Circulating Levels of Interleukin 1-Receptor Antagonist and Risk of Cardiovascular Disease

Meta-Analysis of Six Population-Based Cohorts

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Objective—Interleukin (IL)-1β represents a key cytokine in the development of cardiovascular disease (CVD). IL-1β is counter-regulated by IL-1 receptor antagonist (IL-1RA), an endogenous inhibitor. This study aimed to identify population-based studies on circulating IL-1RA and incident CVD in a systematic review, estimate the association between IL-1RA and incident CVD in a meta-analysis, and to test whether the association between IL-1RA and incident CVD is explained by other inflammation-related biomarkers in the MONICA/KORA Augsburg case–cohort study (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg).

Approach and Results—We performed a systematic literature search and identified 5 cohort studies on IL-1RA and incident CVD in addition to the MONICA/KORA Augsburg case–cohort study for a meta-analysis based on a total of 1855 CVD cases and 18 745 noncases with follow-up times between 5 and 16 years. The pooled standardized hazard ratio (95% confidence interval) for incident CVD was 1.11 (1.06–1.17) after adjustment for age, sex, anthropometric, metabolic, and lifestyle factors (P<0.0001). There was no heterogeneity in effect sizes (I^2=0%; P=0.88). More detailed analyses in the MONICA/KORA study showed that the excess risk for CVD was attenuated by ≥10% after additional separate adjustment for serum levels of high-sensitivity C-reactive protein, IL-6, myeloperoxidase, soluble E-selectin, or soluble intercellular adhesion molecule-1.

Conclusions—Serum IL-1RA levels were positively associated with risk of CVD after adjustment for multiple confounders in a meta-analysis of 6 population-based cohorts. This association may at least partially reflect a response to triggers inducing subclinical inflammation, oxidative stress, and endothelial activation.

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Key Words: biomarker ■ cardiovascular disease ■ cohort study ■ inflammation ■ interleukin-1 receptor antagonist ■ meta-analysis

Proinflammatory processes are important pathophysiological mechanisms involved in the development of cardiovascular disease (CVD).[1] Interleukin 1β (IL-1β) plays a crucial role in this context because it represents one of the most potent inducers of innate immunity and acts as an upstream regulator in the inflammatory cascade.[2,3] Experimental and

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Nonstandard Abbreviations and Acronyms	
AUC	area under the receiver-operating characteristic curve
CANTOS	Canakinumab Anti-Inflammatory Thrombosis Outcome Study
CI	confidence interval
CVD	cardiovascular disease
HR	hazard ratio
IL	interleukin
IL-1RA	interleukin-1 receptor antagonist
IL1RN	gene encoding IL-1RA
KORA	Cooperative Health Research in the Region of Augsburg
MONICA	Multinational Monitoring of Trends and Determinants in Cardiovascular Disease
PRIME	Prospective Epidemiological Study of Myocardial Infarction

clinical studies have demonstrated that IL-1β contributes to the risk of atherosclerosis and cardiovascular events.[4] Thus, IL-1β has also emerged as a promising therapeutic target, for example, in the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcome Study), that uses a neutralizing monoclonal anti-IL-1β antibody to reduce the risk of recurrent cardiovascular events.[3,5]

Interleukin-1 receptor antagonist (IL-1RA) counter-regulates IL-1β as an endogenous inhibitor in vivo by blocking the binding site for IL-1β at the IL-1 receptor I.[2] IL-1β induces the production of IL-1RA to contain immune activation, and the balance between IL-1RA and IL-1β has been implicated as an important control mechanism to prevent inflammation-related tissue damage and reduce cardiometabolic risk.[5,6]

The investigation of IL-1β as a potential biomarker for the prediction of CVD in the general population is complicated by the extremely low systemic levels of this cytokine that preclude reliable quantification.[7] In contrast, circulating levels of IL-1RA are measurable with great precision.[7,8] Systemic IL-1RA levels are elevated in individuals with cardiometabolic risk factors, such as obesity, insulin resistance, and type 2 diabetes mellitus,[9–12] and have been interpreted to reflect higher IL-1β activity in these individuals.[10]

There is preliminary evidence from 2 population-based cohorts using multimarker approaches that higher IL-1RA levels may also be associated with higher risk of CVD, whereas 2 others failed to observe such an association.[13,14] Therefore, it is not clear yet whether circulating IL-1RA is associated with CVD risk independent of established cardiovascular risk factors, whether IL-1RA could be used as a novel biomarker to improve the prediction of CVD, and what the underlying mechanisms are that mediate the upregulation of IL-1RA in the context of cardiometabolic risk.

This study aims (1) to identify population-based studies on circulating IL-1RA and incident CVD by a systematic literature search, (2) to quantify the association between IL-1RA and incident CVD in a meta-analysis, and (3) to identify other inflammation-related biomarkers, which could partially explain this association based on data from the MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease)/KORA (Cooperative Health Research in the Region of Augsburg) Augsburg case–cohort study.

Materials and Methods

The study is based on data from the MONICA/KORA Augsburg case–cohort study and on additional population-based cohort studies identified in a systematic review. The primary outcome was defined as incident CVD (fatal and nonfatal myocardial infarction, sudden death, cardiovascular mortality; however, studies with combined end points, including unstable angina, coronary revascularization, or ischemic stroke, were also eligible). For the meta-analysis, we used inverse-variance fixed effect and DerSimonian–Laird random effects modeling based on study-level data; the DerSimonian–Laird estimator was used to test for heterogeneity. We visually inspected the funnel plot and performed Egger's regression test of funnel plot asymmetry to check for possible publication bias. For all analyses, a P value <0.05 was considered to be statistically significant. Materials and Methods are available in more details in the online-only Data Supplement.

Results

Meta-Analysis of the Association Between IL-1RA and Incident CVD

The literature search for the systematic review using MEDLINE and Embase identified 4202 publications after removal of duplicates (Figure 1). We assessed 21 full-text articles for eligibility and excluded 16 articles because no IL-1RA data were reported and 3 articles[15–17] because the study samples were not population based. The 2 publications that were eligible[13,14] contained data from 3 cohort studies, the FINRISK 1997, the Belfast PRIME (Prospective Epidemiological Study of Myocardial Infarction) Men, and the Rotterdam studies. We contacted the authors of both publications[13,14] to adapt the selection of confounding variables to the MONICA/KORA analysis, to reanalyze data based on extended follow-up times, if possible, and to obtain additional unpublished data that fulfilled our inclusion criteria. Through this contact, we identified the Finnish HEALTH 2000 and FINRISK 2007 surveys (data unpublished) as additional eligible studies for the meta-analysis. Therefore, the meta-analysis included the MONICA/KORA Augsburg study data (see Figure I in the online-only Data Supplement for study design, Table I in the online-only Data Supplement for baseline characteristics, and Table II and Figure II in the online-only Data Supplement for associations between IL-1RA and CVD risk), data from the 3 cohort studies identified through the systematic review (FINRISK 1997, Belfast PRIME Men cohort, Rotterdam Study; updated analyses replaced the data published previously[13,14]), and novel unpublished data from a further 2 studies identified through personal contact as result of the systematic review (HEALTH 2000 and FINRISK 2007; Figure 1).

Material and Methods and Table III in the online-only Data Supplement provide an overview of all 6 study cohorts, including information on sample sizes, end point definitions, follow-up times, and standardized hazard ratios (HRs), for the association between IL-1RA and incident CVD that were used for the meta-analysis. Numbers of cases ranged between 65 and 803 per study, while total sample sizes were between 839 and 6393. Follow-up times ranged from 4.9 to 16.0 years. Standardized HRs were between 1.057 and 1.165 based on the same adjustment strategy for multiple confounders (Table III in the online-only Data Supplement) and showed significantly increased risk of CVD in 3 cohorts.

