Manuscript Number: ATH-D-19-00072R1

Title: Soluble low-density lipoprotein receptor-related protein 1 as a biomarker of coronary risk: predictive capacity and association with clinical events

Article Type: Research paper

Section/Category: Translational Research

Keywords: Biomarker; cardiovascular risk; coronary artery disease; REGICOR; soluble LRP1

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Abstract: Background and aims: To determine whether circulating sLRP1 levels are associated with future coronary events and improve the predictive capacity of the REGICOR (Registre Gironí del Cor) risk function.

Methods: Case-cohort study based on the follow-up of the REGICOR population-based cohort. Of the 5,404 participants aged between 35 and 74 years without previous history of cardiovascular disease, 117 subjects who presented angina, or fatal or non-fatal myocardial infarction were included and 512 individuals were randomly selected as a subcohort (including 14 patients who presented coronary events). sLRP1 levels were measured in basal plasma samples by commercial ELISA. Hazard ratio (HR) was estimated with Cox models adjusted for potential confounding factors. Discrimination and reclassification were analyzed with the c-index and the net reclassification index (NRI), respectively. A Mendelian randomization approach was used to explore the causality of the association between sLRP1 and coronary artery disease (CAD).

Results: The group of participants who presented a CAD event showed higher levels of sLRP1 than the subcohort (2.45 [0.43; 8.31] vs. 2.07 [0.40; 6.65] µg/mL, $p < 0.001$). sLRP1 was significantly associated with CAD events even after adjusting for confounding factors (adjusted HR per standard deviation=1.30, 95% CI: 1.01-1.67, $p = 0.039$). sLRP1 did not increase the predictive capacity or improve cardiovascular risk stratification of the REGICOR function. The LRP1 genetic variants associated with CAD risk were not related to sLRP1 concentration.

Conclusions: Plasma sLRP1 is independently associated with the incidence of coronary events, but it does not improve the predictive capacity of the REGICOR risk function.

HIGHLIGHTS

- Plasma sLRP1 is independently associated with the incidence of coronary events.
- Plasma sLRP1 does not improve risk prediction when added to the REGICOR function
- The *LRP1* variants tested are associated with coronary artery disease.
- The *LRP1* variants tested are not associated with plasma sLRP1 concentration.

ATH-D-19-00072R1

Soluble low-density lipoprotein receptor-related protein 1 as a biomarker of coronary risk: predictive capacity and association with clinical events

Running title: Soluble LRP1 and coronary risk prediction

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Number of figures: 1

Number of tables: 4

ABSTRACT

Background and aims: To determine whether circulating sLRP1 levels are associated with future coronary events and improve the predictive capacity of the REGICOR (Registre Gironí del Cor) risk function.

Methods: Case-cohort study based on the follow-up of the REGICOR population-based cohort. Of the 5,404 participants aged between 35 and 74 years without previous history of cardiovascular disease, 117 subjects who presented angina, or fatal or non-fatal myocardial infarction were included and 512 individuals were randomly selected as a subcohort (including 14 patients who presented coronary events). sLRP1 levels were measured in basal plasma samples by commercial ELISA. Hazard ratio (HR) was estimated with Cox models adjusted for potential confounding factors. Discrimination and reclassification were analyzed with the c-index and the net reclassification index (NRI), respectively. A Mendelian randomization approach was used to explore the causality of the association between sLRP1 and coronary artery disease (CAD).

Results: The group of participants who presented a CAD event showed higher levels of sLRP1 than the subcohort (2.45 [0.43; 8.31] vs. 2.07 [0.40; 6.65] µg/mL, $p<0.001$). sLRP1 was significantly associated with CAD events even after adjusting for confounding factors (adjusted HR per standard deviation=1.30, 95% CI: 1.01-1.67, $p=0.039$). sLRP1 did not increase the predictive capacity or improve cardiovascular risk stratification of the REGICOR function. The *LRP1* genetic variants associated with CAD risk were not related to sLRP1 concentration.

Conclusions: Plasma sLRP1 is independently associated with the incidence of coronary events, but it does not improve the predictive capacity of the REGICOR risk function.

Keywords: Biomarker; cardiovascular risk; coronary artery disease; REGICOR; soluble LRP1.

INTRODUCTION

Low-density lipoprotein receptor-related protein 1 (LRP1) is a ubiquitously expressed cell-bound receptor of the low-density lipoprotein receptor family. It is involved in diverse biological processes, including lipoprotein metabolism and modulation of vascular integrity [1]. Our group has consistently demonstrated that vascular LRP1 plays a pivotal role in human coronary atherosclerosis. This receptor mediates the internalization of matrix-retained aggregated LDL (agLDL), which leads to the intracellular accumulation of neutral lipids in human coronary vascular smooth muscle cells (hcVSMCs) [2, 3]. LDL aggregation is an essential step in the initiation and progression of human atherosclerosis [4]. Indeed, the susceptibility of LDL particles to aggregate is predictive of cardiovascular death, independently of traditional risk factors for coronary artery disease (CAD) [5]. Supporting these findings, LRP1 is overexpressed in human lipid-enriched advanced coronary atherosclerotic plaques [6]. In addition, clinical studies have suggested that different genetic variants of the receptor are independent risk factors for cardiovascular conditions [7], including CAD [8, 9].

LRP1 has a soluble form (sLRP1) that has been detected in the circulation [10]. Comprising the α chain (515 kDa) and a fragment of the β chain (55 kDa) of the cellular receptor, sLRP1 is generated by constitutive or induced intramembrane proteolysis [10, 11]. We have recently demonstrated that sLRP1 release is increased by atherogenic lipoproteins (LDL, VLDL and agLDL) in an *in vitro* model of hcVSMCs and in an *ex vivo* model of human atherosclerotic plaques [12]. In addition, we have reported that circulating sLRP1 is a biomarker of atherosclerotic-related conditions and cardiometabolic disease [12-14]. However, the association between sLRP1 and the risk of CAD and the predictive capacity of sLRP1 have not been addressed at the population level.

Based on our previous findings, here we examined whether circulating sLRP1 levels are associated with future CAD events and also assessed the predictive capacity of sLRP1 beyond classical CAD risk functions in a population-based study. We also explored the causality of the association between sLRP1 and CAD using a Mendelian randomization approach.

PATIENTS AND METHODS

Study participants and study design

We included the follow-up of the participants of the 2005 REGICOR (Registre Gironí del Cor) population survey (n=6,352 individuals) covering a population of ~600,000 in the region of Girona (Catalonia, Spain). The REGICOR protocol was approved by the Institution's ethics committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all REGICOR participants. A detailed design of the study has been previously described [15,16]. The overall participation rate was >72%. Participants aged between 35 and 74 years with no cardiovascular disease (CVD) at baseline were included in the study (n=5,404). A case-cohort study was designed with all CAD cases during follow-up (n=117) and a random subsample of the cohort (subcohort; n=512) (**Figure 1**). As shown in **Supplemental Table S1**, the characteristics of the cohort and subcohort were similar.

