *FCRL3* locus to be associated with antibodies against insulinoma-associated antigen 2 concluding that *FCRL3* "may have general effects in adaptive immunity, in the complex interactions between antigen presenting cells and T cells leading to antibody producing plasma B cells." Unfortunately, we were unable to measure in our sample other antibodies, such as anti-oxidized LDL, antiphospholipid, antinuclear, or anti-heat shock protein antibodies in order to test this hypothesis.

Finally, with regards to anti-apoA-1 IgG and CV disease and mortality (19, 20, 22), *FCRL3* mRNA levels have been reported to be downregulated in patients with myocardial infarction when compared to those with stable angina or healthy subjects (50), suggesting that *FCRL3*-mediated immune dysregulation may also be involved in atheromatous plaque instability and rupture.

Although statistically significant, the effect of the T allele of the lead SNP (rs6427397) on autoantibody levels was modest [OR 1.27 (95% CI 1.15–1.40)] and explained 0.67% of total anti-apoA-1 IgG variation. This is not surprising since incremental effect sizes of genetic variants are rather common in genetic studies of autoimmunity where most risk alleles have ORs less than 1.2 (29, 30, 32) in spite of their low performance regarding disease prediction, these risk variants may provide important etiological information based on associated genomic regions, that could lead to a more sophisticated understanding of the molecular pathways underlying common ADs. Thus, genetic signatures of susceptibility to autoimmunity could provide a basis for assessing heterogeneity in disease progression, in response to targeted immune-modulating interventions, as well as for rational novel drug design.

The fact that the lead SNP in our study relates to an intergenic non-coding variant in the *FCRL3* locus is in accordance with current evidence from high-density genotyping and epigenomic studies which demonstrate that in common ADs, up to 90% of identified causal variants appear to be non-coding, while 60% correspond to immune cell enhancers (3). The current paradigm supports the notion that intergenic regions are densely populated with hundreds of thousands of regulatory elements that modulate cell type-specific gene expression (1, 3). In a study on RA (47), Kochi et al. demonstrated that non-coding SNPs in the promoter region of *FCRL3* have a regulatory effect on expression of the *FCRL3* gene and relate to augmented autoantibody production in subjects with the susceptible genotype. In the same line of thought, our present results indicate that the sequence of events leading to high of anti-apoA-1 IgG levels could be related to an impaired regulation of gene expression programs—including *FCRL3*—a hypothesis that requires further study.

Finally, although we did not retrieve any strictly genome-wide significant association between anti-apoA-1 IgG and HLA-related genes, we did observe an association trend at $P = 7.27 \times 10^{-7}$ that may represent a clinically meaningful signal in future studies and further relates the presence of these autoantibodies to autoimmunity susceptibility genes.

Study limitations are worth noting. First, our genetic analysis was conducted in a single population sample, including only individuals of Caucasian ancestry and will require further validation in independent cohorts, ideally involving other ethnic groups. Since genetic data on anti-apoA-1 IgG are so far inexistent, we were unable to identify a replication cohort for our GWAS. Nevertheless, our findings represent the first attempt to identify the genetic determinants of anti-apoA-1 IgG. Second, as the specific functionality of T allele of rs6427397 is currently unknown, we can only so far extrapolate on the pathophysiological