The meta-analyzed estimate is based on a total of 1855 cases and 18 745 noncases. The pooled standardized and adjusted HR

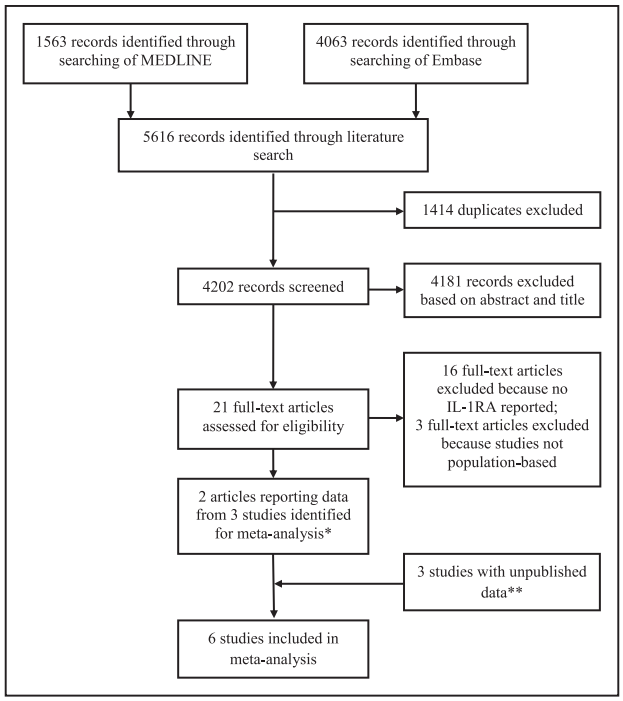

Figure 1. Overview of the literature search for the systematic review and meta-analysis. *One article reported data for 2 cohort studies (FINRISK 1997 and Belfast PRIME Men studies), one article reported data for 1 cohort study (Rotterdam study). **MONICA/KORA Augsburg case–cohort study and 2 studies identified through contact of authors of studies identified in the systematic review (HEALTH 2000, FINRISK 2007). MONICA/KORA indicates Multinational Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg; and PRIME, Prospective Epidemiological Study of Myocardial Infarction.

for incident CVD was 1.11 (95% confidence interval [CI], 1.06–1.17) in both fixed effect and random effects models (P<0.0001; Figure 2). There was no evidence for heterogeneity with I^2 (percentage of variance attributable to study heterogeneity) of 0% (95% CI, 0%–0%; P=0.88). Egger's linear regression test for publication bias yielded no evidence of funnel plot asymmetry (P=0.67; Figure III in the online-only Data Supplement).

No Improvement of CVD Risk Models by IL-1RA

Clinical risk models based on the covariates listed in Table III in the online-only Data Supplement for each study yielded areas under the receiver-operating characteristic curve (AUC) between 0.6760 and 0.8639 (Table). When IL-1RA levels were added to the respective risk models, the AUC values were not significantly improved, with differences in AUC between 0.0005 and 0.0049 across the 6 cohorts.

Impact of Inflammation on the Association Between IL-1RA and CVD Risk

We used a panel of 15 additional biomarkers of subclinical inflammation in the MONICA/KORA case–cohort study to test the hypothesis that the association between IL-1RA and incident CVD may be partially explained by these biomarkers. In models that were adjusted for all aforementioned covariates (model 2) and one of each of these biomarkers, the attenuation of excess risk by IL-1RA was >10% after additional adjustment for high-sensitivity C-reactive protein, IL-6, myeloperoxidase, soluble intercellular adhesion molecule-1, or soluble (s)E-selectin, whereas the HRs based on the additional adjustment for the other biomarkers were rather similar to the HRs in model 2 (Figure 3).

Discussion

This study has 3 main findings: (1) CVD risk increased by 11% per 1-SD increase in serum IL-1RA based on data from >20 000 study participants from 6 population-based cohort studies; (2) this increased CVD risk did not translate into an improved risk prediction over and above classical risk factors as assessed by AUC; and (3) the association between IL-1RA and CVD risk was partially explained by biomarkers of subclinical inflammation, oxidative stress, and cell adhesion.

This study advances our knowledge because the combination of 3 previously published studies (FINRISK 1997, Belfast PRIME Men cohort, and Rotterdam Study)[13,14] and novel data from the MONICA/KORA, FINRISK 2007, and HEALTH 2000 cohorts allowed the assessment of CVD risk based on circulating IL-1RA concentrations in 1855 cases within a total sample of 20 600 individuals. Our meta-analysis yielded a pooled HR of 1.11 (95% CI, 1.06–1.17) based on 6 studies that used the same adjustment strategy for the main cardiovascular risk factors (ie, age, sex, body mass index, blood pressure, total and high-density lipoprotein cholesterol levels, smoking, and prevalent diabetes mellitus). Thus, the effect size was similar to the standardized HR that has been reported for the association between lipoprotein-associated phospholipase A2 and incident CVD,[18] but slightly lower than that for total cholesterol or triglycerides and CVD risk.[19]

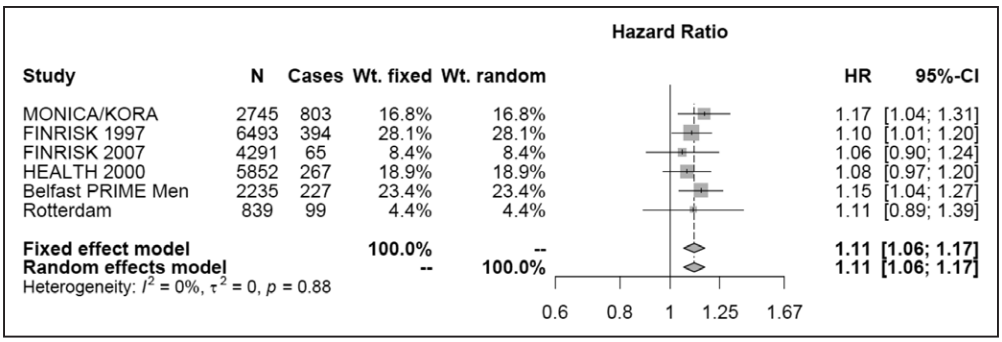

Figure 2. Meta-analysis and pooled estimate for the association between interleukin-1 receptor antagonist (IL-1RA) and incident CVD (Forest Plot). CI indicates confidence interval; HR, hazard ratio; MONICA/KORA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg; N, total number of study participants; and Wt, weight.

Table. Impact of IL-1RA on the Prediction of CVD by Clinical Risk Models

Cohort	AUC for Clinical Model	AUC for Clinical Model+IL-1RA	Difference (95% CI)
MONICA/KORA	0.8210	0.8224	0.0014 (–0.0018 to 0.0034)
FINRISK 1997	0.8562	0.8564	0.0003 (–0.0010 to 0.0015)
FINRISK 2007	0.8567	0.8576	0.0008 (–0.0013 to 0.0030)
HEALTH 2000	0.8639	0.8644	0.0005 (–0.0008 to 0.0017)
Belfast PRIME Men	0.6760	0.6796	0.0035 (–0.0017 to 0.0126)
Rotterdam	0.7003	0.7051	0.0049 (–0.0083 to 0.0181)

Data are given as AUC for clinical risk models (see Table IV in the online-only Data Supplement for an overview of the included variables in the respective cohorts) without and with IL-1RA. AUC indicates areas under the receiver-operating characteristic curve; CI, confidence interval; CVD, cardiovascular disease; IL-1RA, interleukin-1 receptor antagonist; MONICA/KORA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg; and PRIME, Prospective Epidemiological Study of Myocardial Infarction.

