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

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1 **Title Page**

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3 **Full Title**

4 **Impact of CD14 polymorphisms on anti-apolipoprotein A-1 IgG-related coronary artery**
5 **disease prediction in the general population**

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9 **Running Title**

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1 **Panagiotis Antiochos** ¹; **Pedro Marques-Vidal** ¹; **Julien Virzi** ^{2, 3}; **Sabrina Pagano** ^{2, 3};
2 **Nathalie Satta** ^{2, 3}; **Oliver Hartley** ⁴; **Fabrizio Montecucco** ^{2, 5, 6}; **François Mach** ⁵; **Zoltan**
3 **Kutalik** ^{7, 8}; **Gerard Waeber** ¹; **Peter Vollenweider** ^{1*}; **Nicolas Vuilleumier** ^{2, 3*}

4 * These authors contributed equally

5

6 Authors' affiliations:

7 ¹ Department of Internal Medicine, University Hospital of Lausanne, 46 rue du Bugnon, 1011
8 Lausanne, Switzerland

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

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

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

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

17 ⁶ First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, 16143
18 Genoa, Italy.

19 ⁷ Institute of Social and Preventive Medicine, University Hospital of Lausanne, 1010 Lausanne,
20 Switzerland

21 ⁸ Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland

22

1 **CORRESPONDING AUTHOR:**
2 Dr. Panagiotis Antiochos
3 CoLaus Study, Bâtiment des Instituts
4 19, Rue du Bugnon
5 CH-1005 Lausanne
6 Switzerland
7 Tel.: +41 79 556 40 11
8 Fax: +41 21 314 80 37
9 Email: panagiotis.antiochos@chuv.ch
10
11

1 **ABSTRACT**

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

7 **Approach and Results:** In a prospectively-studied, population-based cohort of 5220 subjects
8 (mean age 52.6 ± 10.7 years, 47.4% males), followed over a median period of 5.6 years,
9 subjects positive vs. negative for anti-apoA-1 IgG, presented a total CAD rate of 3.9% vs. 2.8%
10 ($p=0.077$), and a non-fatal CAD rate of 3.6% vs. 2.3% ($p=0.018$) respectively. After multivariate
11 adjustment, the hazard ratio (HR) of anti-apoA-1 IgG were: HR=1.36 [(95% Confidence Intervals
12 (CI) 0.94–1.97), $p=0.105$], and HR=1.53 [(95%CI 1.03–2.26), $p=0.034$], for total and non-fatal
13 CAD, respectively. In subjects with available genetic data for the C260T *rs2569190* SNP in the
14 CD14 receptor gene ($n=4247$), we observed a significant interaction between anti-apoA-1 IgG
15 and *rs2569190* allele status with regards to CAD risk, with anti-apoA-1 IgG conferring the
16 highest risk for total and non-fatal CAD in non-TT carriers, whereas being associated with the
17 lowest risk for total and non-fatal CAD in TT homozygotes (“p-for-interaction”=0.011 and “p-for
18 interaction”=0.033 respectively).

19 **Conclusions:** Anti-apoA-1 IgG are independent predictors of non-fatal incident CAD in the
20 general population. The strength of this association is dependent on a functional polymorphism
21 of the CD14 receptor gene, a finding suggesting a “gene-autoantibody” interaction for the
22 development of CAD.

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24

25 **ABBREVIATIONS**

26 **CAD:** coronary artery disease

27 **Anti-apoA-1 IgG:** anti-apolipoprotein A-1 autoantibodies

28 **TLR:** toll-like receptor

29 **SNP:** single nucleotide polymorphism

30 **DAMP:** danger-associated molecular pattern

31 **HDL:** high-density lipoprotein cholesterol

32 **LDL:** low-density lipoprotein cholesterol

33 **SBP:** systolic blood pressure

34 **FU:** follow-up

35 **eGFR:** estimated glomerular filtration rate

36 **CVRF:** cardiovascular risk factors

37 **HR:** hazard ratio

38 **OD:** optical density

39

1 INTRODUCTION

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

9 Recently, the atherogenic role of autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG),
10 the principal protein component of high-density lipoprotein (HDL) has been investigated in
11 clinical studies, showing that anti-apoA-1 IgG are associated with prevalent and incident CAD in
12 subjects with autoimmune diseases ⁵, subjects at high CV risk ^{6, 10} or following myocardial
13 infarction ^{4,7}, independently of established cardiovascular risk factors (CVRFs). Furthermore, we
14 recently showed that anti-apoA-1 IgG are present in up to one-fifth of individuals in the general
15 population and independently associated with prevalent CAD ¹¹ as well as with all-cause
16 mortality ¹². Nevertheless, their predictive value for incident CAD in the general population has
17 not yet been studied.

18 From a pathophysiological point of view, *in vitro* and *in vivo* studies have demonstrated that anti-
19 apoA-1 IgG *per se* behave as pro-inflammatory, pro-arrhythmogenic and pro-thrombotic
20 molecules, promoting atherogenesis, myocardial necrosis and death in mice. ^{4, 13} Based upon
21 previous published studies, such events could be related to a chronic low-grade inflammatory
22 state, ^{3, 14, 15} associations with elevated high-sensitivity C-reactive protein and with increased
23 uric acid levels, ¹¹ impairment of HDL anti-atherogenic properties, ¹⁶⁻¹⁸ interference with basal
24 heart rate regulation ^{7, 11, 15} or breakdown of self-tolerance. ¹² However, the main
25 pathophysiological mechanism – reported so far – underpinning the pathogenicity of anti-apoA-1
26 IgG is their interaction with innate immune system receptors and the activation of the
27 TLR2/TLR4/CD14 complex. ^{14, 19} In particular, the current paradigm suggests that, due to
28 molecular mimicry of the C-terminal part of ApoA-1 to Toll-like receptor (TLR) TLR2, anti-apoA-1
29 IgG bind to the TLR2/TLR4 complex, and require a functional CD14 receptor for effective
30 intracellular signaling, NF-κB and MAPK downstream activation, and production of pro-
31 inflammatory cytokines. ¹³

32 These findings point to CD14 receptor, the canonical ligand of lipopolysaccharid (LPS), as a
33 major effector of the anti-apoA-1 IgG deleterious properties. A functional single nucleotide
34 polymorphism (SNP) at position C260T (*rs2569190*) of the CD14 receptor gene has been
35 shown to modulate its transcriptional activity. ^{20, 21} Among the three groups of CD14 genotypes
36 for *rs2569190* (CC, CT or TT), TT carriers appear to be protected from CD14 ligand-induced
37 inflammation due to a better ability to adequately control the LPS-mediated TLR/CD14–
38 dependent immune response. ²²⁻²⁴ Indeed, previous studies demonstrated that TT carriers were
39 less at risk for Gram-negative bacterial infection and sepsis death, ^{25, 26} for developing heart
40 failure, ²⁷ as well as atherosclerosis, ²⁸⁻³⁰ although this latter observation is debated. ³¹ However,
41 whether TT carriers are also less susceptible to anti-apoA-1 IgG-related atherosclerosis has not
42 been examined.

43 Thus, our current study had two main aims: firstly, we investigated whether anti-apoA-1 IgG
44 predict incident CAD in the general population. Secondly, because of anti-apoA-1 IgG role as a
45 danger-associated molecular pattern (DAMP), specifically activating CD14-related pathways ⁴.

1¹³, 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.

5 MATERIALS AND METHODS

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

9 RESULTS

10 Association between anti-apoA-1 IgG and incident CAD

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

17 **Table 1** provides baseline characteristics of the final sample according to anti-apoA-1 IgG
18 status. Overall, CVRFs were equally distributed between subjects with positive vs. negative anti-
19 apoA-1 IgG titers. Among the 157 subjects who developed CAD during FU, 132 had a non-fatal
20 event, and 25 a fatal one. Total Incident CAD rate was 3.9% vs. 2.8% ($p=0.077$), while non-fatal
21 incident CAD rate was 3.6% vs. 2.3% ($p=0.018$) for subjects with positive vs. negative anti-
22 apoA-1 IgG titers. No significant differences were observed with regards to fatal incident CAD.

23 **Table 2** summarizes hazard ratios (HR) for the association of anti-apoA-1 IgG with total, non-
24 fatal and fatal incident CAD. In unadjusted models, we retrieved a trend between anti-apoA-1
25 IgG positivity and total incident CAD (HR: 1.39, 95% Confidence Intervals (CI): 0.97–1.99,
26 $p=0.073$), that remained unchanged after adjusting for sex, age, smoking status, diabetes,
27 systolic blood pressure (SBP), LDL and HDL cholesterol, baseline CAD, statin and beta-blocker
28 treatment, and estimated glomerular filtration rate (eGFR) (HR: 1.36, 95%CI: 0.94–1.97,
29 $p=0.105$). The HRs of one SD increase in log-transformed anti-apoA-1 IgG values for total
30 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
31 ($p=0.232$), in the unadjusted and adjusted analysis respectively. Levels of anti-apoA-1 IgG
32 above OD>0.98 (3rd tertile) were significantly associated with total incident CAD in the
33 unadjusted (HR 1.79, 95%CI 1.09–2.95, $p=0.021$) and the adjusted analysis (HR: 1.70, 95%CI:
34 1.03–2.81, $p=0.038$).

35 Furthermore, anti-apoA-1 IgG positivity was significantly associated with non-fatal incident CAD
36 both in the unadjusted (HR: 1.58, 95%CI 1.08–2.31, $p=0.018$) and the adjusted analysis (HR:
37 1.53, 95%CI 1.03–2.26, $p=0.034$). Similarly to what was observed for total incident CAD, the
38 HRs of one SD increase in log-transformed anti-apoA-1 IgG values for non-fatal CAD were: HR:
39 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
40 unadjusted and adjusted analysis respectively. Anti-apoA-1 IgG levels above OD>0.98 (3rd
41 tertile) were strongly associated with non-fatal incident CAD both in the unadjusted (HR: 2.21,
42 95%CI 1.34–3.67, $p=0.002$) and the adjusted model (HR: 2.14, 95%CI 1.29–3.56, $p=0.003$)
43 (**Table 2**). On the other hand, no associations were observed between anti-apoA-1 IgG positivity
44 or tertiles with fatal incident CAD. Sensitivity analyses after exclusion of subjects with baseline
45 CAD or autoimmune disease yielded similar results for the associations between anti-apoA-1

1 IgG and total, non-fatal and fatal CAD (**Supplementary Table II**). Additionally, statistical
2 analyses after excluding adjustment for statin and beta-blocker treatment, or eGFR from the
3 fully adjusted model yielded similar results (**Supplementary Table, III**).

4 5 **Interaction between C260T *rs2569190* polymorphism and anti-apoA-1 IgG for incident** 6 **CAD**

7 Among genotyped subjects (n=4247, **Figure 1**), we further investigated whether the functional
8 C260T *rs2569190* polymorphism in the CD14 receptor gene modulates anti-apoA-1 IgG-related
9 CAD risk. Subjects with missing genetic data tended to have a lower burden of CVRFs and a
10 higher prevalence of anti-apoA-1 IgG positivity (**Supplementary Tables IV & V**).

11 Characteristics of the genotyped sample, according to the C260T *rs2569190* polymorphism
12 allele status are illustrated in **Supplementary Table VI**. All CVRFs were equally distributed
13 among subgroups, with the exception of an increased prevalence of diabetes and statin
14 treatment in the TT subgroup. Importantly, the C260T *rs2569190* polymorphism *per se* was not
15 associated with total, non-fatal or fatal incident CAD, all-cause mortality or anti-apoA-1 IgG
16 positivity (**Supplementary Table VI**).

17 In order to assess differences in anti-apoA-1 IgG-related CAD risk according to the C260T
18 *rs2569190* polymorphism, we created both an additive (CC vs. CT vs. TT) as well as a
19 recessive (CC/CT vs. TT) model and performed a statistical test for the interaction³² between
20 anti-apoA-1 IgG and carriage of the T allele, for total and non-fatal incident CAD risk. As
21 previously, all analyses were adjusted for sex, age, smoking status, diabetes, SBP, LDL and
22 HDL cholesterol, baseline CAD, statin and beta-blocker treatment and eGFR.

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

34 Testing for the interaction between anti-apoA-1 IgG and C260T *rs2569190* polymorphism with
35 respect to CAD in a fully adjusted analysis, indicated that the observed gradient in anti-apoA-1
36 IgG-related CAD risk across the different CD14 genotype subgroups in the additive (CC vs. CT
37 vs. TT) model was statistically significant, for both total and non-fatal CAD risk (“p-for-
38 interaction”=0.011, and “p-for-interaction”=0.033, respectively; **Table 3**), proving substantial
39 heterogeneity in anti-apoA-1 IgG-related CAD risk according to T allele carriage. A forest plot
40 summarizes these findings (**Supplementary Figure 1**). Furthermore, statistical analyses after
41 excluding adjustment for statin and beta-blocker treatment, or eGFR from the fully adjusted
42 model yielded similar results (**Supplementary Table, VII**).

43 **Figure 2** describes Kaplan-Meier curves for total and non-fatal CAD according to anti-apoA-1
44 IgG positivity and C260T *rs2569190* allele status. Participants positive for anti-apoA-1 IgG
45 (panels A and B) presented an increased risk for total and non-fatal CAD compared to those
46 negative for anti-apoA-1 IgG. After splitting the positive anti-apoA-1 IgG group according to
47 homozygous or not carriage of the T allele (CC/CT vs. TT), a decrease in the proportion of total

1 and non-fatal CAD was observed in the anti-apoA-1 IgG positive TT subgroup (panels C and D,
2 green line), falling below the rate of CAD observed in anti-apoA-1 IgG negative subjects (panels
3 C and D, blue line). Conversely, higher proportion of total and non-fatal CAD was observed in
4 anti-apoA-1 IgG positive non-TT carriers (panels C and D, black line), when compared to anti-
5 apoA-1 IgG positive subjects as a whole (panels C and D red line, log-rank: $p=0.023$, and
6 $p=0.017$, for total and non-fatal CAD respectively).
7

1 DISCUSSION

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

10 Firstly, similarly to what has been shown in high risk populations,^{5-7, 33} our current findings
11 argue that anti-apoA-1 IgG positivity is an independent predictor of poor CV outcome in the
12 general population, supporting the notion that preclinical autoimmunity to apolipoprotein A-1
13 may identify a substantial proportion of individuals at increased risk of CAD. In our study, anti-
14 apoA-1 IgG-related CAD risk was highest in subjects carrying at least one C allele (CC/CT) in
15 the functional C260T *rs2569190* polymorphism, a group that represents roughly three quarters
16 of Caucasian populations.³¹ By virtue of being the first study on a “gene-autoantibody”
17 interaction with respect to CAD, our analysis highlights the importance of incorporating genetic
18 data on immune-related polymorphisms when evaluating anti-apoA-1 IgG-related risk and
19 provides insight for future study design on personalized CAD prediction.