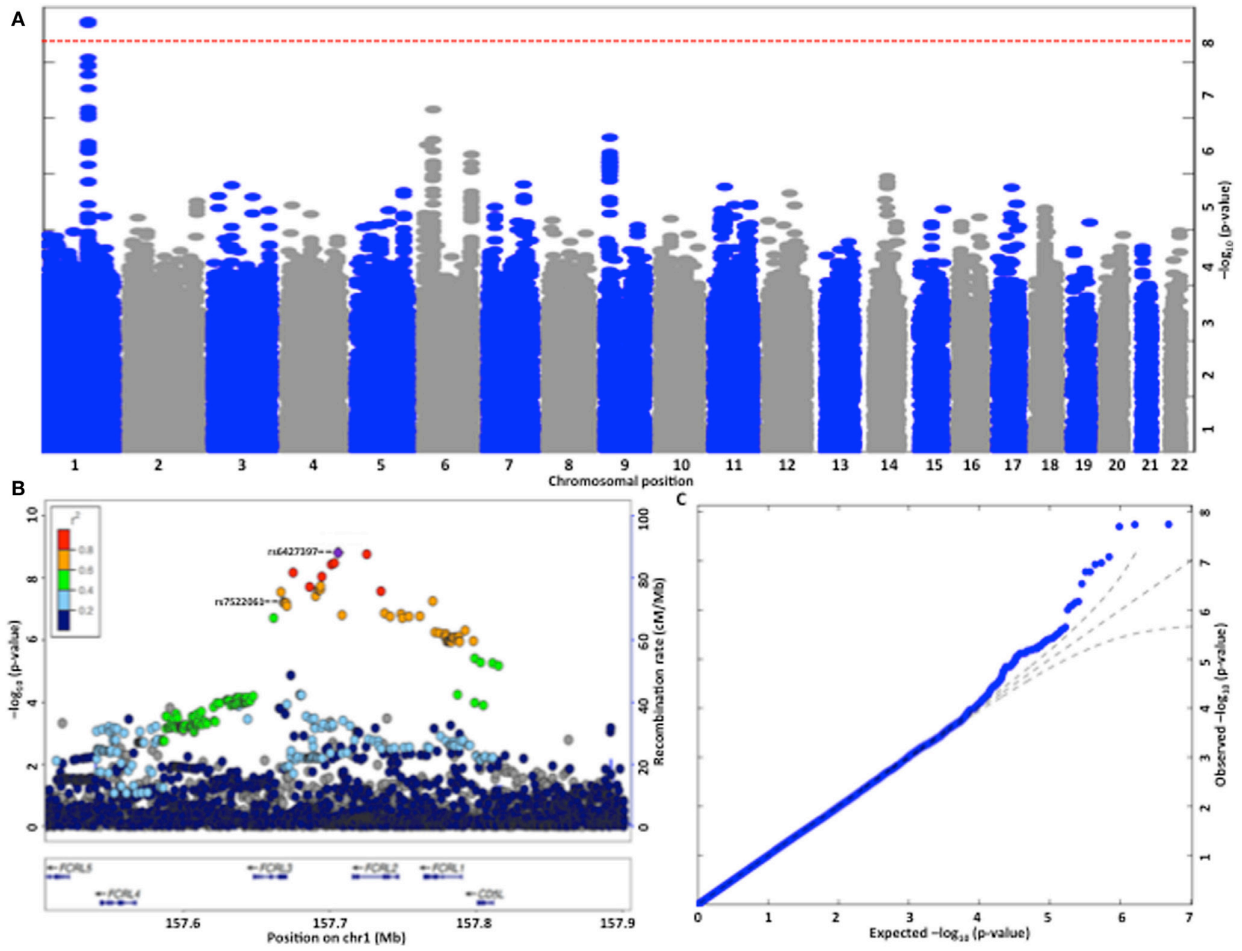

FIGURE 2 | **(A)** Manhattan plot of the genome-wide association analysis for autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG) levels. The *x*-axis is chromosomal position and the *y*-axis is the significance (−log *P*; two-tailed) of association derived by the linear regression. The red line represents genome-wide significance level ($p = 5 \times 10^{-8}$) that was achieved by a single locus located in chromosome 1. **(B)** Regional plot at the Fc receptor-like (FCRL)2/3 locus on chromosome 1 showing single-nucleotide polymorphism (SNP) association strength for anti-apoA-1 IgG levels. To fine map the genome-wide significant association at the FCRL2/3 locus, we re-imputed the 400-kb window around the top HapMap-associated SNP using haplotypes from the HRC reference panel. −log10 *P* values (*y*-axis) are presented according to their chromosomal positions (*x*-axis). The index SNP in the analysis (i.e., SNP with the smallest *P*-value, rs6427397, purple diamond) and the only coding SNP identified (rs7522061) are labeled. The color intensity of each symbol reflects their linkage disequilibrium (r^2) values with the index SNP, red ($r^2 > 0.8$) through to navy blue ($r^2 < 0.2$). Genes in each region, direction of transcription, and genomic coordinates are shown at the bottom. **(C)** Quantile–quantile plot of SNPs for anti-apoA-1 IgG levels. The negative logarithm of the observed (*y*-axis) and the expected (*x*-axis) *P*-value is plotted for each SNP (dot). The black lines indicate the null hypothesis of no true association ($x = y$) with 95% confidence intervals. Significant deviation from the expected (null) *P*-value distribution is evident only in the upper tail area (blue line), corresponding to SNPs with the strongest association.

relevance of the reported association, as related to regulation of the *FCRL3* gene. Indeed, the only genome-wide significant coding variant identified (rs7522061) is a missense variant of the *FCRL3* gene. As we only measured baseline anti-apoA-1 IgG levels, we were not able to assess the dynamic of anti-apoA-1 IgG levels over time in relation with mortality. Knowing whether an increase of anti-apoA-1 IgG would precede clinical events or whether our findings could be affected by transient anti-apoA-1 IgG positivity in a single sample will be important to determine in future studies.

Similarly, one could argue that since the actual difference in anti-apoA-1 IgG levels between the survivors and non-survivors was modest, these results are unlikely to be clinically relevant even though the relative observed difference in anti-apoA-1 IgG values is 9.3% and statistically significant in our sample ($P = 0.007$), as expected from sample power calculation. The fact that the distribution of anti-apoA-1 IgG values is positively skewed (25) and that low CV risk participants are known to present lower levels of anti-apoA-1 IgG than high CV risk subjects (19, 22, 25) could explain these relatively low anti-apoA-1 IgG values in

the general population, despite significant differences between survivors and non-survivors. Finally, the observed anti-apoA-1 IgG values translates in a 19.9% anti-apoA-1 IgG positivity in the sample, which is similar to the reported prevalence of other IgG autoantibodies in the community (7).

Despite these limitations, our study provides—to our best knowledge—the first evidence of a significant association of anti-apoA-1 IgG with all-cause mortality and a major AD susceptibility gene. Although our findings are in accordance and extending previous results, they will need to be replicated in independent, adequately powered, prospective cohorts before any conclusion on potential clinical implications can be drawn.

CONCLUSION

Our study suggests that anti-apoA-1 IgG levels predict all-cause mortality in the general population and are associated with a single locus involving *FCRL3*, a gene known to predispose for ADs. Our findings indicate that preclinical autoimmunity to apoA-1 may identify a substantial proportion of individuals at increased risk of death in the general population, a finding that will need further validation in independent, prospective cohorts.

ETHICS STATEMENT

The study was approved by the Institutional Ethics Committee of the University of Lausanne, and written informed consent was obtained from all participants before inclusion in the study, in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

PA and PM-V contributed in study concept and design, analysis, and interpretation of data, statistical analysis and drafting of the manuscript, and critical revision of the manuscript for important intellectual content. JV, SP, NS, OH, and FM contributed in study concept and design, acquisition of the data, analysis and interpretation of the data, statistical analysis, and critical revision of the manuscript for important intellectual content. FM, ZK, GW, PV, and NV had study supervision, contributed in study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, obtained funding, and provided study supervision, administrative, and technical support. All listed authors gave final approval of the manuscript to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2017.00437/full#supplementary-material.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Impact of CD14 Polymorphisms on Anti-Apolipoprotein A-1 IgG-Related Coronary Artery Disease Prediction in the General Population

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Objective—We aimed to determine whether autoantibodies against apoA-1 (apolipoprotein A-1; anti-apoA-1 IgG) predict incident coronary artery disease (CAD), defined as adjudicated incident myocardial infarction, angina, percutaneous coronary revascularization, or bypass grafting, in the general population. We further investigated whether this association is modulated by a functional CD14 receptor single nucleotide polymorphism.