Follow-up and endpoints

Participants were followed up by re-examination and a structured telephone interview between 2009 and 2011. The composite endpoint included fatal or non-fatal first occurrence of myocardial infarction or angina and CAD mortality. All events were classified by an expert committee following standardized criteria [17,18]. Non-fatal myocardial infarction events were validated with medical records, considering the following International Classification of Diseases (ICD)-9 codes: 410, 411.0, 411.1, 412, 414; and ICD-10 codes: I21-I25, including subtypes. Angina was defined on the basis of the presence of symptoms and objective demonstration of ischemia or presence of coronary stenosis. To identify fatal CAD events, the participant database was linked to the Official Death Registry, considering the following ICD-9 codes: 410–414, and ICD-10 codes: I20-I22, I24, I25.

sLRP1 determination

sLRP1 concentrations were measured in frozen citrated plasma samples using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Usn Life Science Inc. China) and following the manufacturer's instructions. The assay had a within- and between-assay coefficient of variation lower than 10% and 12%, respectively, and a limit of detection of 0.156 ng/mL. According to the manufacturer's specifications, the assay showed no significant cross-reactivity or interference

between LRP1 and analogs. Plasma samples were stored at -80°C from blood sampling (2004-2006) to sLRP1 quantification (2017).

Mendelian randomization approach

Analysis of the CARDIoGRAMplusC4D aggregate results [19] revealed several genetic variants (in a linkage disequilibrium block that included the first and second exons of the gene) in the *LRP1* gene showing a potential association with CAD (**Supplemental Figure S2A**). We selected 2 of these SNPs (rs11172113, $p=1.71 \cdot 10^{-5}$; rs4367982, $p=0.009$). The SNP rs11172113 was selected because it showed the strongest association with CAD in terms of *P*-value. The SNP rs4367982 was randomly selected among those showing an association with CAD with a $p<0.01$. Two SNPs of the same locus but not associated with CAD (rs17547610 and rs1800137) were also randomly selected and included in the study. We then genotyped the 581 participants with available DNA using a TaqMan assay to explore the associations between these variants, CAD risk and sLRP1 concentration. The characteristics of the genotyped and non-genotyped groups were similar (**Supplemental Table S2**).

Other covariates and coronary artery disease risk estimation

Examinations were performed by trained nurses and interviewers using standard questionnaires and measurement methods [20]. Participants were classified as smokers (current or quit<1 year), former smokers (quit≥1 year), or never smokers. Body mass index (BMI) was calculated as weight divided by squared height (kg/m²). Systolic and diastolic blood pressures (SBP and DBP) were measured after 5 min of rest with a calibrated sphygmomanometer following a standardized procedure. Patients were considered hypertensive when previously diagnosed by a physician, under treatment, or presenting SBP ≥140 mmHg or DBP ≥90 mmHg.

Fasting blood samples were drawn using standard procedures, and total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations were measured. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation when triglycerides were <300 mg/dL. Diabetes was defined as history of diabetes, diabetes treatment, or a single fasting glycaemia determination >125mg/dL.

CAD risk was estimated using the calibrated and validated Framingham-REGICOR function [21, 22]. This function is a calibrated version of the original Framingham risk function according to the incidence of CAD and the prevalence of cardiovascular risk factors in Spain.

Sample size determination

The case-cohort design with a subcohort of 512 participants selected from a cohort of 5,404, assuming a significance level of 5%, an incidence of coronary heart disease of 1.9% at 6.2 years (median of follow-up), provided a power of 80% to detect a Hazard Ratio of 1.73 for a unit increment in sLRP1 standard deviation. Sample size calculation was done using “ccsize” from the gap R package [23].

Statistical analysis

Statistical analyses were performed using R version 3.4.0. The simple randomization procedure was implemented in R using the function “sample”. The characteristics of participants were summarized as frequencies (percentage) for categorical variables and as mean (standard deviation) or median (interquartile range -IQR-) for continuous variables. Their distribution was compared among groups by chi-squared for categorical variables, and Student t-test, ANOVA, Mann-Whitney U test or Kruskal-Wallis test for normally distributed and non-normally distributed variables. Spearman's rho coefficient was used to assess the correlation between continuous variables.

The association between sLRP1 and CAD risk was assessed with proportional risk Cox models using the Lin-Ying methodology for case-cohort designs [24]. These models were fitted using the “cch” function from the “survival” R package. The multivariate Cox models included age and sex, and confounder variables. To be considered confounder, a variable had to be associated with both CAD and sLRP1 concentration at P -value<0.05. To replace missing values in the variables included in the multivariate analyses we used 10 multiple imputations using the “mice” package. We compared complete results with multiple imputation results as a sensitivity analysis.

The added predictive value of sLRP1 over the classical Framingham-REGICOR risk function factors (age, sex, total cholesterol, HDL-C, SBP, DBP, diabetes and smoking) was assessed by the improvement in discrimination,

computing the increment modified C-statistic for case-cohort studies [25, 26]. Moreover, a modified categorical and continuous Net Reclassification Index (NRI) taking into account the case-cohort design and censoring the follow-up at 5-years was computed to assess the reclassification capacity of sLRP1 [27]. When computing the categorical NRI, cut-off points of 2.5% and 5% at 5-years were defined, which corresponds to the usual CAD risk points to define moderate and high risk groups [21, 22]. We also calculated the “clinical NRI” considering only the individuals in the moderate risk group and we used the method proposed by Paynter and Cook to correct for potential bias [28].

RESULTS

Baseline characteristics

From the total cohort, we built a subcohort comprising the 117 incident CAD (cases) and 512 randomly selected individuals (including an overlap of 14 patients who presented CAD events in the subcohort). The median follow-up was 6.2 years.

Table 1 shows the characteristics of the study population. As expected, those participants suffering a CAD event during the follow-up period were typically older, more frequently men, and had a significantly poorer cardiovascular risk factor profile than the subcohort members, except for BMI. Compared to the subcohort, cases showed higher plasma sLRP1 concentrations.

The demographic, clinical, and biochemical data of the participants were compared among sLRP1 quartiles (**Table 2**). Participants in the upper quartile had significantly higher BMI, glucose, total cholesterol, LDL-C, triglycerides, REGICOR risk, prevalence of hypertension and incidence of coronary events. In contrast, participants in the upper quartile had lower levels of HDL-C. There were no differences in age, sex or antihypertensive, anti-glycemic and lipid-lowering medication between sLRP1 quartiles.