20 Secondly, these results represent a human validation of the key role of CD14 co-receptor in
21 mediating the anti-apoA-1 IgG pro-atherogenic properties as demonstrated so far in animal and
22 *in vitro* models,^{4, 13} and reinforce the relevance of these preclinical results to the anti-apoA-1
23 IgG-associated CAD risk in humans. Conversely, in line with a recent meta-analysis,³¹ our
24 findings are equivocal and do not provide definite evidence with regards to the association
25 between TT genotype carriage and CAD risk.

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

38 In our study, the fact that TT carriers were less at risk to develop anti-apoA-1 IgG-related CAD
39 compared to non-TT carriers, despite TT homozygote status being previously associated with a
40 higher systemic inflammatory profile,^{20, 21} merits mention. To this respect, several lines of
41 evidence indicate that TT genotype could confer protection against uncontrolled inflammatory
42 response evoked by long-term DAMP exposure through different and mutually non-exclusive
43 mechanisms. Firstly, previous studies indicate that in the context of chronic low grade
44 CD14/TLR4 stimulation, the higher levels of sCD14 ascribed to TT genotype inhibit systemic
45 LPS-mediated inflammatory responses by down-regulating inflammatory cytokines transcription

1 ^{34, 35}, and facilitating CD14-related DAMP clearance ²⁴, thus protecting TT carriers against
2 sustained inflammatory responses, through a negative feedback mechanism. Inversely, lower
3 levels of sCD14 observed in CC carriers have been shown to favor vascular wall inflammation
4 and atherogenesis through impaired plasma clearance of endotoxin. ^{22, 23} C-allele carriers may
5 be less able to prevent anti-apoA-1 IgG-mediated CD14/TLR4 activation, resulting in
6 maintenance of a pro-atherogenic state and a higher risk for developing CAD. ²⁸⁻³⁰ Lastly,
7 increased expression of CD14 on the vascular endothelium of TT homozygotes ³⁵ could also
8 play a role in atherogenesis, in response to CD14 ligands such as anti apoA-1 IgG. ^{22, 23, 34, 35}

9 Several study limitations are noteworthy. Firstly, although great effort was undertaken to
10 maximize the participation rate during follow-up, our results may be subject to attrition bias as
11 drop out rate after mean duration of 5.6 years was about ~20%. Nevertheless, similar losses in
12 follow up are commonly reported in prospective cohorts ³⁶ and are within the conventional
13 participation rate thresholds for cohort studies. ³⁷ Secondly, we didn't directly measure sCD14 in
14 study subjects, in order to confirm the presumed higher sCD14 levels in TT homozygote carriers
15 reported previously. Moreover, as our assay assesses anti-apoA-1 IgG antibodies against
16 native apoA-1, ^{19, 38} we were not able to measure antibodies against modified forms of apoA-1,
17 such as oxidized apoA-1 (or possibly glycated and carbamylated apoA-1). As these modified
18 forms of apoA-1 were shown to be of relevance for HDL functionality and the pathology of
19 atherosclerosis, ³⁹⁻⁴¹ knowing whether they would elicit a humoral response clinically relevant to
20 human physiopathology is still unclear. Thirdly, due to sample availability, we only measured
21 baseline anti-apoA-1 IgG levels and did not assess the dynamic of anti-apoA-1 IgG levels over
22 time in relation with incident CAD. Moreover, we could not test other clinically relevant
23 antibodies, such as anti-oxidized LDL, anti-phospholipid, anti-nuclear or anti-heat shock protein
24 antibodies, which would have been instrumental to better understanding potential associations
25 with innate immune receptor-related genes of interest. Finally, sample size calculation in our
26 study was performed with regards to the primary outcome of detecting a difference in incident
27 CAD in subjects positive vs. negative for anti-apoA-1 IgG. Although the fact that previous
28 evidence suggested an interaction between anti-apoA-1 IgG and the CD14 receptor and that we
29 were able to detect such a – significant – interaction between anti-apoA-1 IgG and the functional
30 C260T *rs2569190* polymorphism in the CD14 receptor gene, it is possible that the current
31 sample size provided less than 80% power for this secondary study outcome. Therefore, this
32 finding of our study requires replication in larger prospective studies.

33 In conclusion, anti-apoA-1 IgG levels are independent predictors of incident non-fatal CAD in
34 the general population. The strength of this association is significantly modulated by the
35 functional C260T *rs2569190* SNP in the CD14 receptor gene, being the highest in non-TT
36 carriers and the lowest in TT homozygotes. These results imply that preclinical autoimmunity to
37 apolipoprotein A-1 should be evaluated carefully as it may help to improve the identification of
38 individuals at increased risk of CAD in the general population, especially in non-TT carriers
39 representing up to 75% of the population. Our findings indicate that “gene-autoantibodies”
40 interaction studies are likely to be required to better assess the CAD risk related to humoral
41 autoimmunity biomarkers in the general population, a concept that requires further investigation
42 in future studies.

43

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2

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Highlights

- **Anti-apoA-1 IgG are independent predictors of non-fatal 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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FIGURE LEGENDS

Figure 1: Study flowchart
Anti-apoA-1 IgG; autoantibodies against Apolipoprotein A-1, CAD; Coronary Artery Disease

Figure 2: Kaplan-Meier curves for cumulative incident coronary artery disease.
Above, Kaplan-Meier curves for cumulative (A) total and (B) non-fatal 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). Below, Kaplan-Meier curves for cumulative (C) total and (D) non-fatal 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 Logrank 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; autoantibodies against Apolipoprotein A-1, CAD; coronary artery disease

TABLES

1 **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, years	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, 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
Non-fatal, n (%)	95 (2.3)	37 (3.6)	0.018
Fatal, n (%)	22 (0.5)	3 (0.3)	0.320

- 1 Data are expressed as mean \pm standard deviation or number of participants and (percentage).
- 2 Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; CAD, coronary artery disease; HDL,
- 3 high density lipoprotein; LDL, low density lipoprotein; SCORE, Systematic Coronary Risk
- 4 Evaluation; CV, cardiovascular; ACEi, Angiotensin Converting Enzyme inhibitor; ARB,
- 5 Angiotensin Receptor Blockers. Statistical analysis for continuous variables by student's t-test or
- 6 Mann-Whitney test (*) depending on the normality assumption. Statistical analysis for
- 7 continuous variables by the chi-squared test.

Table 2: Hazard Ratios of anti-apoA-1 IgG for incident total, non-fatal and fatal CAD in the general population

n=5220		Total Incident CAD (n=159)				Non Fatal Incident CAD (n=134)				Fatal Incident CAD (n=25)			
		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)	0.07 ³	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)	0.15 ⁹	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.)	
1st tertile (0.64<OD≤0.77)		1.18 (0.64–2.19)	0.59 ⁷	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
2nd tertile (0.77<OD≤0.98)		1.16 (0.63–2.16)	0.63 ³	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
3rd tertile (OD>0.98)		1.79 (1.09–2.95)	0.02 ¹	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, smoking, HDL and LDL cholesterol, baseline CAD, statin, beta-blocker treatment and eGFR. CAD, coronary artery disease; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; SD, standard deviation; OD, optical density; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated Glomerular Filtration Rate.

* Subjects with positive Anti-ApoA-1 (n=1040) were divided in tertiles (n=347) of increasing titers: 1st tertile (0.64<OD<0.77), 2nd tertile (0.77<OD<0.98) and 3rd tertile (OD>0.98).

Table 3: Hazard Ratios of anti-apoA-1 IgG for incident total, non-fatal and fatal CAD according to the C260T *rs2569190* polymorphism allele status, in the genotyped population

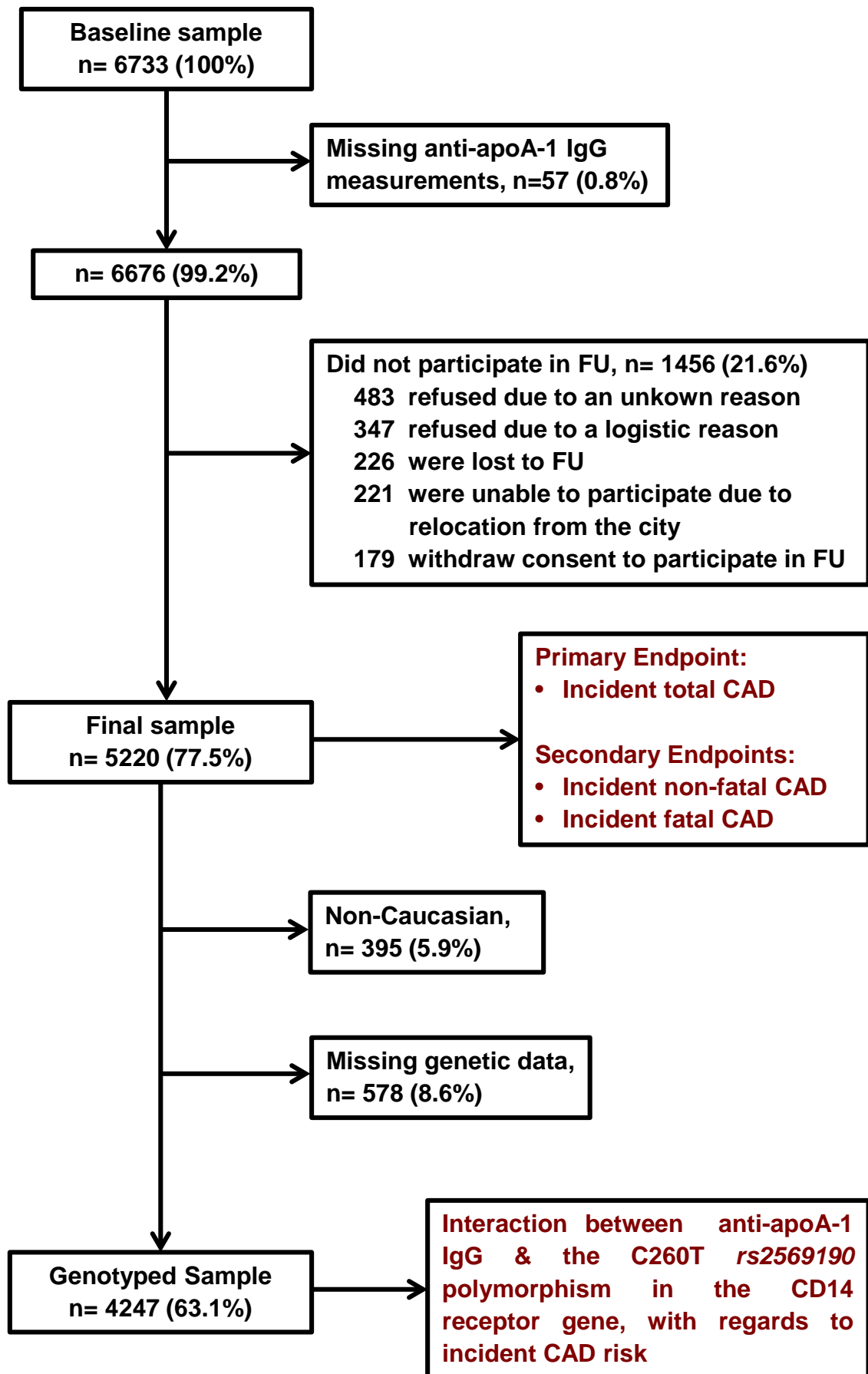
Anti-apoA-1 IgG HR (95% CI) for CAD	Total Incident CAD (n= 132)				Non Fatal Incident CAD (n= 109)				Fatal Incident CAD (n= 23)			
	Unadjusted Model	p- value	Adjusted Model	p- value	Unadjusted Model	p- value	Adjusted Model	p- value	Unadjusted Model	p- value	Adjusted Model	p- value
C260T <i>rs2569190</i> 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 & <i>rs2569190</i> (CC vs. CT vs. TT)		0.064		0.011		0.135		0.033		n/a		n/a
P-value for interaction between anti- apoA-1 IgG &		0.068		0.020		0.126		0.047		n/a		n/a

rs2569190

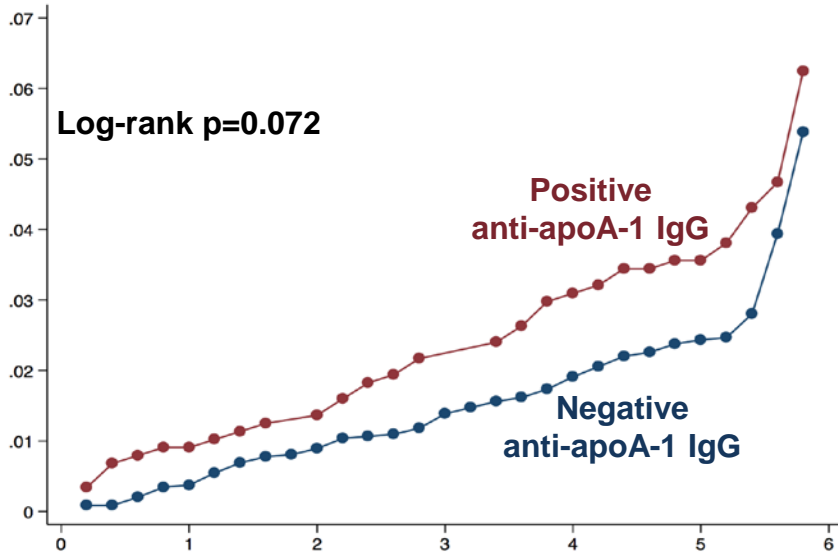
(CC/CT vs. TT)

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, smoking, HDL and LDL cholesterol, baseline CAD, statin, beta-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.

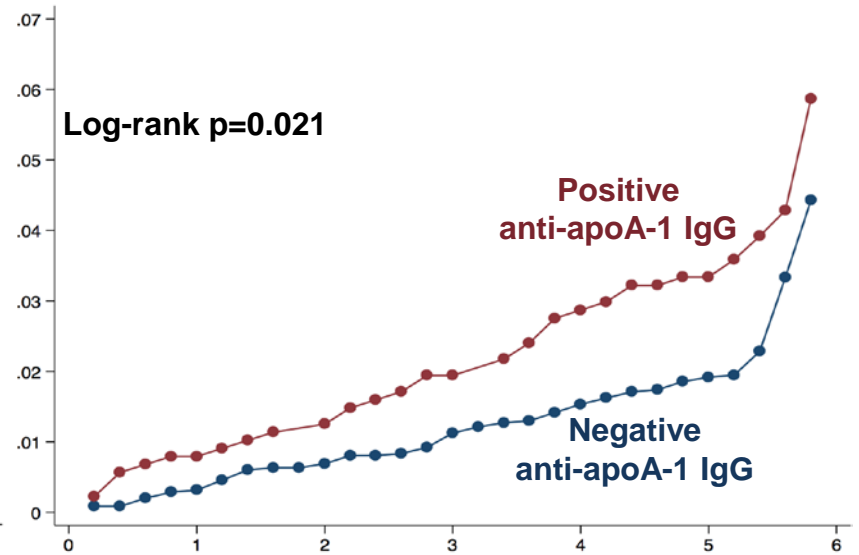
CAD, coronary artery disease; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; HR, hazard ratio; CI, confidence interval; OD, optical density; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated Glomerular Filtration Rate.