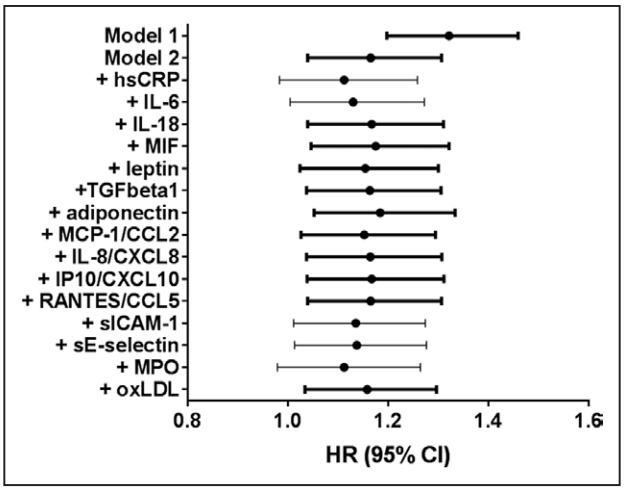

Figure 3. Association between serum interleukin-1 receptor antagonist (IL-1RA) and incident CVD in MONICA/KORA without and with adjustments for further biomarkers of subclinical inflammation. Data are shown as hazard ratios (HR) and 95% CI per 1 SD of log serum IL-1RA. HRs and 95% CIs were 1.32 (1.20–1.46) for model 1 ($P<0.0001$) and 1.17 (1.04–1.31) for model 2 ($P=0.009$). Model 1: adjusted for age, sex, and survey. Model 2: Model 1+body mass index (BMI), systolic blood pressure, total/high-density lipoprotein (HDL) cholesterol, smoking, alcohol consumption, physical activity, parental history of coronary heart disease (CHD), prevalent diabetes mellitus. Model 2+biomarker: We added in separate models as 1 covariate at a time the concentration of one of the following circulating biomarkers to the aforementioned model 2: high-sensitivity C-reactive protein (hsCRP) (log), interleukin (IL)-6 (log), IL-18 (log), macrophage migration inhibitory factor (MIF) (log), leptin (log), transforming growth factor (TGF)-β1, adiponectin (log), monocyte chemotactic protein 1 (MCP1/CCL2), IL-8/CXCL8 (log), interferon-γ-inducible protein (IP-10/CXCL10) (log), regulated on activation, normal T cell expressed and secreted (RANTES/CCL5) (log), soluble intercellular adhesion molecule (sICAM)-1 (log), soluble (s)E-selectin (log), myeloperoxidase (MPO) (log) and oxidized LDL cholesterol (oxLDL). Thin 95% CIs indicate an attenuation of point estimates for excess CHD risk by ≥10% compared with model 2. CI indicates confidence interval; and MONICA/KORA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg.

We identified 3 further relevant studies in our systematic review that we had to exclude because the samples were not population based. The first study was a nested case–control study for 42 candidate biomarkers in individuals with type 2 diabetes mellitus.[16] The sample comprised 1123 cases with incident CVD and 1187 controls from 5 European centers and revealed an odds ratio of 1.13 (95% CI, 1.02–1.25) per SD of IL-1RA.[16] The second study reported a standardized HR (95% CI) of 1.14 (1.03–1.27) for incident myocardial infarction in 3199 study participants with a history of coronary artery disease.[17] The third study investigated 73 consecutive patients undergoing percutaneous coronary intervention.[15] After 18 months of follow-up, IL-1RA levels in the highest quarter were associated with a higher risk of major adverse cardiac events compared with IL-1RA levels in the lowest quarter (19% versus 0%; adjusted $P=0.032$). Taken together, the 3 studies suggest that the positive association between IL-1RA and CVD risk may be similar in the general population compared with that in patients with type 2 diabetes mellitus or preexisting CVD.

The main finding from this meta-analysis also extends observations from the Interleukin-1 Genetics Consortium that investigated the association between gene variants upstream of *IL1RN*, the gene encoding IL-1RA, and coronary heart disease.[20] That study found that each minor allele in a gene score comprising 2 common *IL1RN* gene variants was associated with an increase in circulating IL-1RA of 0.22 SD and with an odds ratio (95% CI) for coronary heart disease of 1.03 (1.02–1.04). Therefore, the odds ratio per 1 SD of genetically upregulated IL-1RA can be calculated as 1.14 (95% CI, 1.09–1.18), which is similar to our pooled estimate.

Despite the agreement of this meta-analysis of prospective, population-based studies, the nested case–control study in individuals with type 2 diabetes mellitus, and the aforementioned genetic data regarding the effect size, the interpretation of the positive association between circulating IL-1RA and incident CVD is less straightforward.[21] First, higher IL-1RA levels in the circulation could have direct detrimental effects and contribute causally to the development of atherosclerosis and CVD. Alternatively, higher IL-1RA levels could reflect a response to proatherogenic processes mediated by IL-1β and other proinflammatory biomarkers and, therefore, serve as an indirect indicator of cardiovascular risk.

The first explanation is mainly supported by the aforementioned genetic study,[20] but it is currently not possible to rule out pleiotropic effects of these *IL-1RN* gene variants on other phenotypes beyond IL-1RA levels that might be responsible for the positive association. The same study also demonstrated increased serum lipid levels (LDL and total cholesterol and triglycerides) associated with the IL-1RA-raising alleles, which could explain 20% to 40% of the excess cardiovascular risk. Importantly, all cohorts that were meta-analyzed in our study adjusted for cholesterol levels, so that the pooled effect estimate was corrected for this potential mediator.

The second explanation that sees IL-1RA as response to proinflammatory triggers is supported by the observation that IL-1RA has no agonist activity on its own and does not induce IL-1 receptor I–mediated signaling in humans at levels that

are 1 000 000-fold greater than those of IL-1α or IL-1β.[2] In addition, experimental studies have demonstrated that IL-1RA deficiency fueled arterial inflammation and atherosclerosis,[22–24] whereas IL-1RA treatment was atheroprotective.[25,26] It is possible that IL-1RA is directly upregulated by the same proinflammatory stimuli that increase the expression of IL-1α, IL-1β, and other proteins that have been characterized as risk indicators or risk factors of CVD (eg, high-sensitivity C-reactive protein, IL-6, myeloperoxidase, soluble intercellular adhesion molecule-1, and soluble (s)E-selectin[27–31]). In addition (and not mutually exclusive), IL-1RA could also be upregulated in response to these aforementioned proteins. Thus, the positive association between circulating IL-1RA levels and risk of CVD would at least partially be based on the coordinated regulatory control of atherogenic immune mediators and IL-1RA as an anti-inflammatory protein. The interpretation of the physiological upregulation of IL-1RA to antagonize proinflammatory stimuli would be facilitated by the ability to precisely measure levels of IL-1 cytokines concomitantly with IL-1RA levels in the circulation or preferably at the sites of atheroma formation. Ultimately, stronger evidence favoring either a direct atherogenic or indirect proinflammatory pathway will emerge from population studies that have repeated measures of these biomarkers with long-term follow-up and a more sophisticated approach to mediation analysis.[32]