Spearman correlations between plasma sLRP1 and cardiovascular risk factors are shown in **Supplemental Table S3**. Plasma sLRP1 levels were directly correlated with BMI, glucose, SBP, total cholesterol, LDL-C and triglycerides. In contrast, an inverse correlation between plasma sLRP1 levels and HDL-C was also observed.

Plasma sLRP1 and coronary events: association and predictive capacity

As shown in **Table 3**, the circulating levels of the sLRP1 were directly associated with CAD events after adjustment for age, sex, SBP, DBP, glycaemia, HDL-C and total cholesterol (Model 2, HR=1.30 for SD of sLRP1; 95% CI: 1.01-1.67; $p=0.039$). This association remained statistically significant after including LDL-C as confounding factor, instead total cholesterol (Model 3, HR=1.40 for SD of sLRP1; 95% CI: 1.11-1.76; $p=0.004$). Finally, due to the previous association reported between hypolipidemic drugs and plasma sLRP1 levels [12], the use of lipid-lowering medication was entered as confounding factor (Model 4). Adjustment for medication use had no effect on the association between circulating sLRP1 levels and coronary risk (HR=1.39 for SD of sLRP1; 95% CI: 1.10-1.76; $p=0.006$).

The addition of plasma sLRP1 to the REGICOR risk function did not improve its predictive capacity: discrimination remained unaltered and reclassification was not significant after the inclusion of sLRP1 (**Table 4**).

Mendelian randomization approach

The association between the four analyzed genetic variants and CAD risk are shown in **Supplemental Figure S2B**. The two variants showing a suggestive association with CAD in the CARDIoGRAMplusC4D consortium also showed a suggestive association in the REGICOR study (P -values 0.036 and 0.085). However, these variants were not associated with plasma sLRP1 concentration (**Supplemental Figure S2C**).

DISCUSSION

In this case-cohort study, we demonstrate for the first time that circulating sLRP1 levels are associated with CAD risk, independently of potential confounding factors. Nevertheless, this soluble receptor does not improve the discrimination and reclassification capacity of the REGICOR function.

Baseline concentration of plasma sLRP1 was independently associated with incidence of angina and fatal or non-fatal myocardial infarction in individuals with no known cardiovascular disease, even after extensive adjustment for potential confounding factors. This result confirms and complements previous data from our group that point to the potential of circulating sLRP1 as a biomarker of atherosclerotic-related conditions. sLRP1 is not constitutively released from

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hcVSMCs, and its shedding is activated by exposure to pro-atherogenic lipoproteins [12]. Smooth muscle cells (SMCs) are the main cellular components of the vascular wall, and recent evidence suggest that up to 40% of the foam cells previously identified as monocyte-derived macrophages in human coronary atherosclerosis are SMCs [29]. Therefore, contrary to established plasma lipid biomarkers that indicate circulating levels of pro- and anti-atherogenic lipoproteins, sLRP1 may reflect the vascular response to pro-atherogenic lipoproteins. It is therefore intuitive that a plasma biomarker that reflects atherosclerotic mechanisms occurring in the vasculature would be independently associated with cardiovascular outcomes. Indeed, plasma levels of sLRP1 are an independent predictor of carotid atherosclerosis in hypercholesterolemic patients, showing higher diagnostic performance than established and emerging lipid parameters, including total cholesterol, LDL-C, non-HDL-C and ApoB [12]. Additionally, we have reported that sLRP1 is a potential biomarker of epicardial fat volume, a surrogate indicator of CAD [30], in the general population and in diabetic patients [13, 14]. The results from the present study suggest that circulating sLRP1 levels indicate the presence of atherosclerosis-related conditions that lead to coronary events during the follow-up period.

Despite the association between sLRP1 and the incidence of coronary events, this soluble receptor did not improve risk prediction in terms of discrimination and reclassification when added to a model including classical risk factors, namely the REGICOR CAD risk function [21, 22]. These results could be explained by the close relation between plasma sLRP1 and cardiovascular risk factors [12] already accounted for in the REGICOR function. Our results are in line with previous findings that demonstrated the modest or the lack of improvement in the prediction of cardiovascular outcomes using novel blood biomarkers beyond readily available clinical data [31-33]. Additional efforts are still needed to identify biological markers that can improve risk stratification.

Our Mendelian randomization approach supports the association between *LRP1* genetic variants and CAD [8, 9, 34, 35], and more specifically corroborates the associations between the variants rs11172113 and rs4367982 and CAD risk reported by the CARDIoGRAMplusC4D consortium [19]. However, we did not observe any association between the *LRP1* variants tested and the plasma sLRP1 concentration. Our results show analogy with previous reports that suggested an independent

behavior of the soluble and tissue forms of the receptor. We and others have proposed that the extracellular levels of sLRP1 do not correlate with the levels of cellular receptor [12, 36], and thus, are not informative of cellular LRP1 membrane levels. Despite these findings, a causal role of sLRP1 in CAD should not be discarded. sLRP1 maintains the binding properties of the cellular receptor, which may affect the functionality of LRP1 ligands in an antagonistic or agonistic fashion [10]. Various *in vitro* models suggest that sLRP1 is a biologically active mediator of several cellular processes linked to atherosclerotic disease. Gaultier et al. [37] demonstrated that purified sLRP1 inhibits the TNF- α -induced activation of p38 MAPK and ERK/MAPK in cells of the nervous system. Other authors proposed that sLRP1 induces the expression of regulatory cytokines, including TNF- α , CCL2, and IL-10, in a murine macrophage cell line [38]. The pro-inflammatory role of sLRP1 has recently been supported by an independent publication [39]. Additionally, extracellular sLRP1 regulates proteolytic activity in the endometrium by decreasing the endocytic clearance of metalloproteinase-2 and -9 [40]. Of note, sLRP1 is functional in the bloodstream. Sagare et al. [41] demonstrated that sLRP1 binds amyloid-beta in the circulation, and they proposed the exogenous administration of recombinant sLRP1 as a novel therapeutic approach for dementia. Accordingly, circulating sLRP1 may be involved in the development of coronary atherosclerotic disease and subsequent cardiovascular events through the regulation of atherosclerotic-related mechanisms. Further functional studies should address this hypothesis.