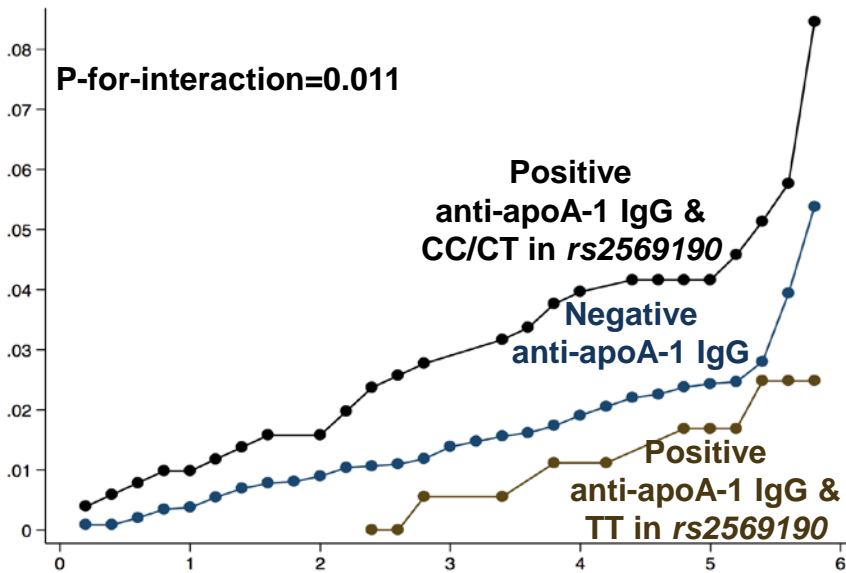
Association between anti-apoA-1 IgG and total incident CAD



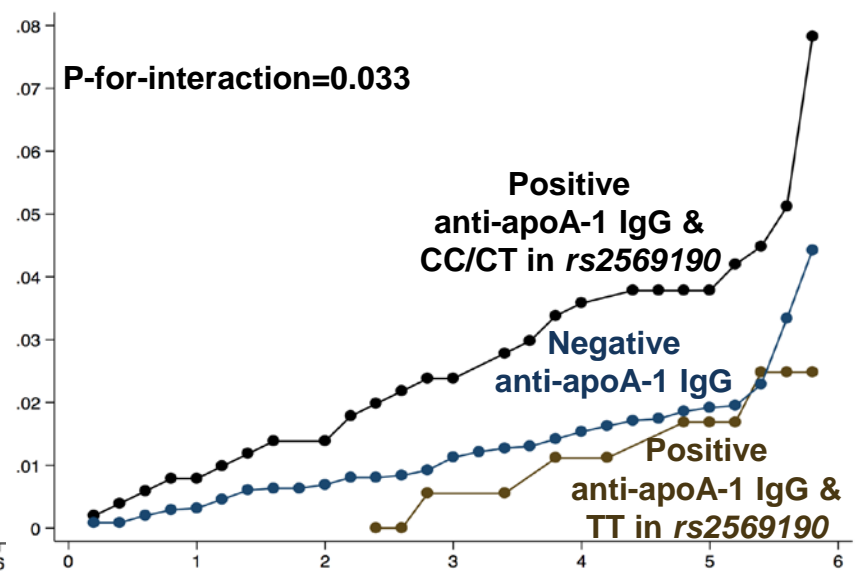
Association between anti-apoA-1 IgG and non-fatal incident CAD



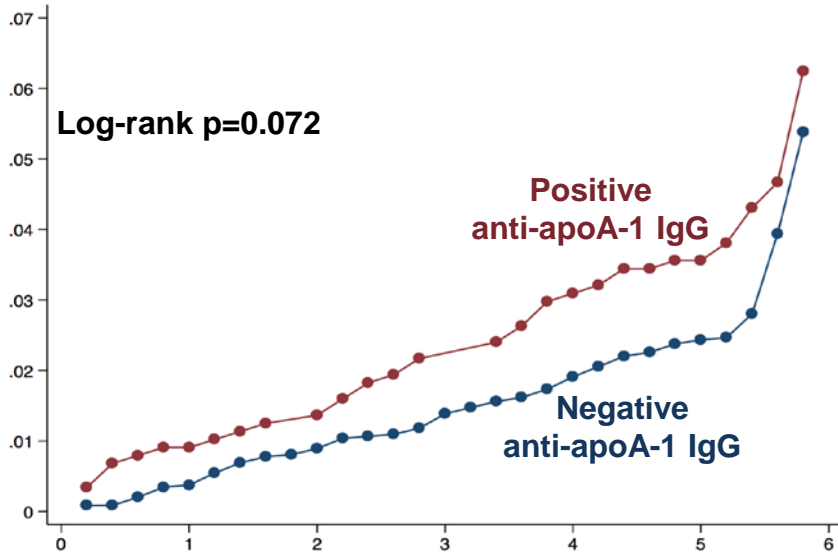
Association between anti-apoA-1 IgG and total incident CAD, according to *rs2569190* allele status



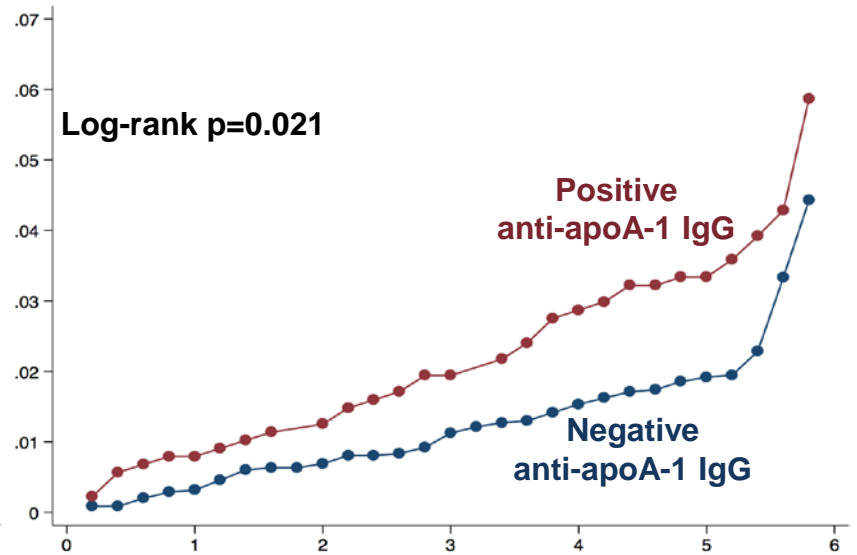
Association between anti-apoA-1 IgG and non-fatal incident CAD, according to *rs2569190* allele status



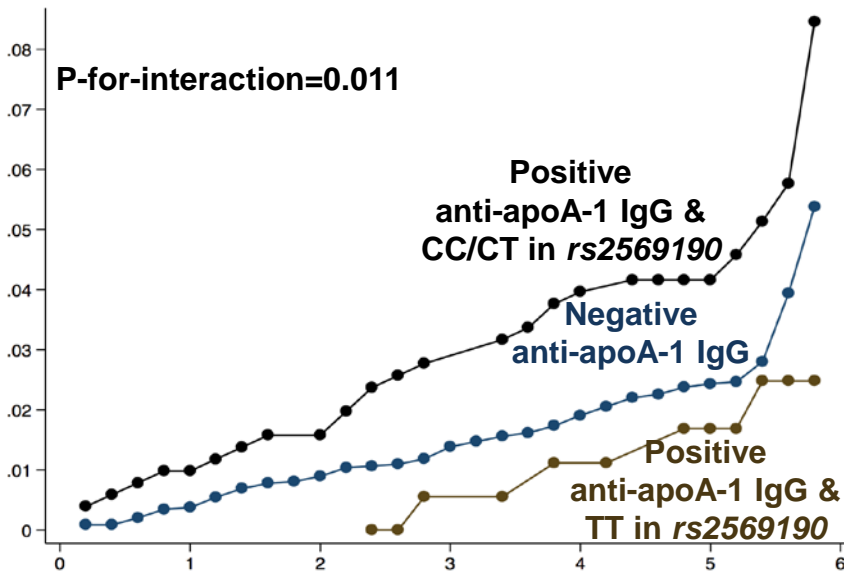
Association between anti-apoA-1 IgG and total incident CAD



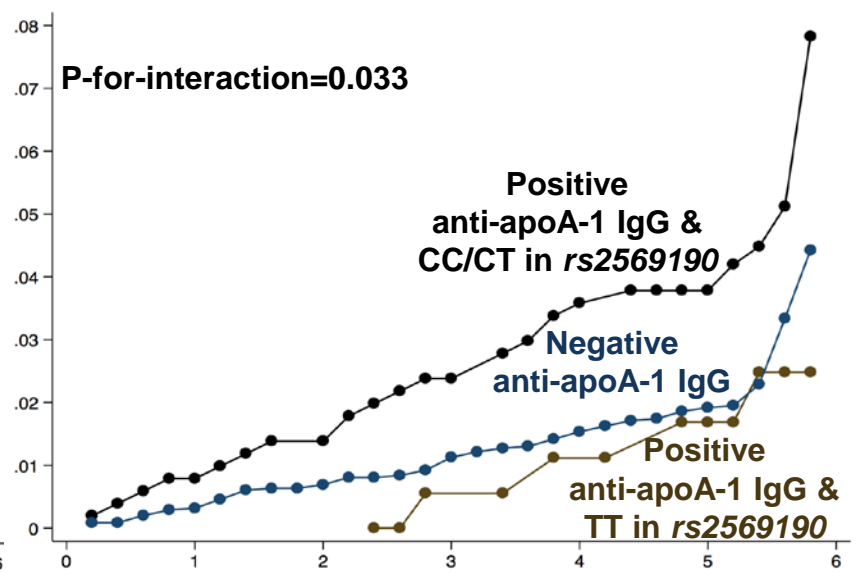
Association between anti-apoA-1 IgG and non-fatal incident CAD



Association between anti-apoA-1 IgG and total incident CAD, according to *rs2569190* allele status



Association between anti-apoA-1 IgG and non-fatal incident CAD, according to *rs2569190* allele status



**Impact of CD14 polymorphisms on anti-apolipoprotein A-1 IgG-related coronary artery disease
prediction in the general population**

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

SUPPLEMENT MATERIAL

Supplemental Table I: Baseline characteristics of subjects included in the analysis vs. subjects missing follow-up data.

Overall sample (n=6676)	Subjects included in the analysis (n=5220)	Subjects missing follow- up data (n=1456)	p-value
Age, years	52.6 ± 10.7	52.6 ± 10.8	0.979
Male sex, n (%)	2496 (47.3)	693 (47.6)	0.841
History of CVD, n (%)	398 (7.5)	90 (6.2)	0.076
Current smoking, n (%)	1382 (26.2)	430 (29.5)	0.011
Diabetes, n (%)	336 (6.4)	100 (6.9)	0.492
Hypertension, n (%)	1756 (33.3)	579 (39.8)	<0.001
Body mass index (kg/m ²)	25.65 ± 4.41	26.34 ± 4.85	<0.001
Total cholesterol (mmol/l)	5.56 ± 1.02	5.62 ± 1.10	0.093
HDL cholesterol (mmol/l)	1.64 ± 0.44	1.60 ± 0.43	0.006
LDL cholesterol (mmol/l)	3.32 ± 0.90	3.37 ± 0.95	0.038
Triglycerides (mmol/l)	1.37 ± 1.14	1.46 ± 1.31	0.010*
SCORE CV risk categories, n (%)	2.07 ± 3.56	2.13 ± 3.40	0.145*
Low risk	3150 (60.4)	817 (57.3)	
Intermediate risk	1429 (27.4)	421 (29.5)	
High risk	394 (7.6)	119 (8.3)	
Very high risk	239 (4.6)	70 (4.9)	0.189
Anti-apoA-1 IgG, n (%)	1040 (19.9)	283 (19.8)	0.920
Anti-apoA-1 OD	0.46 ± 0.33	0.45 ± 0.36	0.377*

Data are expressed as mean \pm standard deviation or number of participants and (percentage). CVD, cardiovascular disease; SBP, systolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; SCORE, Systematic Coronary Risk Evaluation; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; OD, Optical Density. 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 chi-squared test.

Supplemental Table II: Hazard Ratios of anti-apoA-1 IgG for incident total, non fatal and fatal coronary artery disease in the general population, after excluding subjects with a) baseline coronary artery disease, b) baseline autoimmune disease.

Hazard Ratios (95% CI)		Incident CAD (n=111)				Non Fatal Incident CAD (n=93)				Fatal Incident CAD (n=18)			
a) Excluding subjects with baseline coronary artery disease (n=5031)	Unadjusted	p-	Adjusted	p-	Unadjusted	p-	Adjusted	p-	Unadjusted	p-	Adjusted	p-	
	Model	value	Model	value	Model	value	Model	value	Model	value	Model	value	
Positive vs. negative anti-ApoA-1 IgG	1.44 (0.94–2.20)	0.093	1.54 (1.00–2.38)	0.050	1.57 (1.00–2.48)	0.050	1.69 (1.06–2.68)	0.027	0.79 (0.23–2.75)	0.717	0.90 (0.26–3.15)	0.868	
Hazard Ratios (95% CI)		Incident CAD (n=148)				Non Fatal Incident CAD (n=124)				Fatal Incident CAD (n=24)			
b) Excluding subjects with baseline autoimmune disease (n=5100)	Unadjusted	p-	Adjusted	p-	Unadjusted	p-	Adjusted	p-	Unadjusted	p-	Adjusted	p-	
	Model	value	Model	value	Model	value	Model	value	Model	value	Model	value	
Positive vs. negative anti-ApoA-1 IgG	1.37 (0.95–1.99)	0.095	1.42 (0.98–2.08)	0.077	1.62 (1.10–2.40)	0.015	1.64 (1.11–2.46)	0.017	0.36 (0.09–1.55)	0.172	0.43 (0.12–1.86)	0.256	

Results are expressed as adjusted hazard ratios (95% confidence interval). Statistical analysis by Cox proportional hazards regression adjusted for age, sex, systolic blood pressure, diabetes, smoking, HDL cholesterol and LDL cholesterol, statin, beta-blocker treatment and eGFR. CAD, coronary artery disease; OD, optical density; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; eGFR, estimated glomerular filtration rate

Supplementary Table III: Hazard Ratios of anti-apoA-1 IgG for incident total, non-fatal and fatal CAD in the general population.