Approach and Results—In a prospectively studied, population-based cohort of 5220 subjects (mean age 52.6±10.7 years, 47.4% males), followed over a median period of 5.6 years, subjects positive versus negative for anti-apoA-1 IgG presented a total CAD rate of 3.9% versus 2.8% (P=0.077) and a nonfatal CAD rate of 3.6% versus 2.3% (P=0.018), respectively. After multivariate adjustment for established cardiovascular risk factors, the hazard ratios of anti-apoA-1 IgG for total and nonfatal CAD were: hazard ratio=1.36 (95% confidence interval, 0.94–1.97; P=0.105) and hazard ratio=1.53 (95% confidence interval, 1.03–2.26; P=0.034), respectively. In subjects with available genetic data for the C260T *rs2569190* single nucleotide polymorphism in the CD14 receptor gene (n=4247), we observed a significant interaction between anti-apoA-1 IgG and *rs2569190* allele status with regards to CAD risk, with anti-apoA-1 IgG conferring the highest risk for total and nonfatal CAD in non-TT carriers, whereas being associated with the lowest risk for total and nonfatal CAD in TT homozygotes (P for interaction =0.011 and P for interaction =0.033, respectively).

Conclusions—Anti-apoA-1 IgG are independent predictors of nonfatal incident CAD in the general population. The strength of this association is dependent on a functional polymorphism of the CD14 receptor gene, a finding suggesting a gene–autoantibody interaction for the development of CAD.

Visual Overview—An online visual overview is available for this article. (***Arterioscler Thromb Vasc Biol*. 2017;37: 2342-2349. DOI: 10.1161/ATVBAHA.117.309602.)**

Key Words: apolipoprotein A-1 ■ autoimmunity ■ autoantibodies ■ CD14 polymorphism ■ coronary artery disease ■ HDL cholesterol ■ risk stratification

Major discoveries in the pathophysiology of atherosclerosis have established the fundamental role of a chronic inflammatory state in the initiation, progression, and—finally—rupture of the atherosclerotic plaque.[1] During the last decade, humoral autoimmunity and autoantibodies have been recognized as important modulators of vascular inflammation and atherogenesis.[2] Autoantibodies can be active mediators in the development of coronary artery disease (CAD)[3,4] and, as such, serve as biomarkers for the prediction of incident CAD[5–9] and as potential biological targets amenable to immunomodulatory therapies.

Recently, the atherogenic role of autoantibodies against apoA-1 (apolipoprotein A-1; anti-apoA-1 IgG), the principal protein component of high-density lipoprotein (HDL), has been investigated in clinical studies, showing that anti-apoA-1 IgG are associated with prevalent and incident CAD in subjects with autoimmune diseases,[5] subjects at high cardiovascular risk[6,10] or after myocardial infarction,[4,7] independently of established cardiovascular risk factors. Furthermore, we recently showed that anti-apoA-1 IgG are present in up to one fifth of individuals in the general population and independently

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Nonstandard Abbreviations and Acronyms	
Anti-apoA-1 IgG	anti-apolipoprotein A-1 autoantibodies
CAD	coronary artery disease
FU	follow-up
HDL	high-density lipoprotein
HR	hazard ratio
TLR	toll-like receptor

associated with prevalent CAD,[11] as well as with all-cause mortality.[12] Nevertheless, their predictive value for incident CAD in the general population has not yet been studied.

From a pathophysiological point of view, in vitro and in vivo studies have demonstrated that anti-apoA-1 IgG per se behave as proinflammatory, proarrhythmogenic, and prothrombotic molecules, promoting atherogenesis, myocardial necrosis, and death in mice.[4,13] Based on previous published studies, such events could be related to a chronic low-grade inflammatory state,[3,14,15] associations with elevated high-sensitivity C-reactive protein and increased uric acid levels,[11] impairment of HDL antiatherogenic properties,[16–18] interference with basal heart rate regulation,[7,11,15] or breakdown of self-tolerance.[12] However, the main pathophysiological mechanism—reported to date—underpinning the pathogenicity of anti-apoA-1 IgG is their interaction with innate immune system receptors and the activation of the TLR (Toll-like receptor)2/TLR4/CD14 complex.[14,19] In particular, the current paradigm suggests that because of molecular mimicry of the C-terminal part of ApoA-1 to TLR2, anti-apoA-1 IgG bind to the TLR2/TLR4 complex and require a functional CD14 receptor for effective intracellular signaling, NF-κB (nuclear factor-κB) and MAPK (mitogen-activated protein kinase) downstream activation, and production of proinflammatory cytokines.[13]

These findings point to CD14 receptor, the canonical ligand of lipopolysaccharide, as a major effector of the anti-apoA-1 IgG deleterious properties. A functional single nucleotide polymorphism at position C260T (*rs2569190)* of the CD14 receptor gene has been shown to modulate its transcriptional activity.[20,21] Among the 3 groups of CD14 genotypes for *rs2569190* (CC, CT, or TT), TT carriers seem to be protected from CD14 ligand-induced inflammation because of a better ability to adequately control the lipopolysaccharide-mediated TLR/CD14-dependent immune response.[22–24] Indeed, previous studies demonstrated that TT carriers were less at risk for gram-negative bacterial infection and sepsis death[25,26] for developing heart failure,[27] as well as atherosclerosis,[28–30] although this latter observation is debated.[31] However, whether TT carriers are also less susceptible to anti-apoA-1 IgG–related atherosclerosis has not been examined.

Thus, our current study had 2 main aims: first, we investigated whether anti-apoA-1 IgG predict incident CAD in the general population. Second, because of the anti-apoA-1 IgG role as a danger-associated molecular pattern, specifically activating CD14-related pathways,[4,13] we further examined whether the functional C260T *rs2569190* polymorphism in the CD14 receptor gene modulates the anti-apoA-1 IgG–related CAD risk, hypothesizing a protective effect associated with carriage of the T allele.

Materials and Methods

Materials and Methods, including characterization analyses related to anti-apoA-1 IgG assay validation, are available in the online-only Data Supplement.

Results

Association Between Anti-apoA-1 IgG and Incident CAD

Figure 1 demonstrates the flowchart of the study. Of the initial 6733 participants, 5220 had complete clinical and biological data over a median follow-up (FU) time of 5.6 years and were included in the final sample. Participants who did not participate in FU (21.6%) were more likely to be smokers, hypertensive, overweight with a less favorable lipid profile, compared with those included in the analysis. There were no significant differences in anti-apoA-1 IgG levels or prevalence of anti-apoA-1 IgG positivity between the 2 groups (Table I in the online-only Data Supplement).