The strengths of our study are the strict patient characterization and the robust control of potential covariates. We used the REGICOR population cohort, which has been extensively and exhaustively characterized regarding cardiovascular and lifestyle risk factors and incidence of cardiovascular events. This cohort is representative of a population of approximately 600,000 individuals from the Girona province in North-Eastern Iberian Peninsula—an area with a similar cardiovascular risk factor prevalence than the rest of this European region [42], thereby supporting the external validity of the results. We also used a robust and efficient study design that allowed the calculation of discrimination and reclassification statistics [18]. Conversely, some limitations should be noted. First, differential censoring between the cases and the subcohort could introduce some bias. Furthermore although several statistical tools have been developed for case-cohort designs, the comparability between all of them has not been properly assessed. Second, LRP1 is

expressed in a wide range of tissues, and its soluble form can be released by many cell types, including hepatocytes [10], trophoblasts [11] and Schwann cells [37]. Therefore a possible lack of specificity should be taken into account. Additionally, circulating sLRP1 has been proposed as biomarker of conditions such as liver disease [10], autoimmune disease [38], Alzheimer disease-type dementia [43] and idiopathic dilated cardiomyopathy [44]. We cannot exclude the effect of diseases that were not recorded in this study and that may have an effect on plasma sLRP1 levels. Finally, the analyses were performed in participants without a history of cardiovascular disease. Therefore, our results cannot be extrapolated to other populations.

In conclusion, plasma levels of sLRP1 are independently associated with the incidence of CAD. However, sLRP1 failed to improve the discrimination or reclassification of classical risk factors in the REGICOR CAD risk function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FINANCIAL SUPPORT

This work was funded by PERIS from Agència de Gestió d'Ajuts Universitaris i de Recerca [Grant SLT002/16/00088], the Instituto Salud Carlos III, which is co-financed by the European Fund for Regional Development [Grants FIS 94/0540, FIS 99/0013-01, FIS 99/9342, FIS PI020471, FIS PI08/1327, INTRASALUD PI11/01801, CP 06/00100, CES12/025, CP12/03287, ECA07/060, FIS PI14/01729, FIS PI18/01584, RD12/0042/0010/0011/0013/0061], and by the Fundació MARATÓ TV3 [Grant 201521-10; 081630], and the Government of Catalonia through the Agency for Management of University and Research Grants [2009SGR1195, 2014SGR240 and 2017SGR946]. DdG-C was a recipient of a Juan de la Cierva-Incorporación from the Ministerio de Economía y Competitividad [Grant IJCI-2016-29393]. CIBERCV and CIBERESP (CB16/11/00403 to DdG-C and VLI-C; CB16/11/00229 to JM; CB16/11/00246 to RE; CB06/02/0029 to IS) are projects of the Instituto de Salud Carlos III.

AUTHOR CONTRIBUTIONS

Conception and design: (DdG-C, RE, JM, VL-C); Provision of study materials or patients (VL-C, JM); Data analysis and interpretation (DdG-C, RE, AV, IS; SS-B, JM, VL-C); Manuscript writing: (DdG-C, RE, JM, VL-C); Final approval of manuscript: (DdG-C, RE, IS, SS-B, JM, VL-C).

ACKNOWLEDGEMENT

Data on coronary artery disease/myocardial infarction have been contributed by CARDIoGRAMplusC4D investigators and have been downloaded from www.CARDIOGRAMPLUSC4D.org.

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FIGURE LEGENDS

Figure 1. Flowchart of the study participants included in the case-cohort study. In this type of design all the incident cases of interest and a subsample (subcohort) of the participants of the original eligible cohort are included (a subset of participants are considered both cases and members of the subcohort). CVD: cardiovascular disease; CAD: coronary artery disease.

Arnold von Eckardstein

Editor in Chief

Atherosclerosis

Dear Editor in Chief,

Enclosed please find the revised version of our manuscript entitled: "Soluble low-density lipoprotein receptor-related protein 1 as a biomarker of coronary risk: predictive capacity and association with clinical events" (ATH-D-19-00072) resubmitted as an Original Research Paper in Atherosclerosis.

We would like to thank the Reviewers for their critical comments that have strongly improved the manuscript. We have addressed, point-by-point, all the issues raised by the Reviewers. According to their suggestions, we have modified Methods, Results and Discussion sections of the manuscript. As a result, we think that the manuscript has been deeply improved. We have changed each section of the manuscript by including new information that has highly increased the impact of the reported results. Please note that new information has been labeled in red throughout all the manuscript.

All authors have read and approved the submission of the manuscript. The manuscript has not been published and is not being considered for publication elsewhere in whole part or in any language.

We hope that the manuscript will be now acceptable for publication in Atherosclerosis.

We thank you for your time and effort.

Sincerely,

V. Llorente-Cortés (cllorente@santpau.cat)

Jaume Marrugat (jaume@imim.es)

POINT BY POINT RESPONSE TO COMMENTS BY EDITOR AND EDITORIAL OFFICE

The authors should precisely respond to all remarks of the reviewers, particularly, those concerning the statistical analyses. In addition, the randomization process as well as the differences between the case and control study (subgroup) should be reported.

Thanks for the interest in our work. In the new version of the manuscript, we have addressed the remarks and comments raised by the Reviewers on a point by point basis, including the procedure to select the subcohort. We have also analyzed whether there are differences between the subcohort and the original cohort.

Formatting guidelines have been applied to the new version of the manuscript.

POINT BY POINT RESPONSE TO COMMENTS BY REVIEWER # 1

In this interesting sub-study of the REGICOR cohort, Gonzalo-Calvo et al. characterize the prognostic value of Soluble LRP 1 and its potential causal relationship with coronary heart disease. Even though the data might be of interest for the journal, I have some issues with the methods of the sub-study and the presentation of the data:

Thanks for the interest in our work. We have addressed the remarks and comments raised by the Reviewer on a point by point basis.

Major points:

1. In general, the patient selection and methodology of the sub-study has to be presented in more detail.

You state that a random subsample (n= 512) of the original cohort (n=5404) was selected for this sub-study. If randomization was indeed used, the processes (e.g. simple randomization, covariate adaptive randomization etc.) and the used software/calculation need to be described/mentioned in the methods. This is of major importance, since the whole concept of a case-cohort study relies on the random selection of the control group. In addition, did you compare the patients of the sub-cohort with the rest of the eligible cohort in terms of baseline and demographic parameters (e.g. age, frequency of risk factors, laboratory parameters etc.)?

For the mendelian randomization approach you state in your manuscript that you genotyped "the 589 participants with available DNA" (Page 5). Have the rest of the DNA samples been used for other purposes or was DNA purified only for 589 patients? This needs to be addressed in the manuscript. And again, did this sub-cohort for the genetic analysis differ in terms of baseline and demographic parameters with the rest of the patient population?

We used a simple randomization procedure to select 512 participants of the original cohort as a representative subcohort for our case-cohort study. This procedure was implemented in R using the function "sample". Accordingly, additional information has been included in the Methods section.

In the new version of the manuscript, we have compared the characteristics of the whole cohort and the selected subcohort. These results are included as Supplemental Table S1. As shown in this table, the characteristics of the cohort and subcohort are similar and support the representability of the selected group.