Final sample (n=5220)	Total Incident CAD (n=159)				Non Fatal Incident CAD (n=134)				Fatal Incident CAD (n=25)			
	Adjusted Model_1	p-value	Adjusted Model_2	p-value	Adjusted Model_1	p-value	Adjusted Model_2	p-value	Adjusted Model_1	p-value	Adjusted Model_2	p-value
Positive vs. negative	1.36 (0.94–1.97)	0.099	1.37 (0.94–1.98)	0.100	1.53 (1.03–2.26)	0.034	1.54 (1.04–2.28)	0.031	0.58 (0.17–1.98)	0.387	0.56 (0.17–1.91)	0.355
1 SD change in log-transformed anti-ApoA-1 IgG levels	1.08 (0.94–1.25)	0.265	1.09 (0.94–1.26)	0.251	1.12 (0.96–1.30)	0.144	1.13 (0.97–1.32)	0.121	0.89 (0.62–1.29)	0.541	0.87 (0.60–1.28)	0.479
Negative (OD<0.64)	1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)		1 (ref.)	
1st tertile (0.64<OD≤0.77)	1.45 (0.78–2.69)	0.243	1.45 (0.78–2.71)	0.237	1.57 (0.82–3.01)	0.177	1.58 (0.82–3.04)	0.168	0.75 (0.10–5.66)	0.780	0.69 (0.09–5.30)	0.718
2nd tertile (0.77<OD≤0.98)	1.02 (0.52–2.01)	0.958	0.95 (0.48–1.88)	0.882	0.96 (0.44–2.07)	0.910	0.89 (0.41–1.93)	0.766	1.16 (0.27–5.05)	0.839	1.12 (0.26–4.86)	0.880
3rd tertile (OD>0.98)	1.58 (0.96–2.60)	0.074	1.66 (1.01–2.75)	0.047	1.95 (1.17–3.23)	0.010	2.10 (1.26–3.49)	0.004	no subjects	.	no subjects	.
P-value for linear trend		0.254		0.167		0.103		0.022		.		.

Results are expressed as adjusted hazard ratios and (95% confidence interval) for the positive (OD>0.64) vs. negative (OD<0.64) anti apoA-1 antibodies. Statistical analysis by Cox proportional hazards regression adjusted for age, sex, hypertension, diabetes, smoking, HDL cholesterol and LDL cholesterol, baseline CAD, (**Adjusted Model_1**) statin, beta-blocker treatment (**Adjusted Model_2**).

CAD, coronary artery disease; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; SD, standard deviation; OD, optical density; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated Glomerular Filtration Rate.

* Subjects with positive Anti-ApoA-1 (n=1040) were divided in tertiles (n=347) of increasing titers: 1st tertile (0.64<OD<0.77), 2nd tertile (0.77<OD<0.98) and 3rd tertile (OD>0.98).

Supplemental Table IV: Baseline characteristics of subjects with vs. without genetic data for the C260T *rs2569190* polymorphism in the CD14 receptor gene.

Final sample (n=5220)	Final genotyped sample (n=4247)	Subjects without genetic data (n=973)	p-value
Age, years	53.4 ± 10.7	48.9 ± 9.9	<0.001
Male sex, n (%)	2014 (47.0)	482 (48.7)	0.315
History of CVD, n (%)	342 (8.0)	56 (5.7)	0.013
Current smoking, n (%)	1106 (25.8)	276 (27.9)	0.173
Diabetes, n (%)	289 (6.7)	47 (4.8)	0.021
Hypertension, n (%)	1480 (34.5)	276 (27.9)	<0.001
Body mass index (kg/m²)	25.68 ± 4.42	25.51 ± 4.37	0.273
Total cholesterol (mmol/l)	5.59 ± 1.03	5.44 ± 0.99	<0.001
HDL cholesterol (mmol/l)	1.65 ± 0.44	1.60 ± 0.44	0.002
LDL cholesterol (mmol/l)	3.33 ± 0.91	3.25 ± 0.88	0.013
Triglycerides (mmol/l)	1.37 ± 1.12	1.37 ± 1.20	0.162*
SCORE CV risk categories, n (%)			
Low risk	2437 (57.2)	713 (74.7)	
Intermediate risk	1255 (29.5)	174 (18.2)	
High risk	358 (8.4)	36 (3.8)	
Very high risk	208 (4.9)	31 (3.3)	<0.001
Anti-apoA-1 IgG, n (%)	802 (18.81)	238 (24.87)	<0.001
Anti-apoA-1 OD	0.45 ± 0.32	0.51 ± 0.37	<0.001*

Data are expressed as mean ± standard deviation or number of participants and (percentage). CVD, cardiovascular disease; SBP, systolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; SCORE, Systematic Coronary Risk Evaluation; Anti-apoA-1 IgG, anti-apolipoprotein A-1

autoantibodies; OD, Optical Density. 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 chi-squared test.

Supplemental Table V: Baseline characteristics of subjects with vs. without genetic data for the C260T *rs2569190* polymorphism in the CD14 receptor gene or unable to participate in follow up.

Baseline sample (n=6676)	Final genotyped sample (n=4247)	Subjects without genetic data/unable to participate in follow-up (n=2429)	p-value
Age, years	53.4 ± 10.7	51.1 ± 10.6	<0.001
Male sex, n (%)	2014 (47.0)	1175 (48.1)	0.389
History of CVD, n (%)	314 (7.3)	144 (5.9)	0.025
Current smoking, n (%)	1106 (25.8)	706 (28.9)	0.006
Diabetes, n (%)	289 (6.7)	147 (6.0)	0.243
Hypertension, n (%)	1480 (34.5)	855 (35.0)	0.706
Body mass index (kg/m ²)	25.7 ± 4.4	26 ± 4.7	0.005
Total cholesterol (mmol/l)	5.59 ± 1.03	5.55 ± 1.06	0.084
HDL cholesterol (mmol/l)	1.65 ± 0.44	1.60 ± 0.43	<0.001
LDL cholesterol (mmol/l)	3.33 ± 0.91	3.32 ± 0.93	0.756
Triglycerides (mmol/l)	1.37 ± 1.12	1.42 ± 1.27	0.431*
SCORE CV risk categories, n (%)			
Low risk	2428 (57.2)	1530 (64.3)	
Intermediate risk	1249 (29.4)	595 (25)	
High risk	357 (8.4)	155 (6.5)	
Very high risk	208 (4.9)	101 (4.2)	<0.001
Anti-apoA-1 IgG, n (%)	802 (18.8)	521 (21.8)	0.003
Anti-apoA-1 OD	0.45 ± 0.32	0.48 ± 0.36	<0.001*

Data are expressed as mean ± standard deviation or number of participants and (percentage). CVD,

cardiovascular disease; SBP, systolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; SCORE, Systematic Coronary Risk Evaluation; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; OD, Optical Density. 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 chi-squared test.

Supplemental Table VI: Characteristics of the genotyped sample, according to allele status of the C260T *rs2569190* polymorphism in CD14 receptor gene.

C260T <i>rs2569190</i> allele status (CC/CT vs. TT)	Overall (n=4247)		p-value	Anti-apoA-1 IgG positive subjects (n=798)		p-value
	CC/CT (n=3192)	TT (n=1055)		CC/CT (n=585)	TT (n=213)	
Age, years	53.5 ± 10.7	53.5 ± 10.9	0.923	53.2 ± 10.7	53.4 ± 11.3	0.835
Male sex, n (%)	1495 (46.8)	502 (47.6)	0.673	260 (44.4)	108 (50.7)	0.117
History of CAD, n (%)	122 (3.8)	37 (3.5)	0.640	27 (4.6)	8 (3.8)	0.600
Current smoking, n (%)	808 (25.3)	282 (26.7)	0.361	153 (26.2)	51 (23.9)	0.527
Diabetes, n (%)	199 (6.2)	90 (8.5)	0.010	32 (5.5)	15 (7.0)	0.404
Hypertension, n (%)	1099 (34.4)	371 (35.2)	0.663	203 (34.7)	86 (40.4)	0.140
Autoimmune disease, n (%)	80 (2.5)	29 (2.8)	0.666	18 (3.1)	11 (5.2)	0.163
Body mass index (kg/m ²)	25.67 ± 4.46	25.68 ± 4.29	0.973	25.72 ± 4.67	25.90 ± 4.16	0.622
Total cholesterol (mmol/l)	5.59 ± 1.03	5.60 ± 1.02	0.715	5.51 ± 1.00	5.55 ± 1.06	0.654
HDL cholesterol (mmol/l)	1.64 ± 0.44	1.66 ± 0.44	0.501	1.63 ± 0.46	1.65 ± 0.43	0.635
LDL cholesterol (mmol/l)	3.33 ± 0.91	3.34 ± 0.92	0.753	3.27 ± 0.92	3.31 ± 0.88	0.621
Triglycerides (mmol/l)	1.38 ± 1.18	1.36 ± 0.93	0.974*	1.39 ± 1.40	1.27 ± 0.81	0.395*
SCORE CV risk categories, n (%)						
Low risk	1826 (57.3)	602 (57.1)		346 (59.3)	116 (54.5)	
Intermediate risk	952 (29.9)	297 (28.2)		157 (26.9)	65 (30.5)	
High risk	258 (8.1)	99 (9.4)		53 (9.1)	21 (9.9)	

Very high risk	151 (4.7)	57 (5.4)	0.379	28 (4.8)	11 (5.2)	0.682
CV drugs, n (%)						
Aspirin	576 (18.1)	170 (16.1)	0.153	110 (18.8)	29 (13.6)	0.087
Statins	346 (10.8)	143 (13.6)	0.017	60 (10.3)	22 (10.3)	0.976
Beta blockers	185 (5.8)	56 (5.3)	0.553	48 (8.2)	13 (6.1)	0.323
Calcium channel blockers	97 (3.0)	31 (2.9)	0.869	20 (3.4)	5 (2.4)	0.442
ACEi/ARB	241 (7.6)	83 (7.9)	0.737	43 (7.4)	14 (6.6)	0.706
Diuretics	63 (2.0)	22 (2.1)	0.822	12 (2.1)	5 (2.4)	0.798
Anti-apoA-1 IgG, n (%)	585 (18.3)	213 (20.2)	0.179	585 (100.0)	213 (100.0)	.
Anti-apoA-1 OD	0.45 ± 0.31	0.45 ± 0.33	0.937*	0.94 ± 0.29	0.93 ± 0.28	0.633*
Incident CAD, n (%)	100 (3.1)	32 (3.0)	0.872	27 (4.6)	4 (1.9)	0.077
Non-fatal, n (%)	83 (2.6)	26 (2.5)	0.809	24 (4.1)	4 (1.9)	0.131
Fatal, n (%)	17 (0.5)	6 (0.6)	0.890	3 (0.5)	0 (0.00)	0.295
All-cause mortality, n (%)	125 (3.9)	38 (3.6)	0.653	29 (5.0)	13 (6.1)	0.522

Data are expressed as mean ± standard deviation or number of participants and (percentage). Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; CAD, coronary artery disease; HDL, high density lipoprotein; LDL, low density lipoprotein; SCORE, Systematic Coronary Risk Evaluation; CV, cardiovascular; ACEi, Angiotensin Converting Enzyme inhibitor; ARB, Angiotensin Receptor Blockers; OD, optical density. Statistical analysis for continuous variables by student's t-test or Mann-Whitney test (*) depending on the normality assumption. Statistical analysis for continuous variables by the chi-squared test.

Supplementary Table VII: Hazard Ratios of anti-apoA-1 IgG for incident total, non-fatal and fatal CAD according to the C260T *rs2569190* polymorphism allele status, in the genotyped population

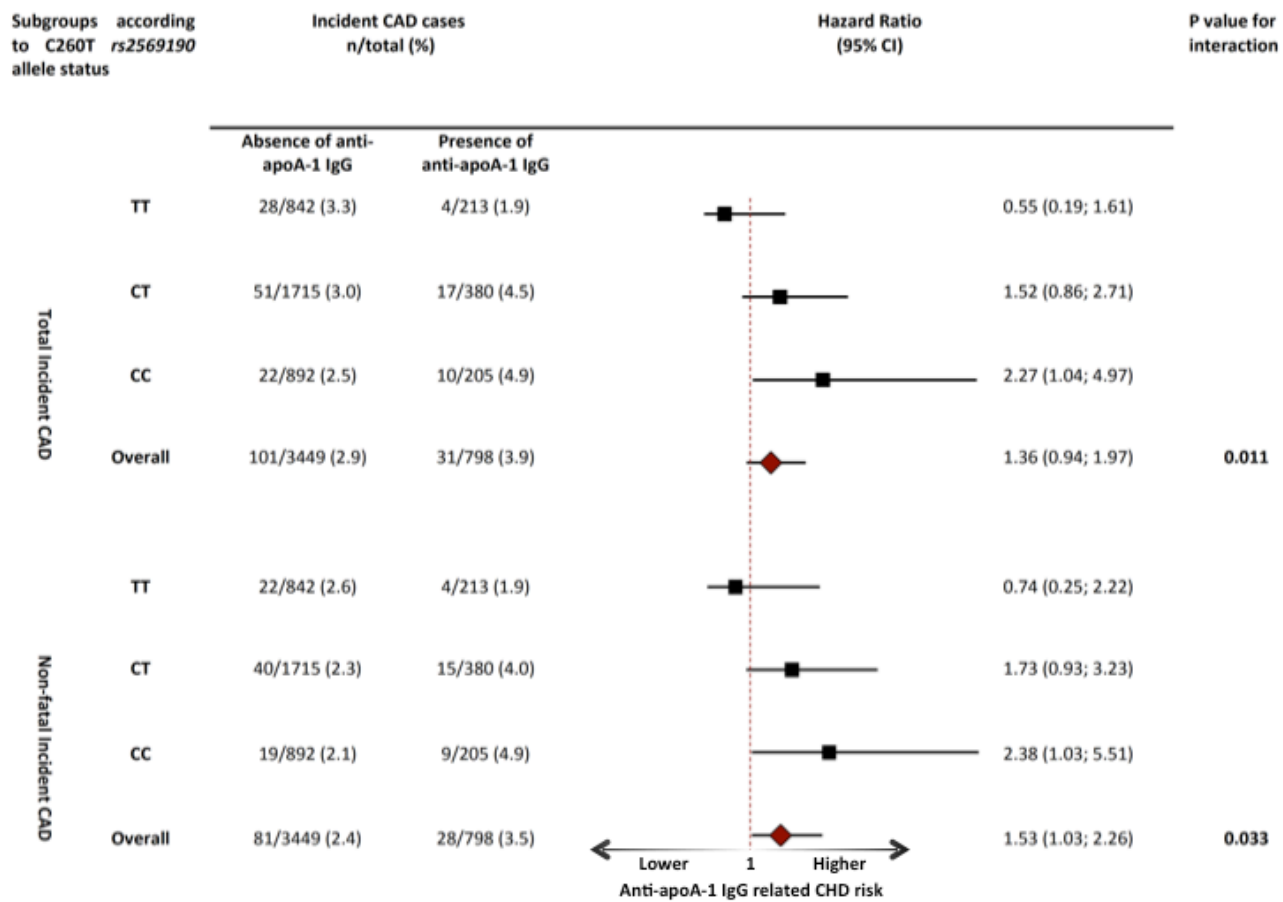
Anti-apoA-1 IgG HR (95% CI) for CAD	Total Incident CAD (n= 132)				Non Fatal Incident CAD (n= 109)				Fatal Incident CAD (n= 23)			
	Adjusted Model_1	p- value	Adjusted Model_2	p- value	Adjusted Model_1	p- value	Adjusted Model_2	p- value	Adjusted Model_1	p- value	Adjusted Model_2	p- value
C260T <i>rs2569190</i> allele status												
CC (n=1097)	2.09 (0.97–4.47)	0.058	2.26 (1.04– 4.92)	0.040	2.13 (0.95–4.80)	0.068	2.36 (1.02– 5.42)	0.044	1.85 (0.18–18.6)	0.602	1.52 (0.15– 15.34)	0.721
CC/CT (n=3192)	1.73 (1.10–2.73)	0.018	1.77 (1.12– 2.80)	0.014	1.87 (1.15–3.05)	0.012	1.96 (1.20– 3.20)	0.007	0.89 (0.24–3.25)	0.858	0.90 (0.24– 3.31)	0.873
CT (n=2095)	1.56 (0.88–2.75)	0.128	1.50 (0.85– 2.67)	0.164	1.73 (0.94–3.20)	0.081	1.72 (0.92– 3.20)	0.087	0.59 (0.11–3.05)	0.528	0.54 (0.10– 2.93)	0.475
TT (n=1055)	0.47 (0.16–1.36)	0.163	0.54 (0.18– 1.58)	0.262	0.59 (0.20–1.76)	0.348	0.71 (0.24– 2.12)	0.536	no subjects		no subjects	
P-value for interaction between anti-apoA-1 IgG & <i>rs2569190</i> (CC vs. CT vs. TT)		0.015		0.010		0.045		0.029		0.183		n/a
P-value for interaction between anti-apoA-1 IgG & <i>rs2569190</i> (CC/CT vs. TT)		0.022		0.025		0.050		0.053		n/a		n/a

