Atherosclerosis 287 (2019) 93–99

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

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

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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.

ARTICLE INFO

Keywords:
Biomarker
Cardiovascular risk
Coronary artery disease
REGICOR
Soluble LRP1

ABSTRACT

Background and aims: We aimed 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: We conducted a 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 with 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 adjustment 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.

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https://doi.org/10.1016/j.atherosclerosis.2019.06.904
Received 18 January 2019; Received in revised form 10 May 2019; Accepted 14 June 2019
Available online 16 June 2019

1. 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.

2. Patients and methods

2.1. 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) (Fig. 1). As shown in Supplemental Table 1, the characteristics of the cohort and subcohort were similar.

2.2. 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.

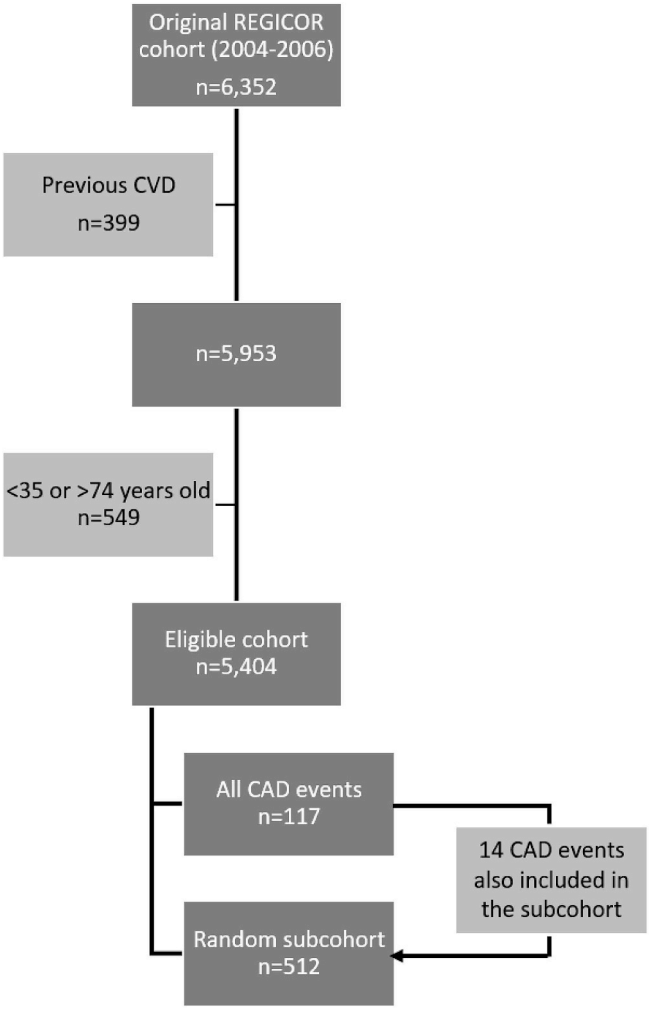

Fig. 1. Flowchart of the study participants included in the case-cohort study. In this type of design, all the incident cases of interest and a subsample (subcohort) of the participants of the original eligible cohort are included (a subset of participants are considered both cases and members of the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

2.3. sLRP1 determination

sLRP1 concentrations were measured in frozen citrated plasma samples using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Uscn 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).

2.4. 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 Fig. 2A). We selected 2 of these SNPs (rs11172113, $p = 1.71 \cdot 10^{-5}$; rs4367982, p = 0.009). SNP rs11172113 was selected because it showed the strongest association with CAD in terms of *p*-value. 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 2).

2.5. 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/m^2). 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 > 125 mg/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.

2.6. 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].

2.7. 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

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.

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].

3. Results

3.1. 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, antiglycemic and lipid-lowering medication between sLRP1 quartiles.

Spearman correlations between plasma sLRP1 and cardiovascular risk factors are shown in Supplemental Table 3. 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.

3.2. Plasma sLRP1 and coronary events: association and predictive capacity

As shown in Table 3, the circulating levels of 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 of 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

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% C, 95% confidence interval.

Table 4
Evaluation of plasma sLRP1 as a potential biomarker to improve 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

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%–10% risk).
NRI, net reclassification improvement.

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).

3.3. Mendelian randomization approach

The association between the four analyzed genetic variants and CAD risk is shown in Supplemental Fig. 2B. 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 Fig. 2C).

4. 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 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 suggests that up to 40% of 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 modest or 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 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 with atherosclerotic disease. Gaultier et al. [37] demonstrated that purified sLRP1 inhibits 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 to 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.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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 VLl-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).

Acknowledgements

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.06.904.

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