In the Mendelian randomization study, we included 581 out of the 615 participants in the study (due to a typing error in the original submission a sample size of 589 was included). In 34 subjects DNA was not available because these participants did not provide DNA or the authorization to perform genetic studies or there were technical problems in the DNA isolation process. We have also analyzed whether there are differences between the group of 581 participants with DNA and the non-genotyped group. The characteristics of the two groups are similar (Supplemental Table S2 in the new version of the manuscript).

Supplemental Table S1. Characteristics of the participants included and not included in the subcohort.

	Subcohort (N=512)	No subcohort (N=5178)	P-value
Age (years)	54.5 (10.9)	54.2 (11.0)	0.555
Male (%)	245 (47.9)	2438 (47.1)	0.775
Total cholesterol (mg/dL)	212 (41.5)	211 (41.9)	0.626
HDL cholesterol (mg/dL)	52.1 (13.6)	52.3 (14.0)	0.727
Systolic blood pressure (mmHg)	126 (18.8)	126 (19.0)	0.648
Diastolic blood pressure (mmHg)	78.8 (9.8)	78.7 (10.4)	0.955
Smoker (%)	113 (22.1)	1234 (24.1)	0.346
Diabetes (%)	77 (15.4)	77 (15.4)	0.471

Data are presented as the mean (SD) for continuous variables and as frequencies (percentages) for categorical variables. HDL: high-density lipoprotein.

Supplemental Table S2. Characteristics of the genotyped and non-genotyped participants.

	Genotyped participants (N=581)	Non-genotyped participants (N=34)	P-value
Age (years)	55.5 (10.9)	58.1 (11.0)	0.179
Male (%)	295 (50.8%)	20 (58.8%)	0.462
Total cholesterol (mg/dL)	215 (42.1)	204 (47.0)	0.179
HDL cholesterol (mg/dL)	51.3 (14.0)	49.7 (13.8)	0.516
Systolic blood pressure (mmHg)	128 (19.4)	131 (20.0)	0.473
Diastolic blood pressure (mmHg)	79.4 (10.2)	79.3 (8.7)	0.938
Smoker (%)	145 (25.0%)	6 (17.6%)	0.442
Diabetes (%)	105 (18.5%)	5 (18.5%)	0.867

Data are presented as the mean (SD) for continuous variables and as frequencies (percentages) for categorical variables. HDL: high-density lipoprotein.

2. The results of the Cox-regression analyses are not sufficiently discussed. They are the most important positive findings of your manuscript and should therefore be presented in detail including the results of the univariable analyses. The NRI/AUC is of interest, but should only be additional information to your data of the cox regression analysis. Other important established risk factors for CAD (which are mentioned in table1)

are further kidney function (Creatinine levels or GFR) and the inflammatory status (hsCRP). Do you have the laboratory data available for these important risk factors?

The results of the univariate analyses were displayed in Table 1 and Table 2 of the original manuscript. In Table 1, we showed that individuals who presented a coronary event in the follow-up had higher sLRP1 levels than those who did not present this type of event (2.45 µg/mL [0.43; 8.31] and 2.07 µg/mL [0.40; 6.65], respectively). In Table 2, we also showed that the incidence of coronary events increased across sLRP1 quartiles (14.8%, 13.1%, 18.2%, and 30.1%, in quartile 1, 2, 3 and 4, respectively). Nevertheless, and attending to Reviewer's comment, we have also added the unadjusted association between sLRP1 and coronary events in Table 3.

We agree with the Reviewer that the association between sLRP1 and CAD risk was one of the main aims of our study. We have highlighted this result in the revised manuscript. A new Table 3 is included and we have extended the discussion of this result. Nevertheless, we have a special interest in the identification of novel biomarkers that could improve the prediction of classical risk functions (in our study the REGICOR function). Thus, we also evaluated the added value of sLRP1 to predict coronary risk. For that reason, we discussed these results in the Discussion section.

Unfortunately, we do not have information related to kidney function and high sensitivity CRP of the participants in this study. Therefore, we were unable to incorporate this information in the multivariate models.

Minor points

1. Legend to Figure 1 should be given in more detail, since case-cohort studies are not really popular and their concept is poorly understood. You should also define in the legend what a "case" and "non-case" is.

According to Reviewer's comment, we have improved the definition of "case" and "non-case" in the legend corresponding to Figure 1.

2. The negative results of the mendelian randomization are of importance and should be thoroughly addressed in the discussion.

According to Reviewer's comment, we have discussed in detail the results from the Mendelian randomization approach in the Discussion section of the revised manuscript.

3. Page 10. Line 45: "We also used a robust study design". Case cohort studies have some restrictions (e.g. compared to prospective cohort studies) and these disadvantages should also be mentioned in the limitations.

In the revised version of the manuscript, we have added the term "efficient" to the description of the study design. We have also added the limitations of this type of studies including the differential censoring between the cases and the subcohort and the need of proper assessment of the statistical tools used.

4. You mainly discuss results of in-vitro and animal studies in the introduction

as well as in the discussion. These publications are interesting, but need to be reduced/comprised since this is a purely clinical study. There are plenty of clinical data available about the association between LRP 1 and atherosclerosis (especially genetic associations) which should be mentioned instead.

Attending to the Reviewer's comment, we have included different studies that have explored the genetic association between *LRP1* variants and cardiovascular disease, and more specifically CAD. We have also reduced the number of references concerning *in vitro* and *in vivo* studies.

5. Out of curiosity, does the inclusion of LDL in the MV cox regression analysis change the association of soluble LRP1 with CAD to a non-significant level?

Attending to Reviewer's comment, we have included LDL-cholesterol in the multivariate Cox regression model instead of total cholesterol (Model 3). We have also included the use of hypolipidemic drugs (Model 4), as suggested by Reviewer 2.

We have run this analysis including the participants with LDL-cholesterol calculated by the Friedwald equation. In those participants with missing data, we have imputed LDL-cholesterol (and other covariates) using a multiple imputation approach. As shown in the new Table 3, the inclusion of LDL-cholesterol, and hypolipidemic drugs, does not significantly change the main results of the study. The HR ranges from 1.30 to 1.58 and the *P*-value is below 0.05 in all the models.

Table 3. Association between sLRP1 plasma levels (per 1 standard deviation increase) and coronary artery disease risk adjusted for confounding factors.

	HR	95% CI	<i>P</i> -value
Results with multiple imputations (N=615)			
Model 1	1.58	1.31-1.91	<0.001
Model 2	1.30	1.01-1.67	0.039
Model 3	1.40	1.11-1.76	0.004
Model 4	1.39	1.10-1.76	0.006
Sensitivity analysis (Data set complete)			
Model 1 (N=615)	1.58	1.31-1.91	<0.001
Model 2 (N=607)	1.29	1.01-1.66	0.043
Model 3 (N=589)	1.23	0.93-1.63	0.153
Model 4 (N=557)	1.26	0.94-1.68	0.121

Model 1: sLRP1; Model 2: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol and total cholesterol; Model 3: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol and LDL-cholesterol; Model 4: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol, LDL-cholesterol and lipid-lowering medication. HR: Hazard ratio; 95% CI: 95% confidence interval.