Our study indicates that the addition of IL-1RA to CVD risk scores consisting of established cardiovascular risk factors failed to improve their accuracy as assessed by the comparison of the respective AUC. It is well known that the AUC of good clinical risk scores can only be increased by a fairly small degree by single biomarkers,[18,33] which is in line with our observation that the increase in AUC, albeit not statistically significant, was largest for the risk scores with the lowest baseline AUC. However, this meta-analysis points toward the role of inflammatory processes in the pathogenesis of CVD and does not preclude a therapeutic value of IL-1 inhibition that can only be assessed with confidence in large randomized controlled trials. The large CANTOS trial will be able to answer the question of whether selective targeting of IL-1β can prevent recurrent major cardiovascular events in patients with stable coronary artery disease and a systemic proinflammatory state.[5]

Our study has several strengths. The large sample size and the absence of heterogeneity between studies allowed us to precisely quantify the association between circulating IL-1RA and CVD risk. The comparable level of adjustment for important confounders helped us to obtain an effect estimate that was not affected by established cardiovascular risk factors, although residual or unmeasured confounding cannot be ruled out. Nevertheless, our meta-analytic approach counters the common criticism of effect size inflation and vibration commonly levied against single studies of biomarkers.[34] The availability of additional 15 biomarkers of inflammation in MONICA/KORA enabled the identification of proinflammatory biomarkers that partially explain the excess cardiovascular risk linked to IL-1RA. Finally, our meta-analysis included both published and previously unpublished data.

Our study has several limitations that should be considered. As our systematic review identified only cohorts from Europe, the results of our meta-analysis are not generalizable to populations with other ethnic backgrounds. We cannot exclude that we may have missed studies because of language restrictions (English, German, Dutch, and French) in our literature search. The difficulty in reliably measuring systemic levels of IL-1α and IL-1β means that we were unable to assess the balance of IL-1 cytokines and IL-1RA as potential risk factors for CVD. In addition, we did not have data on cell surface or circulating levels of the decoy receptor IL-1 receptor II or the IL-1 receptor accessory protein, 2 further members of the IL-1 cytokine and receptor family that downregulate IL-1 activity. Finally, CVD outcome definitions were not identical across studies as outlined in the Materials and Methods section. However, this did not lead to heterogeneity in effect estimates.

We conclude that higher serum levels of IL-1RA are positively associated with incident CVD in the general population. Our findings are in line with the hypothesis that the upregulation of circulating IL-1RA before an incident CVD event may at least partially reflect a response to proinflammatory triggers that also induce subclinical inflammation, oxidative stress, and endothelial activation. The potential clinical relevance of targeting IL-1 cytokines to reduce the risk of CVD is currently being tested in a large randomized clinical trial (CANTOS).

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Disclosures

J. Sudduth-Klinger and D. Peretz were employed by Tethys Bioscience Inc. The other authors declare that they have no conflict of interest.

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Highlights

- Cardiovascular disease risk increases by 11% (95% confidence interval, 6%–17%) per SD of serum interleukin-1 receptor antagonist based on data from >20 000 study participants from 6 population-based cohorts.
- This association is not pronounced enough to result in a significant and clinically relevant improvement in CVD risk prediction.
- The association between interleukin-1 receptor antagonist and CVD risk is partially explained by biomarkers of subclinical inflammation, oxidative stress, and cell adhesion.
- Our data are in line with the hypothesis that the upregulation of circulating interleukin-1 receptor antagonist before the incidence of CVD may at least partially reflect a response to proatherogenic, inflammation-mediated processes.
- These findings corroborate the evidence that cytokines of the interleukin-1 family are implicated in the development of CVD.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Circulating Levels of Interleukin 1-Receptor Antagonist and Risk of Cardiovascular Disease: Meta-Analysis of Six Population-Based Cohorts

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C. Herder et al. Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of 6 population-based cohorts

SUPPLEMENTAL MATERIAL

Supplemental Table I. Baseline Characteristics of the MONICA/KORA Study Population

Characteristic	Incident CVD Cases	Non-Cases	*P*
N	803	1,942	
Age (years)	61 (54; 66)	51 (43; 60)	<0.001
Male sex (%)	69.1	46.5	<0.001
Body mass index (kg/m^2)	28.0 (25.7; 30.7)	26.5 (24.1; 29.5)	<0.001
Systolic blood pressure (mmHg)	141 (130; 156)	130 (120; 143)	<0.001
Diastolic blood pressure (mmHg)	83 (75, 91)	81 (74; 88)	<0.001
Hypertension (% yes)*	65.4	40.4	<0.001
Ratio total/HDL cholesterol	5.1 (4.2; 6.5)	4.2 (3.4; 5.2)	<0.001
Prevalent diabetes (% yes)	15.4	4.2	<0.001
Parental history of CHD (%)			0.001
Negative	51.9	59.7	
Positive	22.0	19.8	
Unknown	26.0	20.5	
Physical activity (% inactive)	69.6	61.4	<0.001
Smoking status (%)			<0.001
Never smoker	35.9	48.7	
Former smoker	33.3	27.1	
Current smoker	30.9	24.2	
Alcohol consumption (%)†			0.796
0 g/day	30.3	31.5	
>0 to <40/20 g/day	43.1	41.9	
≥40/20 g/day	26.7	26.5	
hsCRP (mg/l)	2.3 (1.2; 5.2)	1.4 (0.6; 3.1)	<0.001
IL-1 receptor antagonist (pg/ml)	364 (297; 476)	338 (273; 438)	<0.001

Descriptive analyses of baseline characteristics were carried out for cases and non-cases. Weighting was performed using the survey- and sex-specific sampling weights. Missing values were imputed using 20-fold multiple imputation by chained equations (MICE).[1] We imputed a total of 59 variables including all biomarkers measured as well as important covariables with missing values. Depending on the individual measurement level, different imputation methods were applied. For binary variables, logistic regression imputation was applied. For categorical variables, such as smoking status (current/former/never) or paternal history of diabetes (yes/no/unknown), polytomous regression imputation was used. Continuous variables were, if appropriate, transformed to approximate normal distribution (choosing form natural log, square root and cubic root). Then they were imputed using

predictive mean matching and were afterwards transformed back to their original scale. All variables relevant for later analyses were included to impute the missing values to preserve correlations among variables, also comprising outcome variables and the respective times to event.

Data are given as median (25th percentile; 75th percentile) for continuous variables and as percentages for categorical variables. For continuous variables participants with and without incident CVD were compared using t-tests on regression coefficients based on the SAS procedure SURVEYREG and using Rubin's rules for multiple imputation.[2,3] In case of non-normality, tests were carried out with log-transformed variables and results were presented as geometric means with antilogs of standard errors of the adjusted log-means. Categorical variables were compared using Wald chi-square test based on the SAS procedure SURVEYFREQ and imputation was accounted for using a method proposed by Schafer to combine multiple results of chi-square statistics.[4,5]

*Hypertension is defined based on 1999 ISH-WHO guidelines or medical treatment given that study participants were aware of being hypertensive.
†Alcohol consumption was classified for men as 0, >0 and <40 or ≥40 g per day, and for women as 0, >0 and <20 or ≥20 g per day.

Supplemental Table II. Hazard Ratios (HR) and 95% Confidence Intervals (CI) for the Association between Serum IL-1RA Quarters and Incident CVD in the MONICA/KORA study

	Serum IL-1RA for men*	Serum IL-1RA for women*	N cases / non-cases	Model 1	*Model 2*
Quarter 1	233.0 (85.2; 268.6)	238.1 (85.2; 279.9)	143 / 523	1.00	1.00
Quarter 2	303.1 (268.8; 331.9)	312.9 (280.0; 351.0)	169 / 490	1.17 (0.83; 1.63)	1.04 (0.73; 1.48)
Quarter 3	367.8 (332.0; 426.9)	396.8 (351.1; 465.3)	230 / 485	1.51 (1.12; 2.03)	1.25 (0.90; 1.73)
Quarter 4	528.6 (427.0; 2312.4)	581.1 (465.4; 2312.4)	261 / 444	2.13 (1.58; 2.86)	1.42 (1.02; 1.97)
P_{trend}	-	-	-	<0.0001	0.0109

*Sex-specific serum IL-1RA levels in pg/ml for quarters 1 to 4 are given as median (lower limit; upper limit).