Table 1 provides baseline characteristics of the final sample according to anti-apoA-1 IgG status. Overall, cardiovascular risk factors were equally distributed between subjects with positive versus negative anti-apoA-1 IgG titers. Among the 157 subjects who developed CAD during FU, 132 had a

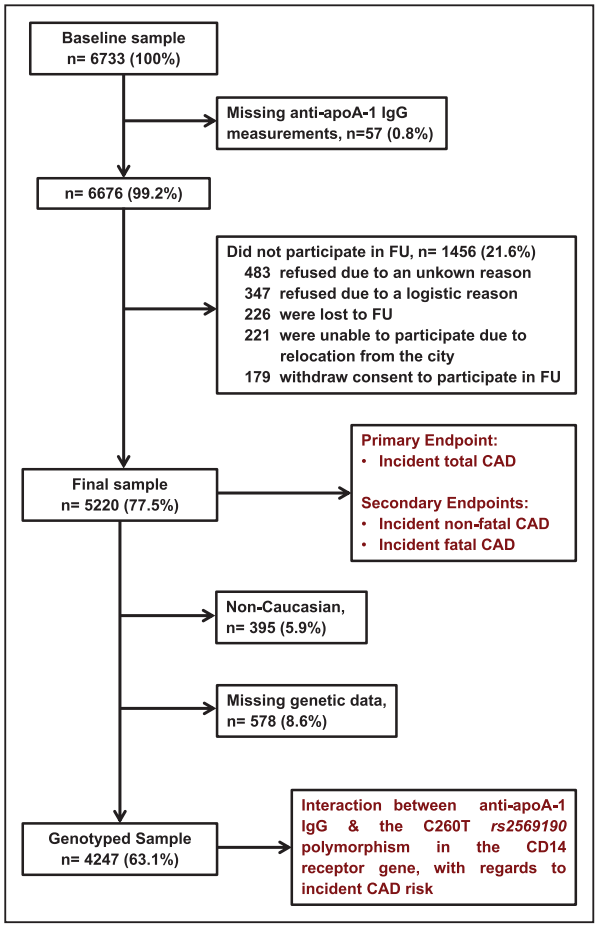

Figure 1. Study flowchart. Anti-apoA-1 IgG indicates autoantibodies against apolipoprotein A-1; CAD, coronary artery disease; and FU, follow-up.

Table 1. Characteristics of the Sample, According to Anti-apoA-1 IgG Status

Total Sample (n=5220)	Anti-apoA-1 IgG		*P* Value
	Absence (n=4180)	Presence (n=1040)	
Age, y	52.7±10.7	52.2±10.7	0.184
Male sex, n (%)	1985 (47.5)	488 (46.9)	0.744
History of CAD, n (%)	146 (3.5)	43 (4.1)	0.321
Current smoking, n (%)	1086 (26.0)	272 (26.2)	0.909
Diabetes mellitus, n (%)	276 (6.6)	58 (5.6)	0.226
Hypertension, n (%)	1389 (33.23)	349 (33.6)	0.841
Autoimmune disease, n (%)	88 (2.1)	32 (3.1)	0.061
Body mass index, kg/m^2	25.6±4.4	25.7±4.6	0.712
Total cholesterol, mmol/L	5.58±1.02	5.50±1.03	0.022
HDL cholesterol, mmol/L	1.64±0.43	1.62±0.46	0.250
LDL cholesterol, mmol/L	3.33±0.90	3.27±0.92	0.068
Triglycerides, mmol/L	1.38±1.12	1.36±1.22	0.663*
SCORE CV risk categories, n (%)			
Low risk	2507 (60.1)	643 (62.0)	
Intermediate risk	1160 (27.8)	269 (25.9)	
High risk	311 (7.4)	83 (8.0)	
Very high risk	196 (4.7)	43 (4.1)	0.487
CV drugs, n (%)			
Aspirin	684 (16.4)	160 (15.4)	0.443
Statins	446 (10.7)	98 (9.4)	0.239
Beta blockers	212 (5.1)	70 (6.7)	0.034
Calcium-channel blockers	120 (2.9)	33 (3.2)	0.605
ACEi/ARB	511 (12.2)	124 (11.9)	0.354
Diuretics	80 (1.9)	19 (1.8)	0.854
Incident CAD rates, n (%)	117 (2.8)	40 (3.9)	0.077
Nonfatal, n (%)	95 (2.3)	37 (3.6)	0.018
Fatal, n (%)	22 (0.5)	3 (0.3)	0.320

Data are expressed as mean±standard deviation or number of participants and (percentage). ACEi indicates angiotensin-converting enzyme inhibitor; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; ARB, angiotensin receptor blockers; CAD, coronary artery disease; CV, cardiovascular; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SCORE, Systematic Coronary Risk Evaluation.

*Statistical analysis for continuous variables by student's *t* test or Mann–Whitney test depending on the normality assumption. Statistical analysis for categorical variables by the χ^2 test.

nonfatal event and 25 a fatal one. Total incident CAD rate was 3.9% versus 2.8% (*P*=0.077), while nonfatal incident CAD rate was 3.6% versus 2.3% (*P*=0.018) for subjects with positive versus negative anti-apoA-1 IgG titers. No significant differences were observed with regards to fatal incident CAD.

Table 2 summarizes hazard ratios (HR) for the association of anti-apoA-1 IgG with total, nonfatal, and fatal incident CAD. In unadjusted models, we retrieved a trend between anti-apoA-1 IgG positivity and total incident CAD (HR, 1.39; 95% confidence intervals (CI), 0.97–1.99; *P*=0.073) that remained unchanged after adjusting for sex, age, smoking status, diabetes mellitus, systolic blood pressure, low-density lipoprotein and HDL cholesterol, baseline CAD, statin and β-blocker treatment, and estimated glomerular filtration rate (HR, 1.36; 95% CI, 0.94–1.97; *P*=0.105). The HRs of 1 SD increase in log-transformed anti-apoA-1 IgG values for total incident CAD were HR, 1.11 (95% CI, 0.96–1.28; *P*=0.159) and HR, 1.09 (95% CI, 0.94–1.27; *P*=0.232) in the unadjusted and adjusted analyses, respectively. Levels of anti-apoA-1 IgG above optical density >0.98 (third tertile) were significantly associated with total incident CAD in the unadjusted (HR, 1.79; 95% CI, 1.09–2.95; *P*=0.021) and the adjusted analysis (HR, 1.70; 95% CI, 1.03–2.81; *P*=0.038).