Results are expressed as adjusted hazard ratios and (95% confidence interval) for positive

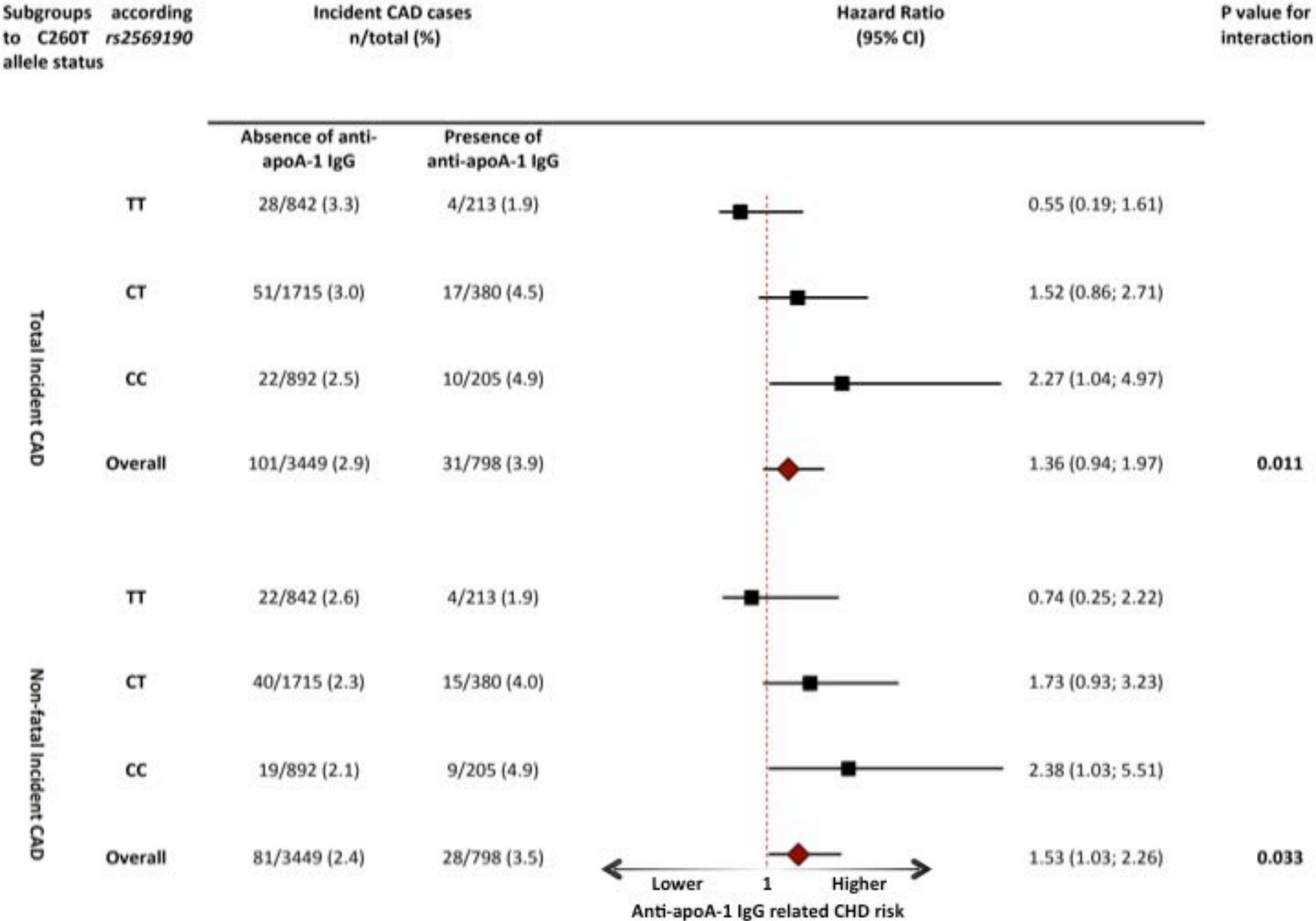
(OD>0.64) vs. negative (OD<0.64) anti apoA-1 IgG. Statistical analysis by Cox proportional hazards regression adjusted for age, sex, hypertension, diabetes, smoking, HDL cholesterol and LDL cholesterol, baseline CAD (**Adjusted Model_1**), statin and beta-blocker treatment (**Adjusted Model_2**). The 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.

CAD, coronary artery disease; Anti-apoA-1 IgG, anti-apolipoprotein A-1 autoantibodies; HR, hazard ratio; CI, confidence interval; OD, optical density; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated Glomerular Filtration Rate.

Supplemental Figure I: Forest plot of subgroup analyses according to the C260T *rs2569190* polymorphism allele status, for incident total and non-fatal coronary artery disease.



Hazard ratios of anti-apoA-1 IgG (and 95% confidence intervals) are shown for the endpoint of incident total and non fatal coronary artery disease, according to C260T *rs2569190* polymorphism allele status. Statistical analysis by Cox proportional hazards regression adjusted for age, sex, hypertension, diabetes, smoking, HDL and LDL cholesterol, baseline CAD, statin, beta-blocker treatment and eGFR. The P value for interaction represents the likelihood of interaction between the C260T *rs2569190* polymorphism allele status and the relative anti-apoA-1 IgG effect for coronary artery disease.



1 **Impact of CD14 polymorphisms on anti-apolipoprotein A-1 IgG-related coronary artery**
2 **disease prediction in the general population**

3
4 **Panagiotis Antiochos, Pedro Marques-Vidal, Julien Virzi, Sabrina Pagano, Nathalie Satta,**
5 **Oliver Hartley, Fabrizio Montecucco, François Mach, Zoltan Kutalik, Gerard Waeber, Peter**
6 **Vollenweider *, Nicolas Vuilleumier ***

7
8 * These authors contributed equally

9

10

11

MATERIALS AND METHODS

12

13

14 **Study population and design**

15 We used clinical and biological data from the CoLaus study, a population-based cohort
16 investigating cardiovascular disease risk in 6733 subjects in the general population aged 35 to
17 75 and living in the city of Lausanne, Switzerland. The recruitment phase of the study took place
18 between 2003 and 2006, where all participants attended the outpatient clinic of the University
19 Hospital of Lausanne. A follow-up visit took place between 2009 and 2012. All study participants
20 at baseline were invited for a follow-up visit at the outpatient clinic of the University Hospital of
21 Lausanne, between 2009 and 2012. Subjects unable to attend had home interviews, were
22 interviewed by phone and/or sent a questionnaire requesting information relevant to study
23 endpoints. Follow-up data collection started after the baseline visit of each participant. The
24 study was approved by the Institutional Ethics Committee of the University of Lausanne and
25 informed consent was obtained from all participants before inclusion in the study, in accordance
26 with the Declaration of Helsinki. A detailed description of the study design, definition of clinical
27 variables and sampling procedures have been described elsewhere.¹

28 Briefly, clinical data, and fasting venous blood samples were collected from each participant by
29 trained field interviewers during a single visit lasting about 60 minutes. Blood pressure and
30 heart rate were measured three consecutive times using an automated sphygmomanometer
31 (Omron® HEM-907, Matsusaka, Japan) and the average of the last two measurements was
32 used. Body weight and height were measured with participants standing without shoes in light
33 indoor clothes. Body weight was measured in kilograms to the nearest 100 g using a Seca®
34 scale, which was calibrated regularly. Height was measured to the nearest 5 mm using a Seca®
35 height gauge. Body mass index (BMI) was calculated as weight (kg)/height (m²). Hypertension
36 was defined as a systolic blood pressure (SBP) ≥140 mm Hg and/or a diastolic blood pressure
37 ≥90 mm Hg and/or the presence of anti-hypertensive treatment. Diabetes mellitus was defined
38 as fasting plasma glucose ≥7.0 mmol/l and/or oral or insulin anti-diabetic treatment. Estimated
39 glomerular filtration rate (eGFR) was calculated by the simplified “Modification of Diet in Renal
40 Disease” prediction equation.² Autoimmune disease was defined as history of rheumatoid
41 arthritis or systemic lupus erythematosus, independently of treatment.

42 Venous blood samples were drawn after an overnight fast, and assays were performed on fresh
43 plasma samples within two hours after blood collection for standard lipid profile, and on
44 unthawed serum aliquots for anti-apoA-1 IgG assessment (see below) that were immediately
45 adequately processed and stored at -80 °C until analysis.³ Standard lipid profile was performed
46 in the CHUV Clinical Laboratory using a Modular P apparatus (Roche Diagnostics, Switzerland).
47 The following analytical procedures (with maximum inter and intra-batch CVs) were used: total
48 cholesterol by the “CHOD-PAP” method (1.6% – 1.7%); HDL-cholesterol by the “CHOD-
49 PAP/PEG/Cyclodextrin” method (3.6% – 0.9%); triglycerides by the “GPO-PAP” method (2.9% –
50 1.5%); glucose by glucose dehydrogenase (2.1% – 1.0%); and serum creatinine by the Jaffe
51 kinetic compensated method (2.9% – 0.7%).

52 **Coronary artery disease and death data collection at baseline and during FU**

53 Trained medical doctors actively searched and collected all medical records related to the
54 coronary artery disease (CAD, defined as myocardial infarction, stable or unstable angina,
55 percutaneous coronary revascularization or bypass grafting) in *all* participants who declared,
56 during the baseline and/or follow-up interviews, to have presented any cardiac event or
57 procedure during their lifetime. CAD that occurred during follow-up was classified as incident.
58 CAD collection in study participants followed a stepwise procedure: 1. The medical record of
59 each participant was checked by hand with the general practitioner and/or the private

60 cardiologist, by both mail surveys and phone interviews. All CAD-related GPs' reports, reports
61 on outpatient contacts with medical specialists and hospital discharge reports were copied and
62 classified. Collected documents further included related laboratory data, electrocardiograms,
63 cardiac imaging data (echocardiography reports, cardiac radionuclide imaging, magnetic
64 resonance imaging, cardiac CT, stress tests) and coronary angiograms. 2. To further search for
65 presence of CAD that may not have been mentioned by the participant during the follow-up visit,
66 the medical databases of: a. the University Hospital of Lausanne, b. regional hospitals (within a
67 radius of 100 kilometers), and c. the pre-hospital emergency care unit of the City of Lausanne,
68 were checked electronically and then also by hand for CAD-related diagnoses, for *all* study
69 participants.

70 Data on deceased study participants were likewise collected in a stepwise procedure, by
71 checking electronically and then also by hand the electronic databases of: a. the University
72 Hospital of Lausanne, b. regional hospitals (within a radius of 100 kilometers), c. population
73 registers of the cities where the participants were living in case of returned mail and/or multiple
74 unsuccessful phone contacts, d. the pre-hospital emergency care unit of the City of Lausanne,
75 e. the forensic medicine department of the University Hospital of Lausanne, and f. the "Office
76 Fédérale de la Statistique", the Swiss governmental agency providing death statistics. If a death
77 was confirmed, physicians of the dead participants were asked to send any medical record
78 related to the death. If all previous steps failed to retrieve the cause of death, the physician in
79 charge when the death occurred and/or a family member of the dead participant were contacted
80 to provide information on the cause of death (verbal autopsy).

81

82 **Study Endpoints**

83 The primary endpoint was total incident CAD as defined by a composite of first-time, fatal or
84 non-fatal myocardial infarction, stable or unstable angina, percutaneous coronary
85 revascularization or bypass grafting for CAD. Separate outcomes of interest included non-fatal
86 as well fatal CAD (definite or probable, in-hospital or out-of-hospital). ⁴ All CAD events were
87 adjudicated separately by two cardiologists, blinded to all study variables, according to a
88 consensus document edited on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the
89 "Universal Definition of Myocardial Infarction". ⁵ All deaths were adjudicated, by 2 internal
90 medicine specialists.

91 In subjects with available genetic data for the functional C260T *rs2569190* polymorphism in the
92 CD14 receptor gene, the secondary study outcome was to test the interaction between anti-
93 apoA-1 IgG and carriage of the T allele, with regards to incident CAD.

94

95 **Assessment of anti-apoA-1 IgG levels**

96 Anti-apoA-1 IgG were measured as previously described, ⁶⁻⁸ using the CoLaus study (2003-
97 2006) frozen serum aliquots, stored at -80 °C. Maxisorp plates (Nunc™, Denmark) were coated
98 with purified, human-derived delipidated apolipoprotein A-1 (20 µg/ml; 50 µl/well) for 1h at 37°C.
99 After being washed, all wells were blocked for 1h with 2% bovine serum albumin (BSA) in a
100 phosphate buffer solution (PBS) at 37°C. Participants' samples were also added to a non-
101 coated well to assess individual non-specific binding. After six washing cycles, a 50 µl/well of
102 signal antibody (alkaline phosphatase-conjugated anti-human IgG; Sigma-Aldrich, St Louis,
103 MO), diluted 1:1000 in a PBS/BSA 2% solution, was added and incubated for 1h at 37°C. After
104 washing six more times, phosphatase substrate p-nitrophenylphosphate disodium (Sigma-
105 Aldrich) dissolved in a diethanolamine buffer (pH 9.8) was added and incubated for 20 min at

106 37°C (Molecular Devices™ Versa Max). As previously published, this assay detects anti-apoA-1
107 antibodies directed against the native lipid-free apoA-1.^{9, 10} Optical density (OD) was
108 determined at 405 nm, and each sample was tested in duplicate. Corresponding non-specific
109 binding was subtracted from mean OD for each sample. The specificity of detection was
110 assessed using conventional saturation tests by Western blot (WB) analysis.