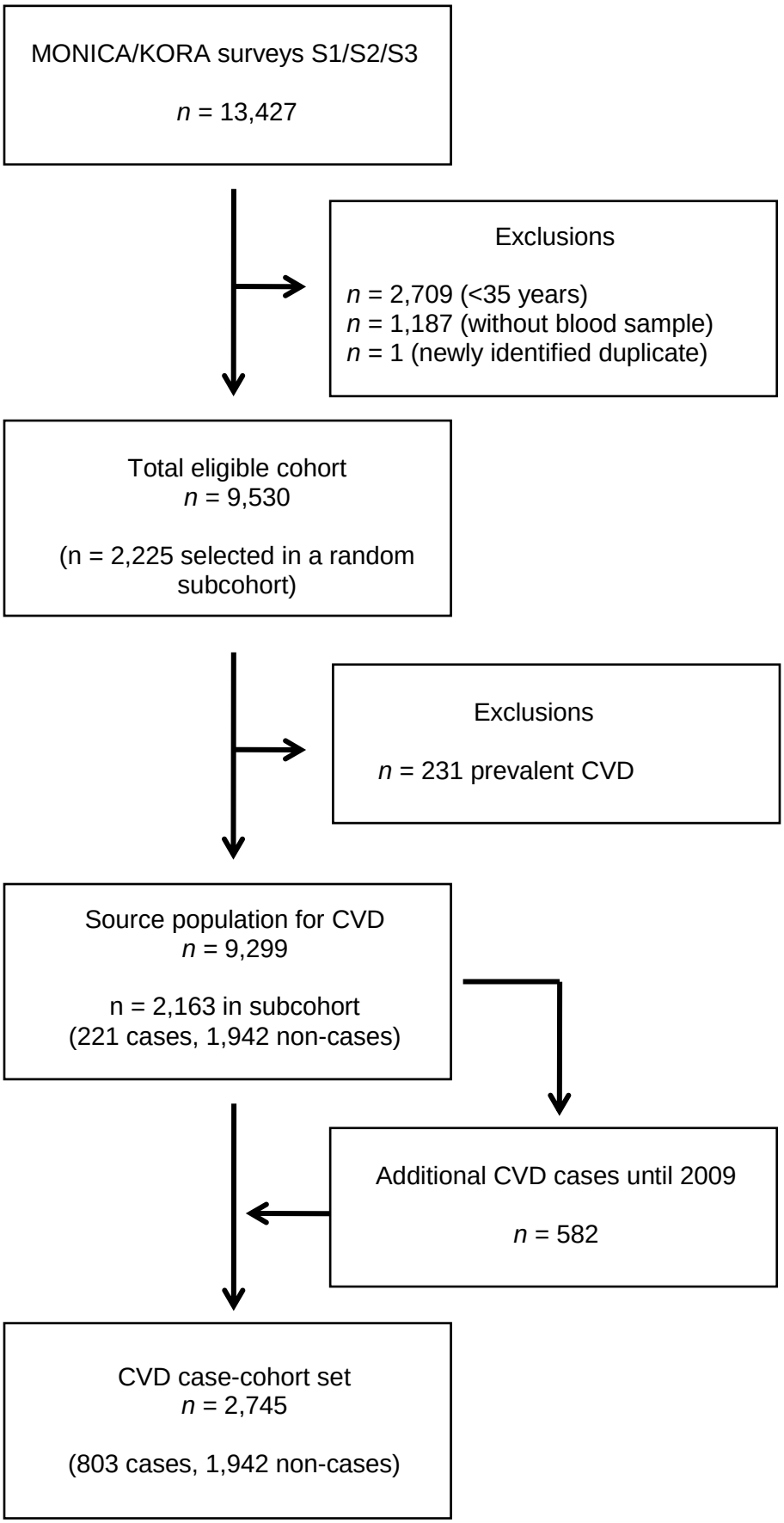
Cox proportional hazard (PH) regression was applied. For the estimation of hazard ratios (HRs) per quarter of IL-1RA, sex-specific quarters of IL-1RA (based on the random sample subcohort) were calculated with the bottom quarter as the reference category. The case-cohort design required correction of the variance estimation based on the sampling weights to give standard error estimates for the parameter estimates. Correction for standard errors was made using the method by Barlow.[6] Incorporation of the additional variation due to imputation was performed using Rubin's rules for multiple imputation.[2] Tests for trend were conducted assigning the median value within each quarter to the corresponding quarter and including this variable in the Cox regression model.

Model 1: adjusted for age, sex and survey.
Model 2: model 1 + BMI, systolic blood pressure, total/HDL cholesterol, smoking, alcohol consumption, physical activity, parental history of CHD, prevalent diabetes.

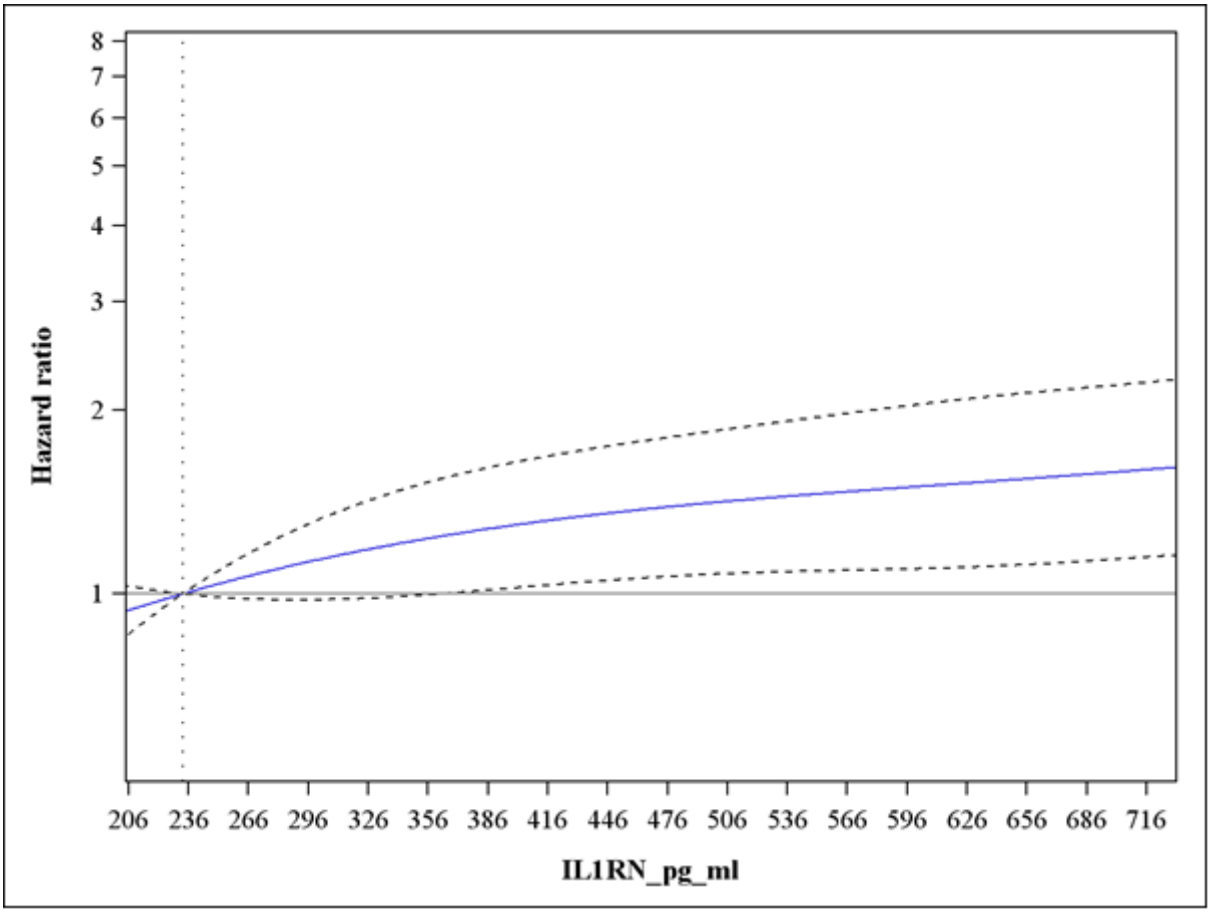
Supplemental Table III. Associations Between IL-1RA and Incident CVD in Six Prospective Cohort Studies