Furthermore, anti-apoA-1 IgG positivity was significantly associated with nonfatal incident CAD both in the unadjusted (HR, 1.58; 95% CI, 1.08–2.31; *P*=0.018) and the adjusted analysis (HR, 1.53; 95% CI, 1.03–2.26; *P*=0.034). Similarly to what was observed for total incident CAD, the HRs of 1 SD increase in log-transformed anti-apoA-1 IgG values for nonfatal CAD were as follows: HR, 1.15 (95% CI, 0.99–1.34; *P*=0.072) and HR, 1.14 (95% CI, 0.97–1.33; *P*=0.109) in the unadjusted and adjusted analyses, respectively. Anti-apoA-1 IgG levels above optical density >0.98 (third tertile) were strongly associated with nonfatal incident CAD both in the unadjusted (HR, 2.21; 95% CI, 1.34–3.67; *P*=0.002) and the adjusted model (HR, 2.14; 95% CI, 1.29–3.56; *P*=0.003; Table 2). On the contrary, no associations were observed between anti-apoA-1 IgG positivity or tertiles with fatal incident CAD. Sensitivity analyses after exclusion of subjects with baseline CAD or autoimmune disease yielded similar results for the associations between anti-apoA-1 IgG and total, nonfatal, and fatal CAD (Table II in the online-only Data Supplement). Additionally, statistical analyses after excluding adjustment for statin and β-blocker treatment or estimated glomerular filtration rate from the fully adjusted model yielded similar results (Table III in the online-only Data Supplement).

Interaction Between C260T *rs2569190* Polymorphism and Anti-apoA-1 IgG for Incident CAD

Among genotyped subjects (n=4247; Figure 1), we further investigated whether the functional C260T *rs2569190* polymorphism in the CD14 receptor gene modulates anti-apoA-1 IgG–related CAD risk. Subjects with missing genetic data tended to have a lower burden of cardiovascular risk factors and a higher prevalence of anti-apoA-1 IgG positivity (Tables IV and V in the online-only Data Supplement).

Characteristics of the genotyped sample according to the C260T *rs2569190* polymorphism allele status are illustrated in Table VI in the online-only Data Supplement. All cardiovascular risk factors were equally distributed among subgroups, with the exception of an increased prevalence of diabetes mellitus and statin treatment in the TT subgroup. Importantly, the C260T *rs2569190* polymorphism per se was not associated with total, nonfatal, or fatal incident CAD, all-cause mortality, or anti-apoA-1 IgG positivity (Table VI in the online-only Data Supplement).

Table 2. Hazard Ratios of Anti-apoA-1 IgG for Incident Total, Nonfatal, and Fatal CAD in the General Population

	Total Incident CAD (n=159)				Nonfatal Incident CAD (n=134)				Fatal Incident CAD (n=25)			
n=5220	Unadjusted Model	*P* Value	Adjusted Model	*P* Value	Unadjusted Model	*P* Value	Adjusted Model	*P* Value	Unadjusted Model	*P* Value	Adjusted Model	*P* Value
Positive vs negative	1.39 (0.97–1.99)	0.073	1.36 (0.94–1.97)	0.105	1.58 (1.08–2.31)	0.018	1.53 (1.03–2.26)	0.034	0.54 (0.16–1.80)	0.313	0.56 (0.17–1.91)	0.356
1 SD change in log-transformed anti-ApoA-1 IgG levels	1.11 (0.96–1.28)	0.159	1.09 (0.94–1.27)	0.232	1.15 (0.99–1.34)	0.072	1.14 (0.97–1.33)	0.109	0.88 (0.61–1.29)	0.520	0.87 (0.60–1.27)	0.474
Anti-ApoA-1 IgG levels*												
Negative (OD<0.64)	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
First tertile (0.64<OD≤0.77)	1.18 (0.64–2.19)	0.597	1.39 (0.74–2.59)	0.879	1.32 (0.69–2.53)	0.406	1.50 (0.78–2.89)	0.227	0.60 (0.08–4.43)	0.613	0.75 (0.96–5.92)	0.788
Second tertile (0.77<OD≤0.98)	1.16 (0.63–2.16)	0.633	0.95 (0.48–1.88)	0.879	1.17 (0.59–2.33)	0.646	0.89 (0.41–1.93)	0.767	1.02 (0.24–4.37)	0.974	1.13 (0.26–4.90)	0.872
Third tertile (OD>0.98)	1.79 (1.09–2.95)	0.021	1.70 (1.03–2.81)	0.038	2.21 (1.34–3.67)	0.002	2.14 (1.29–3.56)	0.003	No subjects		No subjects	
P value for linear trend	0.047		0.160		0.012			0.021				

Results are expressed as adjusted hazard ratios and (95% confidence interval) for subjects positive (OD>0.64) vs negative (OD<0.64) for anti-apoA-1 IgG. Statistical analysis by Cox proportional hazards regression adjusted for age, sex, systolic blood pressure, diabetes mellitus, smoking, HDL and LDL cholesterol, baseline CAD, statin, β-blocker treatment, and eGFR. Anti-apoA-1 IgG indicates anti-apolipoprotein A-1 autoantibodies; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; OD, optical density; and SD, standard deviation.

*Subjects with positive Anti-ApoA-1 (n=1040) were divided in tertiles (n=347) of increasing titers: first tertile (0.64<OD<0.77), second tertile (0.77<OD<0.98), and third tertile (OD>0.98).