6. Page 6. "To be considered confounding, a variable had to be associated with both CAD and quartiles of sLRP1 concentration at P-value <0.05". I guess with spearman correlation which you mention on Page 7. Line 49. If so it belongs to the paragraph "statistical analysis".

The covariates included in the Cox-regression models were chosen based on statistical differences observed in Table 1 (Subcohort versus cases) and Table 2 (quartiles of sLRP1 concentration). In addition to age and sex, the covariates had to be associated with both CAD and sLRP1 levels to be selected as confounding factors. There were a mistake in the previous version of the manuscript. BMI was included as covariate despite this variable showed similar levels between cases and subcohort participants. This mistake has been corrected in the new version of the manuscript. The new models are shown in Table 3. Additionally, we have included information on Spearman correlation analysis in the Methods section.

7. The exact methodology of the multivariable model (how you selected the co-variables) should be described in the statistical analysis or in a separate paragraph and not in various parts throughout the manuscript.

Attending to Reviewer's comment, we have clarified the methodology we used in our multivariate model. Moreover, we have included the multiple imputation approach we have used to deal with missing data in the revised version of the manuscript.

8. When were the measurements of the sLRP1 performed and how long were the samples frozen and at which temperature?

sLRP1 was measured in citrated plasma samples. Samples were frozen at -80°C from blood sampling (2004-2006) to measurement (2017). This information has been included in the Methods section of the new version of the manuscript.

POINT BY POINT RESPONSE TO COMMENTS BY REVIEWER # 2

The manuscript is well-written. The authors have already published extensively in this research field. This manuscript delineates an independent association of baseline sLRP1 and the incidence of CAD in a Spanish population-based cohort.

Thanks for the interest in our work. We have addressed the remarks and comments raised by the Reviewer on a point by point basis.

I have the following comments:

1. In the abstract the follow-up time is given as "10-year incidence of CAD events" however in the results section median follow-up is given as 6.2 years. IQR? Since the cohort was recruited between 2004-2006 it would be helpful to clarify if the CAD events were assessed by the telephone interview in 2009-2011 or a more recent follow-up was used.

We thank the Reviewer for the opportunity to clarify this relevant point. As the Reviewer points out, the median follow-up of the participants in this study was 6.2 years. Although the Cox regression model assumes a proportionality of the risk in the follow-up (HR is equal at 6 years follow-up than at 10 years follow-up), and the usual coronary-cardiovascular risk estimations are made at 10-years, we have deleted the reference to 10-year incidence of CAD to avoid potential misunderstanding.

2. In the methods section the authors specify that LDL-C was calculated by the Friedewald equation if triglycerides were <300mg/dl. In Table 1 the IQR for triglycerides in controls were 32-496 and 42-999 for CAD. The authors thus used total cholesterol in the multivariate model. However, triglycerides should additionally be included into this model due to the correlation of sLRP1 and triglycerides shown in Table S1.

The Reviewer is right. Triglycerides were associated with both sLRP1 levels and CAD risk in the bivariate analysis. Therefore, they could be considered as a confounder variable. Nevertheless, they were not associated with the risk of presenting a coronary event in the multivariate analysis (HR=1.00; 95% CI: 0.99-1.01) and they were correlated with HDL-cholesterol ($\rho=-0.41$).

Additionally, and following the suggestion of the Reviewer, we have explored whether the inclusion of triglycerides modified the effect size of the association between sLRP1 and coronary risk. The HR went from 1.23 to 1.18. On the other hand, triglycerides are not included in the REGICOR risk function (a calibration of the Framingham risk function) and one of our aims was to assess the added predictive value of sLRP1 over the REGICOR risk function.

Thus, we have decided not to include triglycerides in the final multivariate model because: i) the effect size of the association between sLRP1 and CAD risk does not significantly change, ii) this variable is correlated with HDL-cholesterol and introduces colinearity in the model, and iii) this variable is not included in the classical predictive risk function.

3. Table 1 depicts statin use in 9% of the healthy sub cohort (N=498). Since statin use was rarely used in primary prevention in 2004-2006, would it be possible that patients with familial hypercholesterolemia are included in this trial (LDL IQR 48-279)? The authors already published a decrease of sLRP-1 by statins (Ref 15) does adjustment for statin use change the multivariable model?

Reviewer is right. Some of the participants included in this study could present familial hypercholesterolemia. The cohort/subcohort is representative of the general population and the prevalence of this entity in our study could be the same as in the general population (1:250-500). We did not exclude these participants to guarantee the population representability of the study.

Attending to Reviewer's comment, we have included the variable hypolipidemic drug used in the multivariate model. As shown in the new Table 3, the association between sLRP1 and coronary risk remains stable.

Based on a suggestion of Reviewer 1, we have also included LDL-cholesterol in the model, instead of total cholesterol, and we have performed multiple imputations to assign a LDL-cholesterol value to those participants with triglycerides >300 mg/dL. The results of these models are presented below and in the new Table 3.

Table 3. Association between sLRP1 plasma levels (1 standard deviation) and coronary artery disease risk adjusted for confounding factors.

	HR	95% CI	P-value
Results with multiple imputations (N=615)			
Model 1	1.58	1.31-1.91	<0.001
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Model 4 (N=557)	1.26	0.94-1.68	0.121

Model 1: sLRP1; Model 2: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol and total cholesterol; Model 3: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol and LDL-cholesterol; Model 4: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol, LDL-cholesterol and lipid-lowering medication. HR: Hazard ratio; 95% CI: 95% confidence interval.

4. No participants are described as having missing data for the multivariable model. Is this correct?

Thank you for this remark. There were some participants with missing data in the multivariate models. We have reanalyzed the data using multiple imputation methods. The methodology has been included in the statistical analysis section.

POINT BY POINT RESPONSE TO COMMENTS BY REVIEWER # 3

In this paper, the authors investigated the predictive capacity of circulating soluble LRP1 on cardiac risk in the general population. They additionally sought to determine whether circulating sLRP1 levels were determined by two SNPs which were previously linked LRP1 to cardiac disease. This is by a group which provided significant contributions to the field. The manuscript is well written, yet there are a couple of points to be clarified before it can be considered for acceptance:

Thanks for the interest in our work. We have addressed the remarks and comments raised by the Reviewer on a point by point basis.

1. Beginning from the abstract, the authors claim to have investigated the impact of sLRP1 on the 10 years incidence of CAD. According to Figure 1, recruitment took place between 2004 and 2006 and follow-up information was acquired up to 2011. Correspondingly median follow-up was stated as 6.2 years. Please clarify this discrepancy.