Study population	Duration of follow-up	Covariates	HR per SD (95% CI) and *P* value
MONICA/KORA Population-based case-cohort 803 cases, 1942 non-cases	16.0 years	Age, sex, survey, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, smoking, alcohol consumption, physical activity, parental history of CHD, prevalent diabetes	1.165 (1.039; 1.306) *P* = 0.009
FINRISK 1997 Population-based cohort 394 cases, 6099 non-cases	14.8 years	Age, sex, BMI, systolic blood pressure, smoking, alcohol, ratio of total cholesterol to HDL cholesterol, physical activity, prevalent diabetes	1.101 (1.008; 1.203) *P* = 0.034
FINRISK 2007 Population-based cohort 65 cases, 4226 non-cases	4.9 years	Age, sex, BMI, systolic blood pressure, smoking, alcohol, ratio of total cholesterol to HDL cholesterol, physical activity, prevalent diabetes	1.057 (0.899; 1.242) *P* = 0.503
HEALTH 2000 Population-based cohort 267 cases, 5585 non-cases	11.1 years	Age, sex, BMI, systolic blood pressure, smoking, alcohol, ratio of total cholesterol to HDL cholesterol, physical activity, prevalent diabetes	1.104 (0.969; 1.202) *P* = 0.169
Belfast PRIME Men Cohort Population-based cohort 227 cases, 2008 non-cases	10 years	Age, BMI, systolic blood pressure, smoking, non-HDL cholesterol, HDL cholesterol, anti-hypertensive or lipid-lowering medication, prevalent diabetes	1.149 (1.043; 1.266) *P* = 0.005
Rotterdam Study Population-based cohort 99 cases, 740 non-cases	10.6 years	Age, sex, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, smoking, prevalent diabetes, anti-hypertensive medication	1.109 (0.887; 1.386) *P* = 0.364

Supplemental Figure I. MONICA/KORA Case-Cohort Study Population

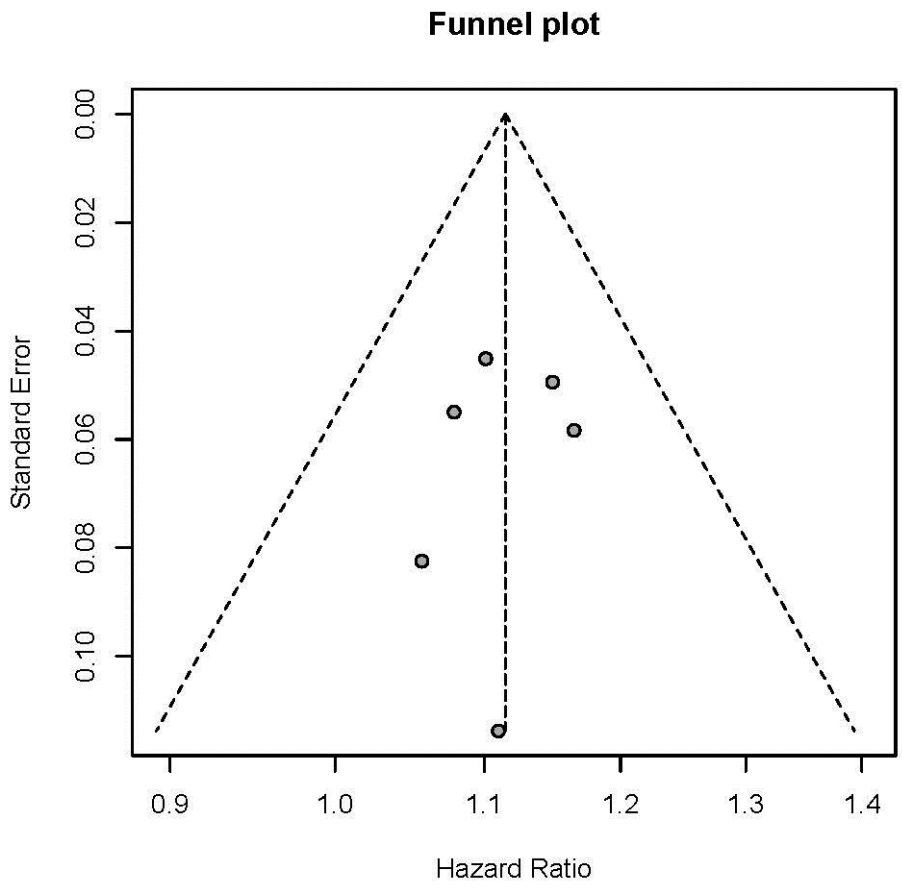


Supplemental Figure II. Dose-Response Relationship of the Association Between Serum IL-1RA and Incident CVD in the MONICA/KORA Study



The figure shows the HR (blue line) and 95% CI (dotted black lines) for the association between IL-1RA levels and incident CVD in model 2. The figure is limited to the IL-1RA range between the 5th and 95th percentiles (206 to 728 pg/ml) with the median of the first IL-1RA quarter (233 pg/ml) as reference (shown for one imputation).

Supplemental Figure III. Funnel Plot to Assess Publication Bias



Egger's linear regression test of funnel plot asymmetry yielded a *P* value of 0.67, so that the hypothesis that the funnel plot is symmetric cannot be rejected (i.e. no evidence for publication bias).

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C. Herder et al. Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of 6 population-based cohorts

Methods

Systematic Review

We performed a systematic review and meta-analysis in accordance with the 'Preferred reporting items for systematic review and meta-analyses' (PRISMA) statement[1] to address the study question if circulating levels of IL-1RA are associated with the risk of CVD independently of traditional cardiovascular risk factors.