To assess differences in anti-apoA-1 IgG–related CAD risk according to the C260T *rs2569190* polymorphism, we created both an additive (CC versus CT versus TT), as well as a recessive (CC/CT versus TT) model and performed a statistical test for the interaction[32] between anti-apoA-1 IgG and carriage of the T allele for total and nonfatal incident CAD risk. As previously, all analyses were adjusted for sex, age, smoking status, diabetes mellitus, systolic blood pressure, low-density lipoprotein and HDL cholesterol, baseline CAD, statin and β-blocker treatment, and estimated glomerular filtration rate.

In the case of the additive model (CC versus CT versus TT), we observed a gradient of risk for anti-apoA-1 IgG with regards to CAD across the 3 predefined C260T *rs2569190* subgroups (Table 3). Specifically, in the subgroup homozygote for the major allele (CC, n=1097), the adjusted anti-apoA-1 IgG HR for total CAD was HR, 2.27 (95% CI, 1.04–4.97; *P*=0.039), while it was HR, 1.52 (95% CI, 0.86–2.71; *P*=0.152) in the heterozygote subgroup (CT, n=2095) and HR, 0.55 (95% CI, 0.19–1.61; *P*=0.275) in the minor allele subgroup (TT, n=1055). Results were similar with regards to the recessive (CC/CT versus TT) model. Notably, in non-TT carriers—representing 75.1% of the cohort—anti-apoA-1 IgG positivity conferred a 1.8-fold risk for total CAD (HR, 1.77; 95% CI, 1.12–2.80; *P*=0.014; Table 3), while change per 1 SD in anti-apoA-1 values yielded a HR of 1.11 (95% CI, 0.92–1.34; *P*=0.285) for total CAD in the fully adjusted model. Results were similar with regards to nonfatal incident CAD (Table 3).

Testing for the interaction between anti-apoA-1 IgG and C260T *rs2569190* polymorphism with respect to CAD in the fully adjusted analysis indicated that the observed gradient in anti-apoA-1 IgG–related CAD risk across the different CD14 genotype subgroups in the additive (CC versus CT versus TT) model was statistically significant for both total and nonfatal CAD risk (*P* for interaction =0.011 and *P* for interaction =0.033, respectively; Table 3), proving substantial heterogeneity in anti-apoA-1 IgG–related CAD risk according to T allele carriage. A forest plot summarizes these findings (Figure I in the online-only Data Supplement). Furthermore, statistical analyses after excluding adjustment for statin and β-blocker treatment or estimated glomerular filtration rate from the fully adjusted model yielded similar results (Table VII in the online-only Data Supplement).

Figure 2 describes Kaplan–Meier curves for total and nonfatal CAD according to anti-apoA-1 IgG positivity and C260T *rs2569190* allele status. Participants positive for anti-apoA-1 IgG (Figure 2A and 2B) presented an increased risk for total and nonfatal CAD compared with those negative for anti-apoA-1 IgG. After splitting the positive anti-apoA-1 IgG group according to homozygous or not carriage of the T allele (CC/CT versus TT), a decrease in the proportion of total and nonfatal CAD was observed in the anti-apoA-1 IgG–positive TT subgroup (Figure 2C and 2D, green line), falling below the rate of CAD observed in anti-apoA-1 IgG–negative subjects (Figure 2C and 2D, blue line). Conversely, higher proportion of total and nonfatal CAD was observed in anti-apoA-1 IgG–positive non-TT carriers (Figure 2C and 2D, black line) when compared with anti-apoA-1 IgG–positive subjects as a whole (Figure 2C and 2D, red line, log-rank: *P*=0.023 and *P*=0.017 for total and nonfatal CAD, respectively).

Table 3. Hazard Ratios of Anti-apoA-1 IgG for Incident Total, Nonfatal, and Fatal CAD According to the C260T *rs2569190* Polymorphism Allele Status, in the Genotyped Population

Anti-apoA-1 IgG, HR (95% CI), for CAD	Total Incident CAD (n=132)				Nonfatal Incident CAD (n=109)				Fatal Incident CAD (n=23)			
	Unadjusted Model	*P* Value	Adjusted Model	*P* Value	Unadjusted Model	*P* Value	Adjusted Model	*P* Value	Unadjusted Model	*P* Value	Adjusted Model	*P* Value
C260T *rs2569190* allele status												
CC (n=1097)	2.08 (0.98–4.42)	0.056	2.27 (1.04–4.97)	0.039	2.19 (0.98–4.87)	0.055	2.38 (1.03–5.51)	0.042	1.39 (0.14–13.42)	0.773	1.55 (0.15–15.81)	0.713
CC/CT (n=3192)	1.67 (1.07–2.60)	0.023	1.77 (1.12–2.80)	0.014	1.84 (1.14–2.95)	0.012	1.95 (1.19–3.19)	0.008	0.91 (0.26–3.17)	0.880	0.90 (0.24–3.32)	0.877
CT (n=2095)	1.55 (0.89–2.68)	0.120	1.52 (0.86–2.71)	0.152	1.75 (0.96–3.16)	0.066	1.73 (0.93–3.23)	0.084	0.76 (0.17–3.43)	0.718	0.54 (0.10–3.00)	0.486
TT (n=1055)	0.58 (0.20–1.65)	0.306	0.55 (0.19–1.61)	0.275	0.74 (0.25–2.14)	0.573	0.74 (0.25–2.22)	0.592	No subjects		No subjects	
P value for interaction between anti-apoA-1 IgG and rs2569190 (CC vs CT vs TT)		0.064		0.011		0.135		0.033		NA		NA
P value for interaction between anti-apoA-1 IgG and rs2569190 (CC/CT vs TT)		0.068		0.020		0.126		0.047		NA		NA

Results are expressed as adjusted hazard ratios and (95% confidence interval) for subjects positive (OD>0.64) vs negative (OD<0.64) for anti-apoA-1 IgG. Statistical analysis by Cox proportional hazards regression adjusted for age, sex, systolic blood pressure, diabetes mellitus, smoking, HDL and LDL cholesterol, baseline CAD, statin, β-blocker treatment, and eGFR. The *P* value for interaction represents the likelihood of interaction between the C260T *rs2569190* allele status and the relative anti-apoA-1 IgG effect for coronary artery disease. Anti-apoA-1 IgG indicates anti-apolipoprotein A-1 autoantibodies; CAD, coronary artery disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low density lipoprotein; and OD, optical density.