This point has also been raised by Reviewer 2. We thank both Reviewers for the opportunity to clarify this relevant point. The median follow-up of the participants in this study was 6.2 years. Although the Cox regression model assumes a proportionality of the risk in the follow-up (HR is equal at 6 years follow-up than at 10 years follow-up), and the usual coronary-cardiovascular risk estimations are made at 10-years, we have deleted the reference to 10-year incidence of CAD in the revised version of the manuscript to avoid potential misunderstanding.

2. In the methods section it is written: "The assay showed no significant crossreactivity or interference between LRP1 and analogs." It should be stated in the manuscript if this based on own experiments or what the source is.

Attending to Reviewer's comment, additional information has been included in the Methods section.

3. It must be stated in the manuscript on which exact considerations you selected the two SNPs (rs11172113 and rs4367982) and not others which were also associated to CAD (page 5 line 17: "several genetic variants").

The SNP rs11172113 was selected because it showed the strongest association with CAD in terms of *P*-value. The SNP rs4367982 was randomly selected among those showing an association with CAD with a *P*-value<0.01. This information has been included in the new version of the manuscript.

4. On Page 6 starting line 4 the REGICOR-Framingham function is mentioned. Please describe it shortly in the manuscript.

A brief description of the REGICOR-Framingham function has been included in the Methods section.

5. Page 6, Statistics section: "The characteristics of participants were summarized as frequencies when they were categorical, as means and standard deviation when they showed a continuous normal distribution, or as quartiles otherwise." In all the tables "mean [IQR]" is used. Please clarify and adapt this sentence accordingly.

Thank you for this comment. We have clarified this point in the revised version of the manuscript.

6. Page 6, Statistics section: The "classical risk factors" must be listed.

According to Reviewer's comment, the classical risk factors and other potential confounding factors have been listed in the new version of the manuscript.

7. Page 8, first paragraph: In the methods, the authors wrote they were going to adjust for classical risk factors and those risk factors which were both statistically significantly associated with both sLRP1 and CAD. Smoking is usually considered a classical risk factor, yet not included. Triglycerides as well as LDL-C meet according to table 1 and table 2 the criteria of confounding factors but are not included. Please clarify this. In a previous paper by the group (reference 15), the strength of association between sLRP1 and classical lipid parameters ("study 2") was in descending order LDL-C>total cholesterol>triglycerides>HDL-C. Correspondingly, apolipoprotein B was the strongest novel lipid marker associated with sLRP1. Given these previous results and the hypothesis that shedding occurs due to exposure to pro-atherogenic lipoproteins, it is mandatory to adjust this analysis for LDL-C (with imputation if TG > 300) or ApoB as a surrogate if the aim is causal inference. Since, as cited, sLRP1 could exert pro-inflammatory functions which could mechanistically contribute to the progression of atherosclerosis, it might not only "reflect the vascular response to proatherogenic lipoproteins" but also amplify the effect of pro-atherogenic lipids. This should be tested with an interaction term between LDL-C and sLRP1. To sum all of this up, the selection of the potential confounding factors should be based on subject matter knowledge and not on statistical significance alone.

We have clarified the concerns raised by the Reviewer in the revised version of the manuscript. First, smoking was not included in the multivariate model to assess the association between sLRP1 and CAD because it was not associated with sLRP1, and therefore does not meet the definition of a confounder variable. However, smoking was included in the multivariate predictive model to assess the added value of sLRP1 as it is a variable of the Framingham-REGICOR risk function. Second, we have included new multivariate models adjusted for LDL-cholesterol in the revised version of the manuscript. The association between sLRP1 and CAD remains consistent in these models (new Table 3). Third, the Reviewer is right and triglycerides were associated with both sLRP1 levels and CAD risk in the bivariate analysis. Therefore, they could be considered as a confounder variable. However, they were not associated with the risk of presenting a coronary event in the multivariate analysis (HR=1.00; 95% CI: 0.99-1.01). Moreover, they were correlated with HDL-cholesterol ($p=-0.41$) and are not

included in the REGICOR risk function. Following the suggestion of the Reviewer, we have explored whether the inclusion of triglycerides modified the size effect of the association between sLRP1 and coronary risk and the HR went from 1.23 to 1.18. Consequently, we have decided not to include triglycerides in the final multivariate model because i) the effect size of the association between sLRP1 and CAD risk does not significantly change, ii) this variable is correlated with HDL-cholesterol and introduces colinearity in the model, and iii) this variable is not included in the classical predictive risk function.

8. Page 8, line 11: For a better interpretability, the NRI for events and non-events should be added separately to table 3. Please clarify what is meant by the clinical NRI in table 3 as it is not mentioned in the methods section. In table 3, the categorical NRI as well as the clinical NRI are significant in the control group but not in the cases. The authors should describe and discuss this.

Attending to Reviewer's comment, we have reanalyzed the NRI censoring the follow-up at 5 years to adapt this analysis to the follow-up of our cohort. As shown in the new Table 4, the results were no longer statistically significant. In addition, we have also included the definition of the "clinical NRI" in the Statistical analysis section in the revised manuscript.

9. Page 8, Mendelian randomization: Figure 2 is not very informative to the reader. It could be added as supplemental figure. The data from supplemental tables 2 and 3 should be presented in figure 2.

Attending to Reviewer's suggestion, Figure 2 is included as Supplemental Figure S1 in the revised version. Data from supplemental Tables 2 and Table 3 have also been included in the Supplemental Figure S1.

STATEMENT OF ORIGINALITY

I certify that this is an original paper and it has not been previously published, is under submission, or currently in press elsewhere.

A handwritten signature in blue ink, reading "Vicenta Llorente Cortés". The signature is written in a cursive style with a long horizontal stroke at the bottom.

Vicenta Llorente Cortés

AUTHOR DECLARATION TEMPLATE

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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Figure 1
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TABLES

Table 1. Characteristics of patients with coronary events (cases) and of the subcohort without events.