The study protocol is given as an Appendix to this Methods section. Briefly, we searched for original publications from population-based prospective studies, that reported data on the association between serum or plasma levels of IL-1RA protein and incident CVD (fatal and non-fatal myocardial infarction, sudden death, cardiovascular mortality). Combined endpoints including unstable angina, coronary revascularisation or stroke were also acceptable. Further main eligibility criteria were at least minimal adjustment for age and sex; and reporting of effect measures as relative risk/risk ratio (RR), odds ratio (OR) or hazard ratio (HR) of CVD according to levels of IL-1RA. We used MEDLINE and Embase as data sources, but also screened cited references in eligible publications and included unpublished data from the MONICA/KORA Augsburg case-cohort study. Search terms were ((cardiovascular OR coronary OR myocardial OR CHD OR CVD) AND (interleukin-1 receptor antagonist OR IL-1ra OR IL1ra)) OR ((cardiovascular OR coronary OR CHD OR CVD OR myocardial) AND (biomarker OR marker OR biomarkers OR markers) AND incident AND risk AND (predictive OR prospective)). The search was limited to publications in English, German, Dutch and French and performed on December 30, 2016 without any restrictions regarding publication time. Results of the literature search were summarised in a flowchart.

We contacted the authors of the retrieved eligible studies[2,3] to clarify items on the respective data extraction forms and asked for re-analysis of data to obtain a comparable level of adjustment for potential confounders in line with analyses in the MONICA/KORA Augsburg case-cohort study and, if possible, use data with extended follow-up times compared to the original publications. We also asked for further studies which might be eligible for the meta-analysis, which added a further two unpublished studies to the analysis.

Meta-analysis

For the meta-analysis we used inverse-variance fixed effect and DerSimonian-Laird random effects modeling based on study-level data; the DerSimonian-Laird estimator was used to test for heterogeneity.[4] To check for possible publication bias we visually inspected the funnel plot and performed Egger's regression test of funnel plot asymmetry.[5]

For all analyses, a *P* value <0.05 was considered to be statistically significant. Imputation and meta-analysis were performed using R version 3.2.3 and R packages mice (version 2.25) and meta (version 4.2-0).[6-8] All other statistical calculations were performed with SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA).

Characteristics of the Study Populations

- MONICA/KORA Augsburg Case-Cohort Study

The Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) / Cooperative Health Research in the Region of Augsburg (KORA) studies served as the database for a prospective case-cohort study in initially healthy, middle-aged men and women.[9,10] Briefly, three independent population-based MONICA/KORA Augsburg surveys (S), with a total number of 13,427 participants (6,725 men, 6,702 women) aged 25-64 (S1) or 25-74 years (S2-S3), were conducted in 1984/85 (S1), 1989/90 (S2) and 1994/95 (S3). All study participants were prospectively followed within the framework of KORA. The case-cohort design used in the present study has been described previously in detail[11-13].

Due to the low incidence of cardiovascular disease (CVD) under the age of 35, we restricted the source population to 10,718 persons (5,382 men, 5,336 women) between 35-74 years of age at baseline who participated in at least one of the three surveys. After exclusion of 1,187 subjects with missing blood samples, 231 participants with self-reported prevalent CVD and one duplicate identified during the course of the study (i.e. one subject who participated in two surveys), the source population for the present study comprised 9,299 subjects (4,506 men, 4,793 women).

For the case-cohort study, a random sample subcohort of 2,163 subjects (1,154 men, thereof 161 incident CVD cases as defined below and 1,009 women, thereof 60 incident CVD cases) was drawn from the source population, stratifying by sex and survey. This random sample was enriched with all additional incident CVD cases (582 cases) which occurred during the follow-up time until 2009. The final case-cohort set comprised 2,745 participants aged 35-74 years, including 1,548 men (555 incident CVD cases) and 1,197 women (248 incident CVD cases). Mean follow-up time (SD) was 16.0 (5.8) years.

All participants provided written informed consent. The study was approved by the ethics committee of the Bavarian Chamber of Physicians and complies with the principles outlined in the Declaration of Helsinki.

- FINRISK Studies

FINRISK surveys are population-based studies conducted every five years since 1972 to monitor the risk of chronic diseases.[2,14] For each survey, a representative random sample was selected from inhabitants in the age range of 25 to 74 years from five geographical regions in Finland. The survey included a questionnaire and a clinical examination, at which a blood sample was drawn, with linkage to national registers of cardiovascular and other health outcomes.[2] The current study included eligible individuals from two independent FINRISK surveys conducted in 1997 (FINRISK 1997) and in 2007 (FINRISK 2007).

All the participants gave written informed consent and the survey was conducted in accordance with the Declaration of Helsinki. Ethics approval was received from the Ethics Committee of the National Public Health Institute.

- Health 2000 Study

Health 2000 is a population-based national survey on the health and functional capacity of Finnish individuals (http://www.terveys2000.fi/julkaisut/baseline.pdf). A nationally representative sample of 10,000 individuals was drawn of the population aged ≥18 years. The survey included an interview about medical history and health-related lifestyle habits and a clinical examination (for individuals of ≥30 years of age), at which a blood sample was drawn. Study participants were restricted to be aged ≤80 years at baseline.

All the participants gave written informed consent and the survey was conducted in accordance with the Declaration of Helsinki. Ethics approval was received from the Ethics Committee of the National Public Health Institute.

- Belfast PRIME Men Study

The Belfast Prospective epidemiological Study of Myocardial Infarction (PRIME) men study is a population-based study of coronary events.[15] Men aged 50 to 59 years were recruited between 1991 and 1994. The baseline examination included standardised questionnaires, anthropometric measurements and blood sampling. Participants were followed up by letters and telephone contact if necessary in order to complete a clinical event questionnaire. All possible events were validated using hospital or general practitioners' notes. Death certificates were obtained for supporting information on cause of death.[2]

Approval for the study was obtained from the local ethics committee; all participants gave informed consent and the study was compliant with the guidelines contained within the Declaration of Helsinki.[15]

- Rotterdam Study

The Rotterdam Study is a population-based cohort study in Ommoord, a district of Rotterdem, the Netherlands.[16] Participants aged ≥55 years entered the study between 1990 and 1993, after which follow-up examinations were conducted in 1993-1994, 1997-1999, 2002-2004 and 2009-2011. This study is based on data from the third visit (clinical examination, questionnaires and blood sampling) in 1997-1999.[3] Follow-up information on CVD was obtained through continuous monitoring of the cohort through automated coupling of the study database with medical records from general practitioners and from letters and discharge reports of medical specialists.

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports in the Netherlands. A written informed consent was obtained from all participants.

Definition of CVD Endpoints, Measurement of IL-1RA and Statistical Analysis in the Study Populations

Details are summarised in the following table.

Table. Summary of CVD Endpoint Definitions, IL-1RA Measurements and Statistical Analyses in the Study Cohorts Entering the Meta-Analysis

Study population	CVD endpoint	Measurement of IL-1RA	Statistical analysis
MONICA/KORA	Combined coronary heart disease endpoint that included incident non-fatal myocardial infarction (MI) as well as fatal MI and sudden death. Incident events were identified through MONICA/KORA follow-up questionnaires or through the MONICA/KORA registry of acute myocardial infarction.[17,18]	Serum samples: measurement of IL-1RA using ultrasensitive molecular counting technology (MCT; Singulex, Alameda, CA).[19,20] The IL-1RA assay used a recombinant IL-1RA protein (280-RA-050/CF) from R&D Systems (Minneapolis, MN, USA), a mouse monoclonal anti-human IL-1RA capture antibody (WH0003557M1) from Sigma-Aldrich (St. Louis, MO, USA) and a biotinylated polyclonal goat anti-IL-1RA detection antibody (BAF280; R&D Systems). Intra- and inter-assay CVs were 6% and 8%, respectively.	The association between serum IL-1RA and incident CVD was assessed using multivariable Cox proportional hazard (PH) regression. Results are given as HR (95% CI) per standard deviation of natural log-transformed IL-1RA levels. The accuracy of the different models to assess 10-year event risk was evaluated by AUC. Performance measures were calculated applying methods appropriate for survival data and case cohort design,[21-23] which are implemented as SAS macros available from http://ncook.bwh.harvard.edu/sas-macros.html.[24] For the correct assessment of predictive model performance, 1,000 bootstrap and 1,000 out-of-bag (OOB) samples were drawn from the original case-cohort set. Missing values were imputed in each of these 2,000 data sets separately using MICE. AUC were calculated according to an approach proposed by Wahl et al.[25] with 95% CI calculated using a bootstrap-based approach by Jiang et al.[26] to evaluate the results in the OOB samples.
FINRISK 1997	First occurrence of a major cardiovascular event during follow-up, which included the first fatal or non-fatal definite or possible myocardial infarction or coronary death, unstable angina, cardiac revascularisation, ischaemic stroke and unclassifiable	Serum samples, Quantikine ELISA (R&D Systems), intra- and inter-assay CVs of 3.59% and 5.68%, respectively.[27]	The association between serum IL-1RA and incident CVD was assessed using multivariable Cox PH regression. Results are given as HR (95% CI) per 1 standard deviation of IL-1RA levels. AUC were calculated using the R package 'validstats' (http://individual.utoronto.ca/osaarela/).[28]