Discussion

The main finding of the present study is that anti-apoA-1 IgG are independently associated with nonfatal incident CAD in the general population, with the anti-apoA-1 IgG–related CAD risk being strongly modulated by the C260T *rs2569190* CD14 gene polymorphism. Indeed, after taking CD14 single nucleotide polymorphisms into account, we observed a significant anti-apoA-1 IgG–related CAD risk gradient, dependent on carriage of the C260T *rs2569190* T allele, with non-TT carriers being at significantly increased risk for both total and nonfatal CAD compared with TT homozygotes. Our results extend current knowledge not only in the field of anti-apoA-1 IgG but also in the field of personalized CAD prediction in different ways.

First, similarly to what has been shown in high-risk populations,[5–7,33] our current findings argue that anti-apoA-1 IgG positivity is an independent predictor of poor CV outcome in the general population, supporting the notion that preclinical autoimmunity to apoA-1 may identify a substantial proportion of individuals at increased risk of CAD. In our study, anti-apoA-1 IgG–related CAD risk was highest in subjects carrying at least one C allele (CC/CT) in the functional C260T *rs2569190* polymorphism, a group that represents roughly 3 quarters of White populations.[31] By virtue of being the first study on a gene–autoantibody interaction with respect to CAD, our analysis highlights the importance of incorporating genetic data on immune-related polymorphisms when evaluating anti-apoA-1 IgG–related risk and provides insight for future study design on individualized CAD prediction.

Second, these results represent a human validation of the key role of CD14 coreceptor in mediating the anti-apoA-1 IgG proatherogenic properties as demonstrated to date in animal and in vitro models[4,13] and reinforce the relevance of these preclinical results to the anti-apoA-1 IgG–associated CAD risk in humans. Conversely, in line with a recent meta-analysis,[31] our findings are equivocal and do not provide definite evidence with regards to the association between TT genotype carriage and CAD risk.

Third, our data highlight the importance of considering the individual genetic information on innate immune receptors for proper assessment of CAD risk associated with biomarkers of humoral autoimmunity. To the best of our knowledge, none of the genetic studies published to date took into account biomarkers (including autoantibodies) for CAD risk prediction, and none of the publications exploring the auto-antibodies–associated CAD risk prediction evaluated the impact of individual genetic background on such risk. By demonstrating a potentially important gene–environment interaction between anti-apoA-1 IgG and the CD14 receptor gene in the pathogenesis of atherosclerosis, our findings may explain the discordant findings regarding both the role of CD14 polymorphisms in CAD prediction[31] and the contrasting results of humoral auto-autoimmunity in CAD risk assessment.[33] Overall, our results provide a proof-of-concept that combining genetic data together with serum biomarkers is likely to be required for the implementation of precision medicine in the field of CAD prediction.

In our study, the fact that TT carriers were less at risk to develop anti-apoA-1 IgG–related CAD compared with