111 As previously described, elevated levels of anti-apoA-1 IgG were set at an OD cut-off of
112 OD>0.64, corresponding to the 97.5th percentile of a reference population.⁶⁻⁸ In order to limit the
113 impact of inter-assay variation, we further calculated an index consisting in the ratio between
114 sample net absorbance and the positive control net absorbance × 100. The index value
115 corresponding to the 97.5th percentile of the normal distribution was 37. Accordingly, to be
116 considered as positive (presenting elevated anti-apoA-1 IgG levels), samples had to display
117 both an absorbance value >0.64 OD and an index value ≥37%.

118 As described before,^{9, 10} our ELISA principally detects immunoreactivity (anti-apoA-1 IgG)
119 against unmodified form of apoA-1. In order to further determine whether our assay is
120 specifically dedicated to detect antibodies against native apoA-1 devoid of post-translational
121 modifications (PTM) and does not cross-react with other forms of modified apoA-1, we used our
122 human purified apoA-1 to generate carbamylated apoA-1, glycated apoA-1, and oxidized apoA-
123 1 to be tested as coating antigen in our ELISA, comparing the respective signals produced by
124 Passing-Bablok analyses.

125 ApoA-1 *carbamylation* was performed according to the protocol by Holzer et al.¹¹ Briefly, native
126 human purified ApoA-1 was carbamylated with potassium cyanate (50 mmol/L) in phosphate
127 buffered saline (pH 7.4) containing 100 µmol/L diethylenetriaminepentaacetic acid (DTPA) for 4
128 hours at 37°C. As a control, the same protocol was applied in the absence of potassium
129 cyanate. All preparations were passed through a column (MWCO 10,000 Da) to remove excess
130 reagents and used immediately for experiments. The apoA-1 carbamylation state was then
131 verified using commercial ELISA (OxiSelect Protein Carbamylation Sandwich Elisa kit from Cell
132 Biolabs; ref. STA-877). The quantity of carbamylated apoA-1 generated by this protocol was
133 33.8 ng/ml against 2.9 ng/ml with the control procedure.

134
135 ApoA-1 *glycation* was performed according to the protocol by Nobécourt et al.¹² Briefly, native
136 human purified apoA-1 was exposed to methylglyoxal (MG) (6mmol/l) in phosphate buffered
137 saline containing 100 µmol/L etilendiaminetetracetic acid (EDTA) for 24 hours at 37°C under 5%
138 CO₂. As a control, the same protocol was applied in the absence of MG. All preparations were
139 passed through a column (MWCO 10,000 Da) to remove excess reagents and used
140 immediately for experiments. The apoA-1 glycation state was then verified using commercial
141 ELISA (OxiSelect Methylglyoxal Competitive Elisa kit; Cell Biolabs ref. STA-811). The quantity
142 of MG glycated apoA-1 generated with this protocol was 138.9µg/ml against 0.07 µg/ml with the
143 control procedure.

144 ApoA-1 *oxidation* was performed according to the protocol by DiDonato et al.¹³ Briefly, native
145 human purified apoA-1 was oxidized in 60 mmol/l Na[PO₄] buffer (pH 7.4) using CuSO₄
146 (10µmol/l) with 40 µmol/l of H₂O₂ for 24 hours at 37°C. As a control, the same protocol was
147 applied in the absence of CuSO₄ and H₂O₂. All preparations were passed through a column
148 (MWCO 10,000 Da) to remove excess reagents and used immediately for experiments. The
149 amount of apoA-1 oxidation state was first verified by the mobility shift on SDS-PAGE gels, and
150 visualized by WB. As shown in **Supplemental Figure II**, the expected higher MW apoA-1

151 bands induced by oxidation-mediated apoA-1 dimers formation were achieved by combining
152 H₂O₂ and CuSO₄, allowing to use this procedure to generate oxidized apoA-1 for further
153 experiments.

154 These results indicate that i) our *in vitro* procedure generated the expected PTM, and that ii) the
155 native apoA-1 used in the present study does not contain substantial amount of carbamylation,
156 glycation or oxidation.

157 In order to further explore a theoretical potential influence of carbamylation, glycation or
158 oxidation of apoA-1 in our study, we further performed Passing-Bablok analyses using
159 carbamylated, glycated, oxidized apoA-1. Using these modified forms of apoA-1 in our in house
160 ELISA assay, we compared the immunoreactivity signals obtained, with those derived using
161 native apoA-1 on a subset of n=63 randomly selected CoLaus subjects and displaying a anti-
162 apoA-1 IgG positivity rate of 17% (11/63), closely corresponding to the anti-apoA-1 IgG positivity
163 rate retrieved on the whole CoLaus cohort (19%).

164 As shown in **Supplemental Figure III**, using *carbamylated* apoA-1 as coating antigen in our
165 ELISA induced a significant proportional bias of + 22% (slope: 1.22; 95%CI: 1.13-1.33), but no
166 systematic bias (intercept:-0.004; 95%CI:-0.04-0.04). Indeed, taking the same anti-apoA-1 IgG
167 positivity definition, using carbamylated apoA-1 induced a positivity rate of 27% with n=7
168 discordant cases. Six were false positives ((FP): samples that were negative when using native
169 apoA-1) and one was false negative ((FN): sample that was tested positive using native apoA-
170 1), translating into a significant anti-apoA-1 IgG positivity rate discordance of 64% (0/11 vs.
171 7/11; p=0.003). These results indicate that using carbamylated apoA-1 provides a significant
172 different signal than using native apoA-1 in our ELISA.

173 As shown in the **Supplemental Figure IV**, using *glycated* apoA-1 as coating antigen in our
174 ELISA, induced a non-significant proportional positive bias of + 8% (slope: 1.08,95%CI:0.97-
175 1.24), and a small statistically significant, but minor bias of 0.07 arbitrary units (Intercept: -0.07;
176 95%CI:-13 to -0.02). These results indicate that our ELISA is insensitive to apoA-1 MG-induced
177 glycation status.

178 Lastly, as depicted in **Supplemental Figure V**, using *oxidized* apoA-1 as coating antigen in our
179 ELISA showed that the two methods provide identical results with a no proportional bias (slope:
180 1.003; 95%CI:0.82-1.34) and no systematic bias (intercept :-0.0006; 95%CI:-0.09-0.09) when
181 compared to using human purified native apoA-1. These results indicate that our ELISA is
182 insensitive to apoA-1 oxidation status.

183 Lastly, in order to further eliminate the possibility of anti-apoA-1 IgG being directed against
184 glycated, carbamylated, oxidized apoA-1 or PTM-induced cross-linked multimers of apoA-1, we
185 adapted our ELISA protocol, performing additional WB and Liquid Chromatography (LC) – Mass
186 Spectrometry (MS)/MS analyses on pooled serum derived from n=3 study patients tested
187 positive and n=3 tested negative for anti-apoA-1 IgG.

188 For the *WB analysis*, one microgram of purified delipidated apoA-1 devoid of PTM was resolved
189 by 10% polyacrylamide gel electrophoresis under reducing conditions and transferred to a
190 polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore IPVH 00010), which was then
191 blocked for 1 hour at RT with non-fat dry milk 5% in tris-buffered saline with tween 20 (T-TBS).

192 Membranes were incubated with pooled sera derived from three anti-apoA-1 IgG positive
193 (OD_{405nm} value: 1.7 AU) patients and three anti-apoA-1 IgG negative patients (OD_{405nm} value:
194 0.2 AU) diluted (1:50) in non-fat dry milk 5% in T-TBS 2 hours at room temperature. A goat anti-
195 human IgG or rabbit anti-goat horseradish peroxidase conjugated (Dako, Agilent) was used as
196 secondary antibody, diluted 1:7000 in non-fat dry milk 5% in T-TBS for 1 hour at room
197 temperature. The detection was performed using the BM Chemiluminescence Blotting Substrate
198 (POD from Roche). The blot was exposed to horseradish peroxidase-conjugated anti-human Fc
199 IgG to reveal the anti-apoA-1 IgG binding and with horseradish peroxidase-conjugated anti-goat
200 Fc IgG for the commercially available goat anti-human apoA-1 IgG used as positive control.

201 As shown in **Supplemental Figure VI**, pooled sera from the anti-apoA-1 IgG positive patients
202 displayed a strong signal at 61 kD (panel A), whereas sera derived from anti-apoA-1 IgG
203 negative patients hardly provided a signal on WB (panel B). Polyclonal goat anti-human apoA-1
204 IgG displayed the expected 29kD MW band (panel C), as well as the one at 61 KD, in
205 accordance to our previous experiments.⁹

206 We then submitted the identified 61kD MW band to LC-MS/MS analyses. According to our *in-gel*
207 *digestion* protocol, gel pieces were dehydrated with 100 µl of 50 mM ammonium bicarbonate
208 (AB) in 30% acetonitrile (ACN) for 10 min. This solution was removed and gel pieces were then
209 incubated for 35 minutes at 56°C in 100 µl of 10 mM DTT in 50 mM AB. DTT solution was then
210 replaced by 100 µl of 55 mM iodoacetamide in 50 mM AB and the gel pieces were incubated for
211 30 min at room temperature in the dark. Gel pieces were then washed for 30 minutes with 100
212 µl of 50mM AB and for 30 min with 100 µl of 50 mM AB and 30% ACN. Gel pieces were then
213 dried for 45 minutes in a Speed-Vac Concentrator. Dried pieces of gel were rehydrated for 45
214 minutes at 4°C in 50 µl of a solution of 50 mM AB containing trypsin at 6.25 ng/µl. Extraction of
215 the peptides was performed with 50 µl of 1% trifluoroacetic acid (TFA) for 30 minutes at room
216 temperature with occasional shaking. The TFA solution containing the proteins was transferred
217 to a polypropylene tube. A second extraction of the peptides was performed with 70 µl of 0.1%
218 TFA in 50% ACN for 30 minutes at room temperature with occasional shaking. The second TFA
219 solution was pooled with the first one. The pooled extracts were completely dried by evaporation
220 under speed-vacuum. LC-ESI-MS/MS was performed on a Orbitrap XL Mass Spectrometer
221 (Thermo Fisher Scientific) equipped with a NanoAcquity system from Waters. Peptides were
222 trapped on a home-made 5 µm 200 Å Magic C18 AQ (Michrom) 0.1 × 20 mm pre-column and
223 separated on a home-made 5 µm 100 Å Magic C18 AQ (Michrom) 0.75 × 150 mm column with
224 a gravity-pulled emitter. The analytical separation was run for 40 minutes using a gradient of
225 H₂O/FA 99.9%/0.1% (solvent A) and CH₃CN/FA 99.9%/0.1% (solvent B) from 5% to 35% A in
226 20 minutes at a flow rate of 220 nL/min. For MS survey scans, the OT resolution was set to
227 60000 and the ion population was set to 5 × 10⁵ with an m/z window from 400 to 2000. Four
228 precursor ions were selected for collision-induced dissociation (CID) in the LTQ. For this, the ion
229 population was set to 1 × 10⁴ (isolation width of 2 m/z). The normalized collision energies were
230 set to 35% for CID. Then, for *protein identification*, peak lists (MGF file format) were generated
231 from raw data using the MS Convert conversion tool from ProteoWizard The peaklist files were
232 searched against the *Homo sapiens* database (UniProtKB, release 2017-03, 20184 entries)
233 using Mascot (Matrix Science, London, UK; version 2.5.1). Trypsin was selected as the enzyme,
234 with two potential missed cleavage sites. Precursor ion tolerance was set to 10 ppm and
235 fragment ion tolerance to 0.6 Da. Variable amino acid modifications were oxidized methionine,
236 carbamylated lysine, and glycated lysine, arginine and tryptophan. Fixed amino acid
237 modification was carbamidomethyl cysteine. The Mascot search was validated using Scaffold

238 4.7.5 (Proteome Software). Protein identifications were accepted if they could be established at
239 greater than 95.0 % probability and contained at least two identified peptides.

240 Results are shown in **Supplemental Figure VII**. Analysis of this 61 kD band identified apoA-1
241 with a total of 29 identified peptides spectrum matched (PSM), corresponding to 12 unique
242 peptide sequences, representing a protein sequence coverage of 47% (highlighted in yellow).
243 Among these 29 PSM, 3 PSM with the same sequence ((K)WQEE M_{ox} ELYR(Q)) were
244 identified with an oxidized methionine (**Supplemental Figure VII**, red frame). The amount of
245 oxidation could not be quantified exactly using this LC-MS/MS system, but as only one oxidized
246 methionin was found, the oxidation status of apoA-1 is likely to be insignificant and most
247 probably generated by the WB and LC-MS/MS process given apoA-1 susceptibility to oxidation.
248 Moreover, among these 29 PSM, no carbamylation or glycation were detected. Of interest, 8 of
249 these PSM corresponded to the C-terminal sequence spanning aa 240 to 265 (data not shown).
250 On this C-terminal sequence no oxidation or other PTM were identified.

251 These WB & LC-MS/MS findings indicate that the apoA-1 band at 61 KD recognized on WB by
252 pool sera derived from patients tested positive for anti-apoA-1 IgG is very unlikely to result from
253 recognition of glycated, carbamylated, oxidized cross-linked multimers of apoA-1. The most
254 likely explanation for this phenomenon is that anti-apoA-1 IgG preferentially recognize lipid-low
255 apoA-1. However, as the lipid content of our apoA-1 preparation is not assessable by LC-
256 MS/MS, this hypothesis warrants further study.

257 Taken together, these supplementary characterization analyses point to two main conclusions.
258 The first one is that our native human purified apoA-1 does not contain substantial amount of
259 carbamylation, glycation or oxidation, confirming previous studies that demonstrate that the
260 immune response to apoA-1 measured in our assay is well directed against unmodified apoA-1,
261 devoid of PTM.^{9, 10} The second one is that that our assay is not significantly influenced by
262 glycation or oxidation of apoA-1.