	Subcohort (n=498)	Cases (n=117)	P-value
Age (years)	54 [35; 74]	62 [39; 74]	<0.001
Male (%)	233 (46.8)	82 (70.1)	<0.001
Body mass index (Kg/m ²)	27.3 [18.7; 68.7]	27.9 [17.1; 42.4]	0.379
Smoker (%)	106 (21.3)	45 (38.8)	<0.001
Diabetes (%)	61 (12.5)	33 (28.9)	<0.001
Glucose (mg/dL)	94 [49; 223]	100 [76; 373]	<0.001
Systolic blood pressure (mmHg)	123 [75; 195]	136 [100; 207]	<0.001
Diastolic blood pressure (mmHg)	78 [53; 120]	83 [52; 115]	0.001
Hypertension (%)	208 (42.9)	79 (70.5)	<0.001
Total cholesterol (mg/dL)	206 [84; 369]	224 [124; 338]	<0.001
HDL-C (mg/dL)	50 [20; 103]	44 [22; 92]	<0.001
LDL-C (mg/dL)	132 [48; 279]	150 [61; 242]	0.001
Triglycerides (mg/dL)	95 [32; 496]	122 [42; 999]	<0.001
Antihypertensive medication (%)	92 (18.8)	44 (37.9)	<0.001
Antiglycemic medication (%)	27 (5.5)	19 (16.5)	<0.001
Lipid-lowering medication (%)	44 (9.0)	27 (23.5)	<0.001
sLRP1 (µg/mL)	2.07 [0.40; 6.65]	2.45 [0.43; 8.31]	<0.001
REGICOR risk (%)	2.84 [0.17; 25.20]	6.02 [1.35; 40.30]	<0.001

Data are presented as the median [IQR] for continuous variables and as frequencies (percentages) for categorical variables. HDL: high-density lipoprotein; LDL: low-density lipoprotein; REGICOR: Registre Gironi del Cor; sLRP1: soluble low-density lipoprotein receptor-related protein 1.

Table 2. Characteristics of the patients stratified by quartiles of sLRP1 plasma level.

	Q1 (n=155)	Q2 (n=153)	Q3 (n=154)	Q4 (n=153)	P-value	P-trend
Age (years)	53 [35; 74]	57 [36; 74]	57.0 [35; 74]	57.0 [35; 74]	0.494	0.131
Male (%)	73 (47.1)	79 (51.6)	79 (51.3)	84 (54.9)	0.594	0.200
Body mass index (Kg/m ²)	26.0 [17.1; 68.7]	27.3 [18.7; 41.2]	27.6 [19.8; 41.7]	28.4 [21.2; 43.5]	<0.001	<0.001
Smoker (%)	34 (21.9)	32 (21.2)	39 (25.3)	46 (30.1)	0.232	0.245
Diabetes (%)	16 (10.5)	27 (18.0)	28 (18.4)	23 (15.8)	0.200	0.209
Glucose (mg/dL)	92 [49; 171]	95 [72; 289]	94 [70; 229]	97 [66; 373]	0.009	0.002
Systolic blood pressure (mmHg)	119 [92; 193]	124 [75; 180]	125 [88; 207]	129 [94; 191]	0.005	0.002
Diastolic blood pressure (mmHg)	77 [55; 104]	80 [56; 107]	79 [52; 120]	79 [53; 115]	0.072	0.043
Hypertension (%)	62 (41.1)	72 (48.0)	74 (49.0)	79 (54.5)	0.144	0.025
Total cholesterol (mg/dL)	194 [84; 309]	209 [136; 318]	207 [108; 369]	228 [113; 356]	<0.001	<0.001
HDL-C (mg/dL)	54 [20; 102]	50 [30; 96]	50 [23; 97]	46 [22; 103]	<0.001	<0.001
LDL-C (mg/dL)	121 [48; 219]	135 [68; 223]	134 [66; 279]	155 [52; 277]	<0.001	<0.001
Triglycerides (mg/dL)	83 [35; 228]	98 [43; 289]	100 [32; 321]	136 [47; 999]	<0.001	<0.001
Antihypertensive medication (%)	32 (20.8)	36 (23.8)	32 (21.1)	36 (24.2)	0.841	0.628
Antiglycemic medication (%)	6 (3.9)	17 (11.3)	12 (7.9)	11 (7.4)	0.120	0.448

Lipid-lowering medication (%)	18 (11.9)	18 (12.0)	14 (9.2)	21 (14.2)	0.604	0.747
REGICOR risk (%)	2.48 [0.17; 17.20]	3.58 [0.45; 21.50]	3.23 [0.27; 40.30]	5.13 [0.26; 25.20]	<0.001	<0.001
Coronary event (%)	23 (14.8)	20 (13.1)	28 (18.2)	46 (30.1)	0.001	<0.001
sLRP1 (µg/mL)	1.39 [0.40; 1.64)	1.89 [1.64; 2.12)	2.35 [2.13; 2.69)	3.21 [2.69; 8.31]	<0.001	<0.001

Data are presented as the median [IQR] for continuous variables and as frequencies (percentages) for categorical variables. HDL: high-density lipoprotein; LDL: low-density lipoprotein; REGICOR: Registre Gironi del Cor; sLRP1: soluble low-density lipoprotein receptor-related protein 1.

Table 3. Association between sLRP1 plasma levels (per 1 standard deviation increase) and coronary artery disease risk.

	HR	95% CI	P-value
Results with multiple imputations (n=615)			
Model 1	1.58	1.31-1.91	<0.001
Model 2	1.30	1.01-1.67	0.039
Model 3	1.40	1.11-1.76	0.004
Model 4	1.39	1.10-1.76	0.006
Sensitivity analysis (Data set complete)			
Model 1 (n=615)	1.58	1.31-1.91	<0.001
Model 2 (n=607)	1.29	1.01-1.66	0.043
Model 3 (n=589)	1.23	0.93-1.63	0.153
Model 4 (n=557)	1.26	0.94-1.68	0.121

Model 1: sLRP1; Model 2: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol and total cholesterol; Model 3: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol and LDL-cholesterol; Model 4: sLRP1, age, sex, systolic blood pressure, diastolic blood pressure, glycaemia, HDL-cholesterol, LDL-cholesterol and lipid-lowering medication. HR: Hazard ratio; 95% CI: 95% confidence interval.

Table 4. Evaluation of plasma sLRP1 as a potential biomarker to improve the reclassification and discrimination capacity of the REGICOR algorithm terms.

Reclassification	NRI [95% CI]	P-value
Continuous NRI, %	9.3 [-14.4; 33.7]	0.416
Categorical NRI, %	2.3 [-2.6; 7.2]	0.358
cases	1.3 [-3.3; 6.0]	0.575
subcohort	1.0 [-0.3; 2.2]	0.144
Clinical NRI, %	6.0 [-4.1; 16.1]	0.241
cases	2.6 [-6.2; 11.4]	0.561
subcohort	3.4 [-1.1; 8.0]	0.137
Discrimination	c-Index [95% CI]	P-value
Without sLRP1	81.5 [77.8; 85.2]	
With sLRP1	81.6 [77.9; 85.2]	
Difference	0.1 [-0.2; 0.4]	0.443

NRI: Net reclassification improvement. For the categorical NRI, 5% and 10% were used as cut-off points to define low, intermediate and high risk groups. Clinical NRI refers to the reclassification in the intermediate category risk (5% to 10% risk).

ATH-D-19-00072R1

We confirm that we have read

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