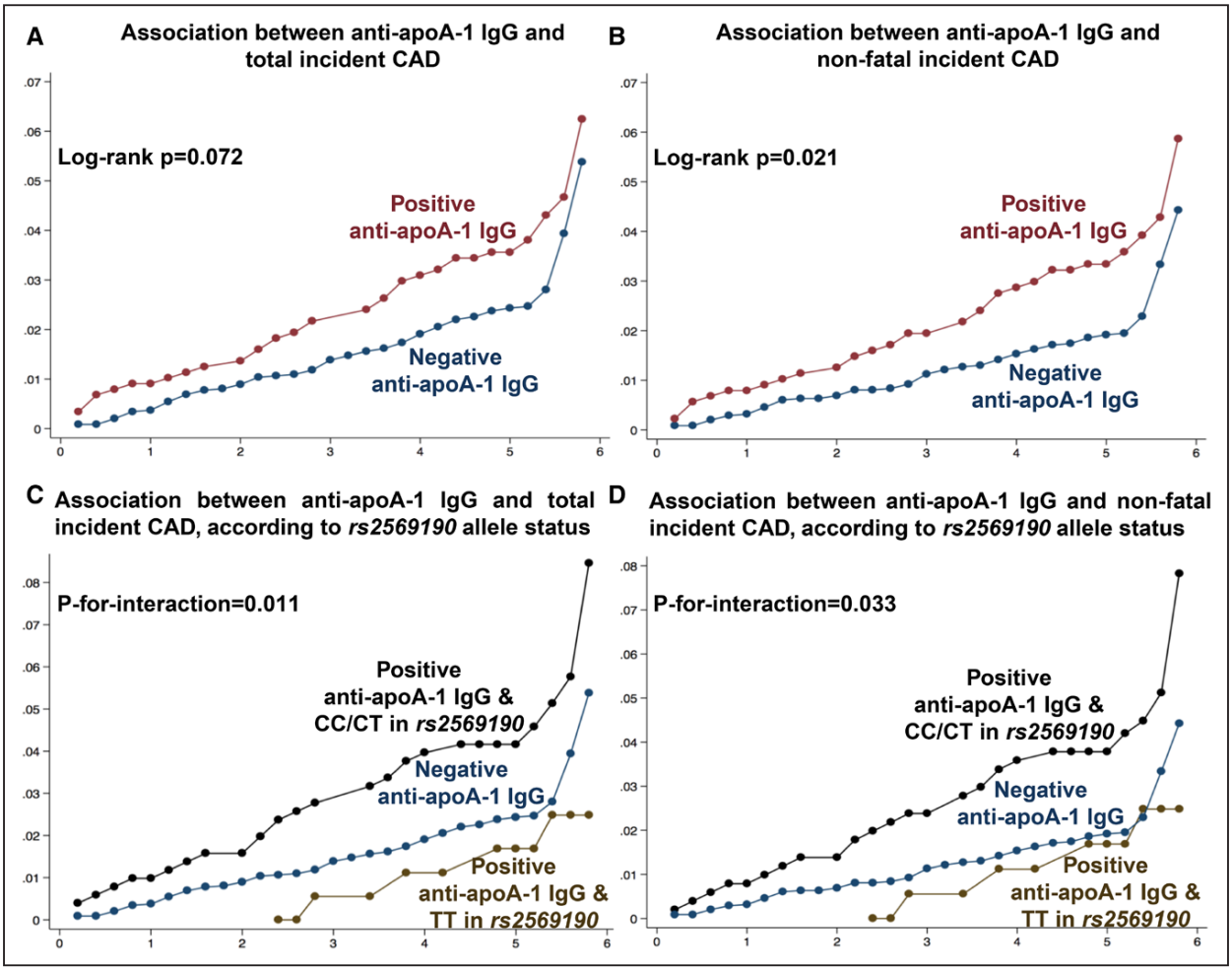

Figure 2. Kaplan–Meier curves for cumulative incident coronary artery disease. **Top**, Kaplan–Meier curves for cumulative (**A**) total and (**B**) nonfatal incident CAD according to anti-apoA-1 IgG status (red line, positive for anti-apoA-1 IgG; blue line, negative for anti-apoA-1 IgG). **Bottom**, Kaplan–Meier curves for cumulative (**C**) total and (**D**) nonfatal incident CAD according to anti-apoA-1 IgG and C260T *rs2569190* allele status (black line, positive for anti-apoA-1 IgG and carrying the C allele (CC/CT); green line, positive for anti-apoA-1 IgG and carrying the T allele (TT); blue line, negative for anti-apoA-1 IgG). Data are expressed as the cumulative proportion of the sample presenting with incident CAD (*y* axis) during study years (*x* axis). Statistical analysis by Log rank test, for the comparison between anti-apoA-1 IgG negative subjects (blue line) vs anti-apoA-1 IgG positive-TT carriers (green line) vs anti-apoA-1 IgG positive-non-TT carriers (black line). Anti-apoA-1 IgG indicates autoantibodies against apolipoprotein A-1; and CAD, coronary artery disease.

non-TT carriers, despite TT homozygote status being previously associated with a higher systemic inflammatory profile,[20,21] merits mention. To this respect, several lines of evidence indicate that TT genotype could confer protection against uncontrolled inflammatory response evoked by long-term danger-associated molecular pattern exposure through different and mutually nonexclusive mechanisms. First, previous studies indicate that in the context of chronic low-grade CD14/TLR4 stimulation, the higher levels of sCD14 ascribed to the TT genotype inhibit systemic lipopolysaccharide-mediated inflammatory responses by downregulating inflammatory cytokines transcription[34,35] and facilitating CD14-related danger-associated molecular pattern clearance,[24] thus, protecting TT carriers against sustained inflammatory responses through a negative feedback mechanism. Inversely, lower levels of sCD14 observed in CC carriers have been shown to favor vascular wall inflammation and atherogenesis through impaired plasma clearance of endotoxin.[22,23] C-allele carriers may be less able to prevent anti-apoA-1 IgG–mediated CD14/TLR4 activation, resulting in maintenance of a proatherogenic state and a higher risk for developing CAD.[28–30] Finally, increased expression of CD14 on the vascular endothelium of TT homozygotes[35] could also play a protective role in atherogenesis, in response to CD14 ligands such as anti-apoA-1 IgG.[22,23,34,35]

Several study limitations are noteworthy. First, although great effort was undertaken to maximize the participation rate during FU, our results may be subject to attrition bias as dropout rate after mean duration of 5.6 years was ≈20%. Nevertheless, similar losses in FU are commonly reported in prospective cohorts[36] and are within the conventional participation rate thresholds for cohort studies.[37] Second, we did not directly measure sCD14 in study subjects to confirm the presumed higher sCD14 levels in TT homozygote carriers reported previously. Moreover, as our assay assesses

anti-apoA-1IgG antibodies against native apoA-1,[19,38] we were not able to measure antibodies against modified forms of apoA-1, such as oxidized apoA-1 (or possibly glycated and carbamylated apoA-1). Because these modified forms of apoA-1 were shown to be of relevance for HDL functionality and the pathology of atherosclerosis,[39–41] knowing whether they would elicit a humoral response clinically relevant to human physiopathology is still unclear. Third, because of sample availability, we only measured baseline anti-apoA-1 IgG levels and did not assess the dynamic of anti-apoA-1 IgG levels over time in relation with incident CAD. Moreover, we could not test other clinically relevant antibodies, such as anti-oxidized low-density lipoprotein, anti-phospholipid, antinuclear or antiheat shock protein antibodies, which would have been instrumental to better understanding potential associations with innate immune receptor–related genes of interest. Finally, sample size calculation in our study was performed with regards to the primary outcome of detecting a difference in incident CAD in subjects positive versus negative for anti-apoA-1 IgG. Although the fact that previous evidence suggested an interaction between anti-apoA-1 IgG and the CD14 receptor and that we were able to detect such a—significant—interaction between anti-apoA-1 IgG and the functional C260T *rs2569190* polymorphism in the CD14 receptor gene, it is possible that the current sample size provided <80% power for this secondary study outcome. Therefore, this finding requires replication in larger prospective studies.

In conclusion, anti-apoA-1 IgG levels are independent predictors of incident nonfatal CAD in the general population. The strength of this association is significantly modulated by the functional C260T *rs2569190* single nucleotide polymorphism in the CD14 receptor gene, being the highest in non-TT carriers and the lowest in TT homozygotes. These results imply that preclinical autoimmunity to apoA-1 should be evaluated carefully as it may help to improve the identification of individuals at increased risk of CAD in the general population, especially in non-TT carriers representing ≈75% of the population. Our findings indicate that gene–autoantibodies interaction studies are likely to be required to better assess the CAD risk related to humoral autoimmunity biomarkers in the general population, a concept that requires further investigation.

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Highlights

- Anti-apoA-1 (apolipoprotein A-1) IgG are independent predictors of nonfatal incident coronary artery disease in the general population.
- Anti-apoA-1 IgG could represent a potential novel target for immune-modulating preventive strategies for coronary artery disease.
- The strength of the association between anti-apoA-1 IgG and coronary artery disease is dependent on a functional polymorphism of the CD14 receptor gene.
- Our findings suggesting a gene–autoantibody interaction for the development of CAD, an observation that requires further study.

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