263 **Genotyping of the C260T *rs2569190* polymorphism in the CD14 receptor gene.**

264 After exclusion of non-Caucasian participants (n=395) and those with missing genetic data
265 (n=578), the final genotyped sample for the C260T *rs2569190* polymorphism consisted of 4247
266 individuals (**Figure 1**). Genotyping was performed using the Affymetrix GeneChip® Human
267 Mapping 500K array set, excluding SNPs with call rate <70% and individuals with call rate
268 <90%. The imputation dataset included 390,631 genotyped SNPs with call rate>0.9, Hardy-
269 Weinberg P-value>10⁻⁷ and minor-allele frequency (MAF)>1%. Imputation was performed using
270 IMPUTE version 0.2.0 and CEU haplotypes from HapMap release 21. The C260T *rs2569190*
271 polymorphism was imputed with an r²-hat=0.994. The minor allele in the CoLaus study was T
272 (49.3%) and the major allele C (50.7%), which is consistent with previous reports in Caucasian
273 populations.¹⁴

274

275 **Statistical analyses**

276 Univariate analysis of continuous variables was performed using the student's t-test or the non-
277 parametric Mann-Whitney test as appropriate, and results were expressed as mean ± standard
278 deviation (SD). Analysis of discrete variables was performed using chi-square test and results
279 were expressed as number of participants and (percentage). Survival curves for incident CAD
280 were produced using the Kaplan-Meier method and compared using the Logrank test. Patients
281 who had no events were censored at the time of death, loss to follow-up or the end of the study

282 period. Multivariate analysis of the associations between anti-apoA-1 IgG and incident CAD was
283 performed using Cox proportional hazards adjusting for sex, age, smoking status, diabetes,
284 hypertension, low (LDL) and high density lipoprotein cholesterol (HDL), baseline CAD, statin
285 and beta-blocker treatment and eGFR. Results were expressed as hazards ratio (HR) and 95%
286 confidence interval (CI). Adjusted HRs for incident CAD were firstly estimated for anti-apoA-1
287 IgG positivity as well as across tertiles of increasing anti-apoA-1 IgG values, with anti-apoA-1
288 IgG negative subjects used as the reference group. As anti-apoA-1 IgG concentration
289 distribution is skewed, values were further natural log transformed and standardized (mean=0
290 and SD=1) and HR for incident CAD were also assessed per one SD change. The same
291 analyses were repeated in genotyped subjects according to CD14 SNP subgroups and a
292 statistical interaction test was performed to assess differences between these genotype
293 subgroups.¹⁵ Sensitivity analyses were performed after exclusion of subjects with baseline CAD
294 and autoimmune disease.

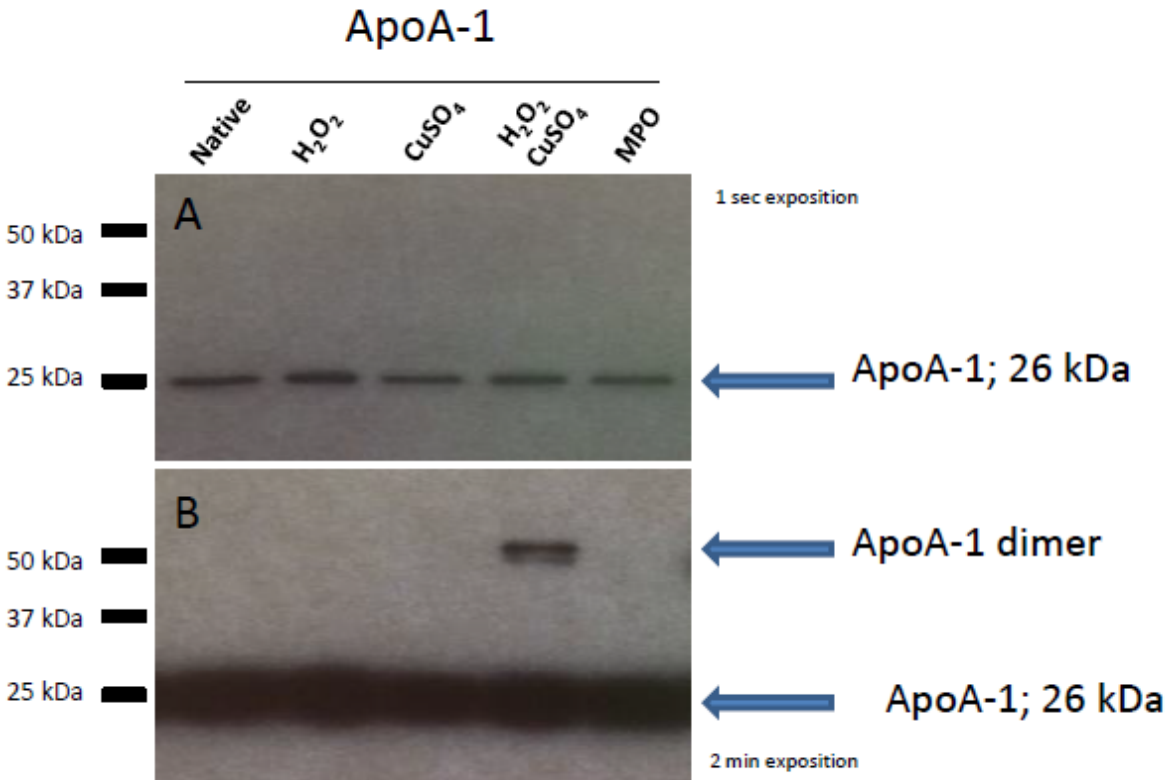
295 In genotyped subjects, we first assumed an additive model (CC vs. CT vs. TT), dividing the
296 sample into three subgroups according to the C260T *rs2569190* allele status. As previously
297 suggested,^{14, 16} we then assumed a recessive model (CC/CT vs. TT), where C-allele carriers
298 show similar, neutral CD14 gene expression, while homozygotes for the minor allele present
299 increased CD14 gene expression.^{17, 18} In both cases, a statistical interaction test was performed
300 to assess the heterogeneity of anti-apoA-1 IgG-related CAD risk, according to carriage of the T
301 allele.¹⁵ Results were expressed as HR (95%CI) within each subgroup and presented as a
302 forest plot.¹⁵ Taking into account the incident CAD rate in CoLaus (157 events or 3%) and a
303 two-sided alpha of 5%, we required 35-59 incident CAD events in subjects positive for anti-
304 apoA-1 IgG to detect an HR of anti-apoA-1 IgG for CAD of 1.5-1.7 with >80% power. Similarly,
305 considering the incident nonfatal CAD rate of 2.5% in our study and two-sided alpha of 5%, we
306 required 32-55 incident non-fatal CAD events in subjects positive for anti-apoA-1 IgG to detect
307 an HR of 1.5-1.7 with >80% power. All analyses were performed using STATA 13.0 (Stata Corp,
308 College Station, Texas, USA). A two-tailed test with $p < 0.05$ was considered statistically
309 significant.

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FIGURES

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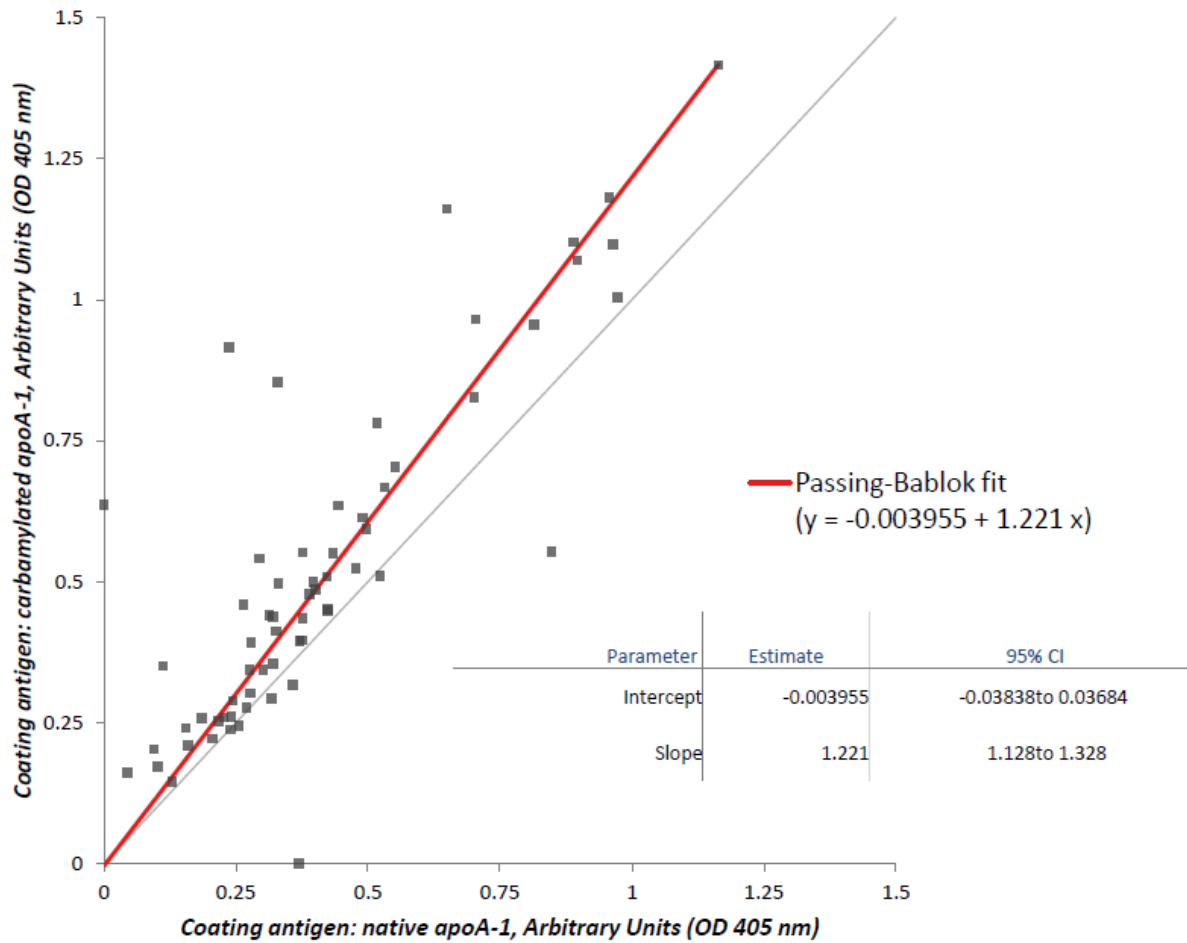


318

319 **Figure 1. ApoA-1 oxidation Western Blot. Panel A)** one-second exposition, **B)** two-minutes exposition in
320 order to visualize the oxidation-induced apoA-1 dimerization. The expected higher molecular weight
321 apoA-1 bands induced by oxidation-mediated apoA-1 dimers formation were achieved by combining
322 H₂O₂ and CuSO₄.

323

324

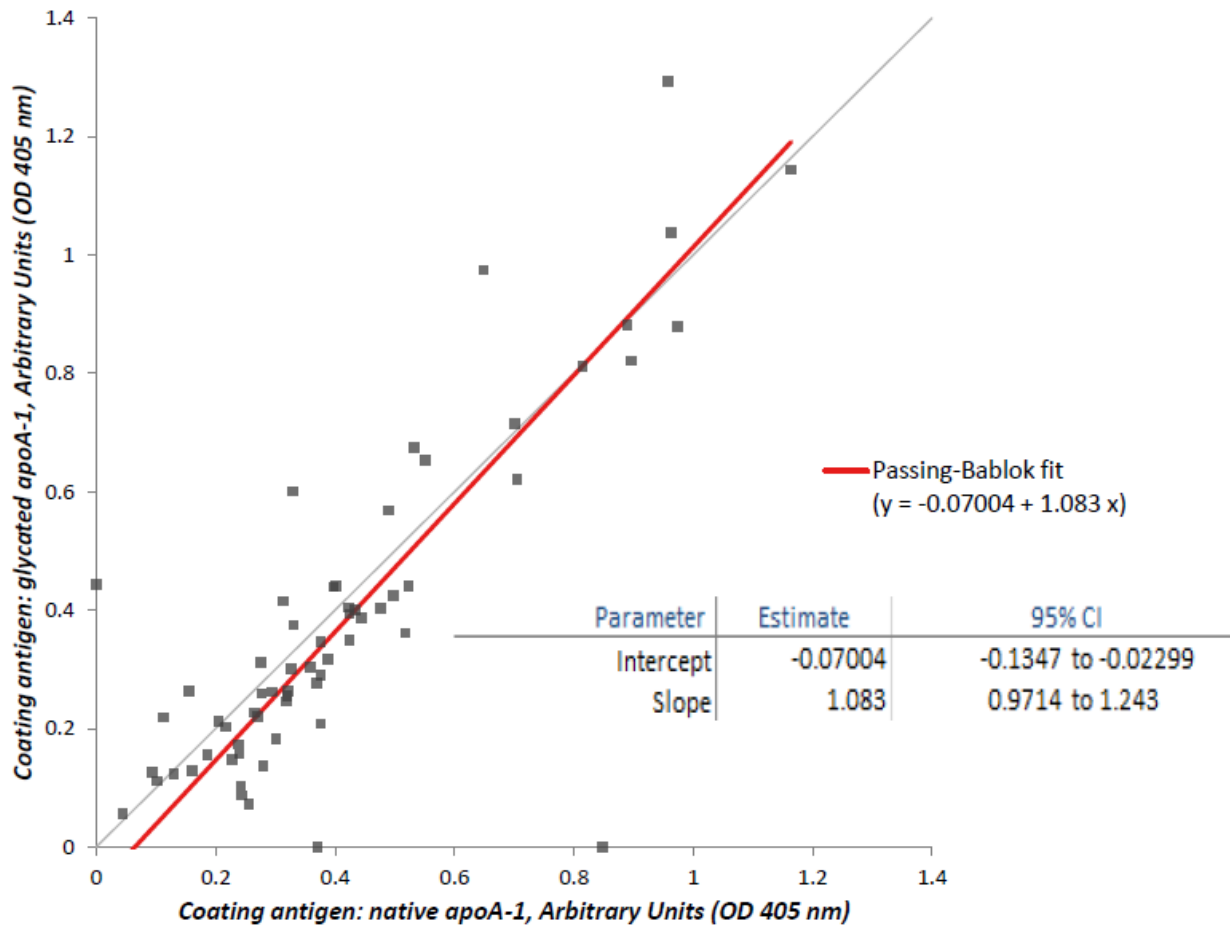


325

326 **Figure 2. Passing-Bablok analysis comparing the signals obtained using native versus carbamylated**
327 **apoA-1.** The grey line indicates the identity line, the red line indicates the correlation obtained. Using
328 carbamylated apoA-1 as coating antigen in our ELISA, induced a significant proportional bias of + 22%
329 (slope: 1.22; 95%CI: 1.13-1.33), but no systematic bias (intercept: -0.004; 95%CI: -0.04-0.04).

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332

333 **Figure 3. Passing-Bablok analysis comparing the signals obtained using native versus glycosylated apoA-1.**

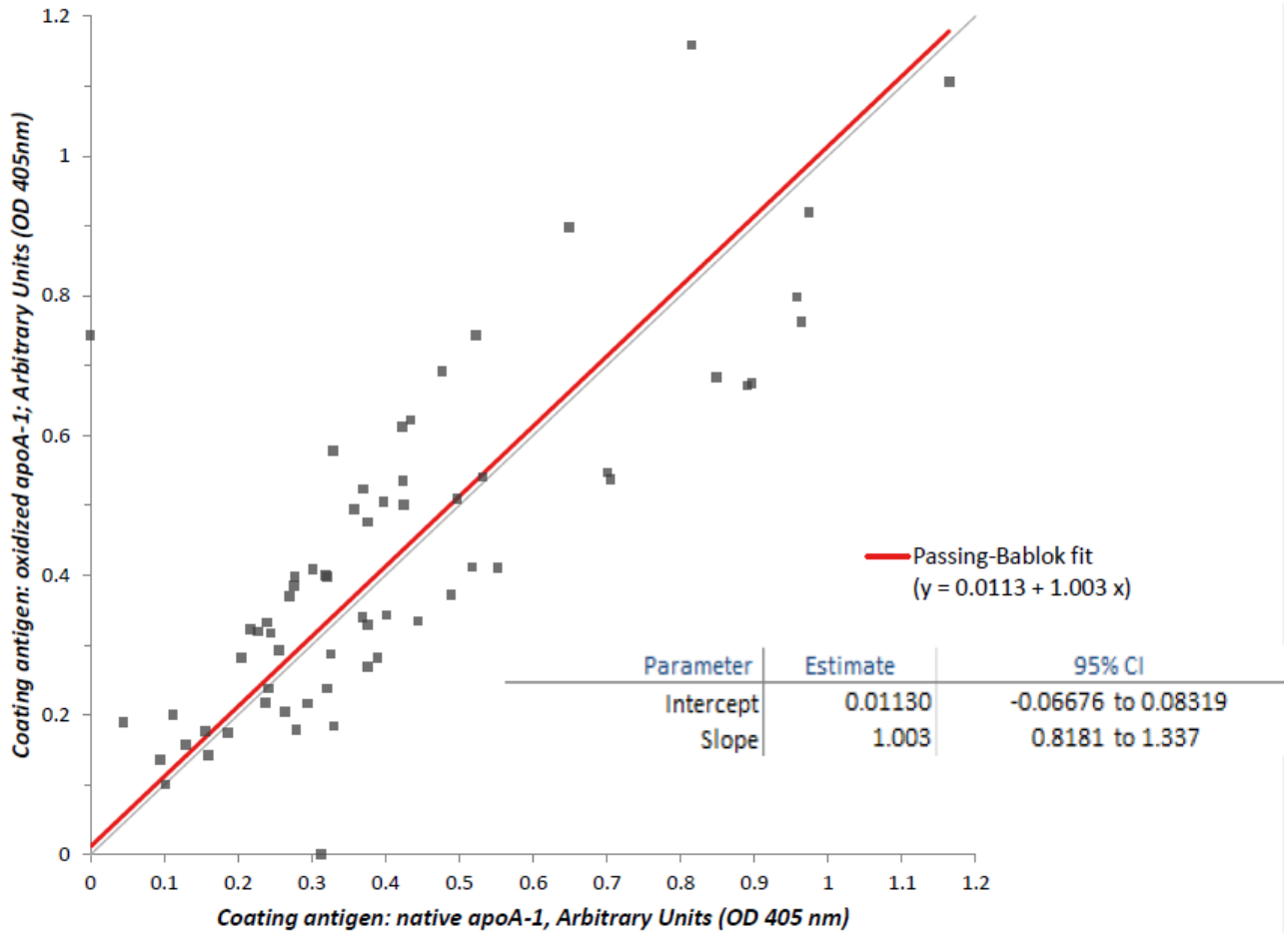
334 The grey line indicates the identity line, the red line indicates the correlation obtained. Using glycosylated

335 apoA-1 as coating antigen in our ELISA, induced a non-significant proportional positive bias of + 8%

336 (slope: 1.08; 95%CI: 0.97-1.24), and a small statistically significant, but minor bias of 0.07 arbitrary units

337 (Intercept: -0.07; 95%CI: -13 to -0.02).

338



340

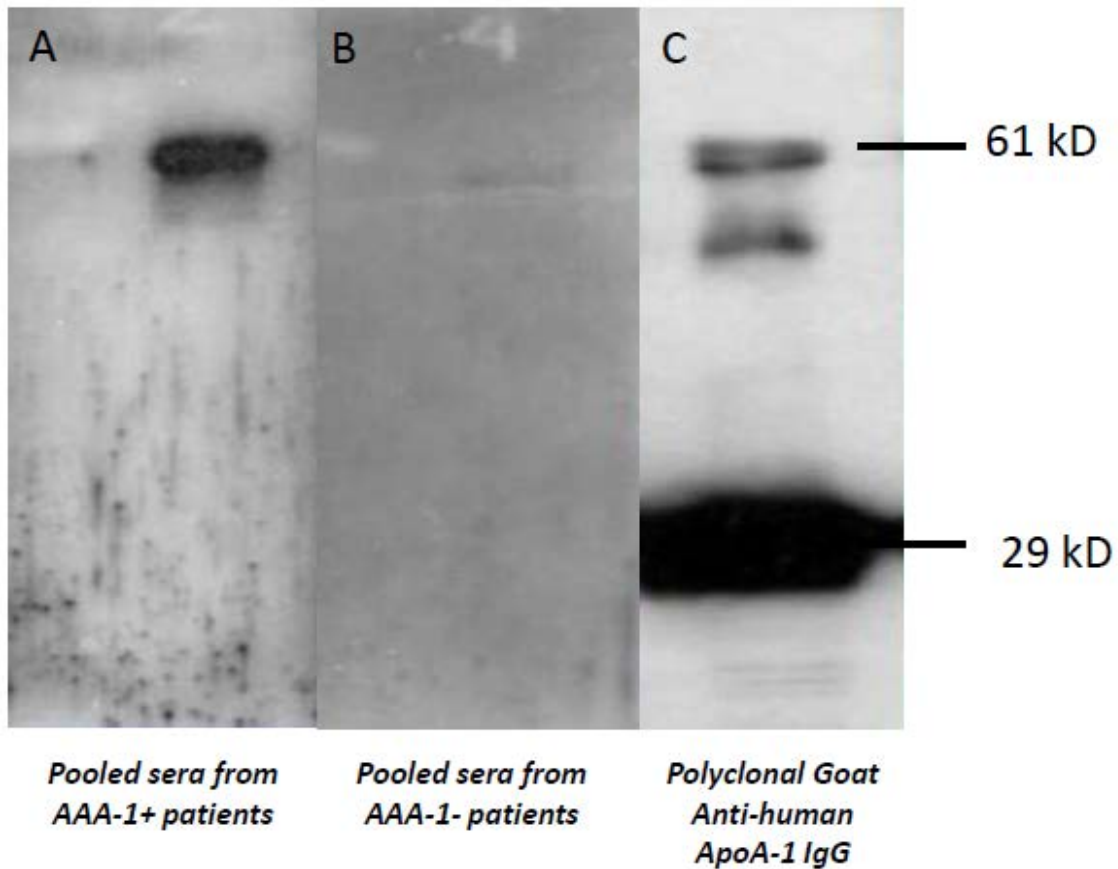
341 **Figure 4. Passing-Bablok analysis comparing the signals obtained using native versus oxidized apoA-1.**

342 The grey line indicates the identity line, the red line indicates the correlation obtained. Using oxidized

343 apoA-1 as coating antigen in our ELISA, induced a non significant proportional bias of 0.3% (slope: 1.003;

344 95%CI: 0.82-1.34) and no systematic bias (intercept: -0.0006; 95%CI:-0.09-0.09).

345



347

348 **Figure 5. Western blot of anti-apoA-1 IgG.** Human apoA-1 was migrated on polyacrylamide gel (10%)
 349 and then exposed to pooled sera (dilution 1:50) of patients tested either positive (panel A) or negative
 350 for anti-apoA-1 IgG (panel B). The sera from the anti-apoA-1 IgG positive patients displayed a strong
 351 signal at 61 kD (panel A), whereas the pooled sera derived from anti-apoA-1 IgG negative patients hardly
 352 provided a signal on WB (panel B). Polyclonal goat anti-human apoA-1 IgG displayed the expected 29kD
 353 molecular weight band (panel C), as well as a band at 61 KD, further submitted to liquid chromatography
 354 – mass spectrometry analyses.

355

APOA1_HUMAN (100 %), 30778.5 Da
Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1
12 exclusive unique peptides, 15 exclusive unique spectra, 29 total spectra, 125/267 amino acids (47 % coverage)

```

M K A A V L T L A V   L F L T G S Q A R H   F W Q Q D E P P Q S   P W D R V K D L A T
V Y V D V L K D S G   R D Y V S Q F E G S   A L G K Q L N L K L   L D N W D S V T S T
F S K L R E Q L G P   V T Q E F W D N L E   K E T E G L R Q E M   S K D L E E V K A K
V Q P Y L D D F Q K   K W Q E E M E L Y R   Q K V E P L R A E L   Q E G A R Q K L H E
L Q E K L S P L G E   E M R D R A R A H V   D A L R T H L A P Y   S D E L R Q R L A A
R L E A L K E N G G   A R L A E Y H A K A   T E H L S T L S E K   A K P A L E D L R Q
G L L P V L E S F K   V S F L S A L E E Y   T K K L N T Q
    
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Valid	...	Sequence	Prob	Masc...	Masc...	Masc...	NTT	Modifications	Observed	Actual Mass	Charge	Delta ...	Delta ...	Ret...
✓	✓	(K)DLATVYVDV(LK)(D)	100 %	40.5	29.2	27.7	2		618.35	1'234.68	2	0.0010	0.84	1760
✓	✓	(K)DLATVYVDV(LK)(D)	100 %	37.8	29.2	28.0	2		618.35	1'234.68	2	0.0010	0.84	1760
✓	✓	(R)DYVVSQFEGS(LGK)(Q)	100 %	117.0	31.1	102.0	2		700.84	1'399.66	2	0.0016	1.1	1390
✓	✓	(K)LLDNWDSVTSF(LK)(L)	100 %	105.1	32.4	99.6	2		806.90	1'611.78	2	0.0027	1.7	1690
✓	✓	(K)LLDNWDSVTSF(LK)(L)	100 %	86.7	32.4	86.7	2		806.90	1'611.78	2	0.0040	2.5	1680
✓	✓	(R)QEMSKDLEEVK(A)	100 %	35.8	30.6	33.5	2		668.33	1'334.64	2	0.0014	1.0	1050
✓	✓	(K)WQPYLDDFQK(K)	100 %	46.5	31.2	34.7	2		626.81	1'251.61	2	-0.00039	-0.31	1290
✓	✓	(K)WQPYLDDFQK(K)	100 %	38.3	30.9	34.2	2		626.81	1'251.61	2	0.0012	0.95	1290
✓	✓	(K)WQPYLDDFQK(W)	100 %	36.8	31.6	22.5	2		690.86	1'379.71	2	-0.00067	-0.48	1200
✓	✓	(K)WQPYLDDFQK(W)	98 %	31.2	31.6	19.7	2		690.86	1'379.71	2	-0.00067	-0.48	1200
✓	✓	(K)WQPYLDDFQK(W)	95 %	28.7	31.6	19.9	2		460.91	1'379.71	3	-0.00050	-0.36	1200
✓	✓	(K)WQEE ⁺ MELYR(Q)	100 %	43.8	25.8	43.8	2	Oxidation (+16)	650.29	1'298.56	2	0.00058	0.45	1200
✓	✓	(K)WQEE ⁺ MELYR(Q)	100 %	36.0	25.8	36.0	2	Oxidation (+16)	650.29	1'298.56	2	0.00058	0.45	1200
✓	✓	(K)WQEE ⁺ MELYR(Q)	100 %	33.0	25.8	31.7	2	Oxidation (+16)	650.29	1'298.56	2	0.00058	0.45	1200
✓	✓	(K)VEPLRAELQEGAR(Q)	100 %	35.3	30.8	31.0	2		734.40	1'466.79	2	0.0012	0.84	1150
✓	✓	(K)VEPLRAELQEGAR(Q)	95 %	32.1	30.9	22.3	2		489.94	1'466.78	3	-0.00074	-0.50	1150
✓	✓	(R)THLAPYSDEL(R)(Q)	100 %	38.1	31.3	27.9	2		651.33	1'300.64	2	0.0012	0.89	1120
✓	✓	(R)THLAPYSDEL(R)(Q)	97 %	30.7	31.3	19.7	2		434.56	1'300.64	3	0.0022	1.7	1730
✓	✓	(K)AKPALEDL(R)(Q)	100 %	43.7	25.0	36.3	2		506.79	1'011.57	2	-0.00063	-0.63	1080
✓	✓	(K)AKPALEDL(R)(Q)	100 %	32.7	25.0	25.1	2		506.79	1'011.57	2	0.0016	0.16	1080
✓	✓	(K)AKPALEDL(R)(Q)	98 %	28.0	25.0	20.8	2		506.79	1'011.57	2	-0.00063	-0.63	1080
✓	✓	(R)QGLLPVLESF(K)(V)	100 %	55.3	27.4	49.0	2		615.86	1'229.70	2	0.00032	0.26	1640
✓	✓	(R)QGLLPVLESF(K)(V)	100 %	53.2	27.4	41.8	2		615.86	1'229.70	2	0.00032	0.26	1640
✓	✓	(R)QGLLPVLESF(K)(V)	100 %	51.0	27.3	31.6	2		615.86	1'229.70	2	0.00093	0.76	1620
✓	✓	(R)QGLLPVLESF(K)(V)	99 %	30.9	26.9	21.3	2		615.86	1'229.71	2	0.0033	2.7	1800
✓	✓	(K)VSFLSALEEYTK(K)	100 %	76.1	30.9	72.3	2		693.86	1'385.71	2	0.0018	1.3	1670
✓	✓	(K)VSFLSALEEYTK(K)	100 %	66.9	30.9	65.3	2		693.86	1'385.71	2	0.0018	1.3	1670
✓	✓	(K)VSFLSALEEYTK(K)	100 %	63.8	30.7	59.9	2		693.86	1'385.71	2	0.00048	0.34	1680
✓	✓	(K)VSFLSALEEYTK(K)	100 %	58.3	30.7	56.7	2		693.86	1'385.71	2	0.00048	0.34	1680

359 **Figure 6. Liquid Chromatography – Mass Spectrometry Analyses of the 61kD apoA-1 band. Above :**
 360 **Highlighted in yellow the 12 exclusive amino acid sequences identified in the 61kD apoA-1 band.**
 361 **Highlighted in green, the only oxidized methionin found. Below :** Among the 29 peptides spectrum

362 matched (PSM), 3 PSM with the same sequence ((K)WQEE **M_{ox}** ELYR(Q)) were identified with an oxidized
363 methionine (sequences in the red frame), but no carbamylation or glycation were detected.

364

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