	death.[2]		
FINRISK 2007	Same as FINRISK 1997.	Serum samples, Quantikine ELISA (R&D Systems), intra- and inter-assay CVs of 2.2% and 10.3%, respectively.[27]	Same as FINRISK 1997
HEALTH 2000	Same as FINRISK 1997.	Same as FINRISK 1997.	Same as FINRISK 1997
Belfast PRIME Men Cohort	Same as FINRISK 1997.	Same as FINRISK 1997.	The association between IL-1RA and incident CVD was estimated using Cox regression models. Results are given as HR (95% CI) per 1 standard deviation of IL-1RA levels. AUC were calculated with bootstrap sampling distribution for C-index and C-index improvement.
Rotterdam Study	Myocardial revascularisation, fatal and non-fatal myocardial infarction and CVD mortality.[29]	Fasting plasma samples, multiplex immunoassay at Rules-Based Medicine, Austin, TX (www.myriadrbm.com) with intra- and inter-assay CVs of <4% and <13%, respectively.[3]	Cox proportional hazard models were used to assess the association between plasma IL-1RA and incident CVD. Results are given as HR (95% CI) per 1 standard deviation of IL-1RA (natural log-transformed). AUC were calculated using the 'cindex' function in the R package 'dynpred'. The difference in C-statistic between the base model and the model with IL-1RA was corrected for optimism using 1000 bootstraps.

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Appendix: Study protocol for the systematic review and meta-analysis

Review question

Are circulating levels of interleukin 1 receptor antagonist (IL-1RA) associated with the risk of cardiovascular disease (CVD) independently of traditional cardiovascular risk factors?

Searches

- All steps of the literature search and eligibility assessment will be carried out by two investigators independently.
- Data sources: MEDLINE, Embase, screening of cited references in eligible publications.
- Additional datasource: unpublished data from the MONICA/KORA study and the Finnish HEALTH 2000 and FINRISK 2007 surveys.
- Search terms: ((cardiovascular OR coronary OR myocardial OR CHD OR CVD) AND (interleukin-1 receptor antagonist OR IL-1ra OR IL1ra)) OR ((cardiovascular OR coronary OR CHD OR CVD OR myocardial) AND (biomarker OR marker OR biomarkers OR markers) AND incident AND risk AND (predictive OR prospective))
- Limits: no further limits; search on December 30, 2016.

Types of study to be included

Eligibility criteria:
- Publication languages: English, German, Dutch, French
- Original publication on human subjects
- Prospective studies
- Association between serum or plasma levels of IL-1RA protein and risk for CVD is reported as relative risk/risk ratio (RR), odds ratio (OR) or hazard ratio (HR) for incident CVD according to levels of IL-1RA by means of Cox proportional hazard (or logistic) regression models with comparable adjustment.

Condition or domain being studied

Incident CVD

Participants/population

Eligibility criteria: Population-based studies

Intervention(s), exposure(s)

-Exposure: Different IL-1RA protein levels in blood between incident cases and non-cases/controls

-Eligibility criteria: Studies investigating the association of IL1RA and incident CVD are only included if they present at least age- and sex-adjusted results because IL-1RA levels are known to be strongly associated with both age and sex.

Comparator(s)/control

Non-CVD cases from the general population

Outcome(s)

-Primary outcomes
Incident CVD (fatal and non-fatal myocardial infarction, sudden death, cardiovascular mortality). Combined endpoints including unstable angina, coronary revascularisation or ischaemic stroke will be acceptable.
Effect measures:
Relative risk/risk ratio (RR), odds ratio (OR) or hazard ratio (HR) of CVD according to levels of IL-1RA.

-Secondary outcomes
None

Data extraction

Study selection:
- Records retrieved will be imported from MEDLINE and Embase into MS Word and duplicates will be removed.
- The retrieved records and abstracts (if available) will be screened for eligibility and those that clearly do not meet the inclusion criteria (e.g. wrong topic, no IL-1RA protein assessed, studies not population-based) will be excluded. The number of studies excluded in this step will be documented.
- Full texts of all other retrieved records will be screened and further exclusions will be made based on the eligibility criteria. The number of studies excluded in this step will be documented and grouped by reasons for exclusion.
- The eligible studies retrieved by investigator 1 and investigator 2 during the literature search will be compared. Disagreements between investigators will be resolved by consensus. If consensus cannot be reached, a third investigator will make the decision.
- In order to report the described literature search steps in the manuscript, a PRISMA flow chart will be created.

Data extraction I:
A pilot form for the data extraction will be created in MS Word and tested by the investigators using data from two exemplary studies. The results will be discussed and a common form agreed on for the further data extraction. The following items will be included in the form:
• first author
• journal
• publication year
• study type (design)
• name of study cohort
• year of study or baseline investigation, respectively
• years of follow-up
• country/setting
• number of participants (CVD cases, comparison group, total)
• age (range and/or mean (SD))
• sex (percent male/female, female/male only)
• ethnicity
• participants (e.g. from which study, inclusion/exclusion criteria)
• comparison group (selection, characteristics, matching)
• definition of CVD
• exposure (IL-1RA: how was it measured, including in which medium, i.e. serum or plasma, how large were the inter- and intra-assay CVs if reported)
• variables adjusted for (separately for all reported models)
• modelling of exposure: description of applied regression model (Cox PH, logistic, Poisson), description of reported estimates (OR, RR or HR), compared categories (e.g. quarters of IL-

1RA, per SD of IL-1RA, log-transformed), plus the corresponding ranges, means or medians of levels within the classes, with measuring unit
• results: based on reported models and planned analyses, results will be entered into a separate EXCEL sheet for the analysis (see data extraction II)

Missing information:
If important information that should be extracted from the studies (e.g. number of participants) is missing, the corresponding authors will be contacted. If the information cannot be retrieved from the author, we will handle this as follows:
If information about variables is missing without offending the inclusion criteria, this will be accounted for in the assessment of study quality (e.g. in terms of poor reporting) but the study will still be included.
In case it is unclear whether two studies report results from the same population, we will contact the authors for clarification.

Data extraction II (extraction results):
Based on how results are reported in the included studies (which association estimates for which comparisons, adjustment for which covariables), we will decide which results are to be extracted for the final analyses and an EXCEL sheet for data entry plus a clarification sheet will be developed.

Strategy for data synthesis

Statistical assessment of heterogeneity between study-specific association estimates:
- Chi-squared Q-test for heterogeneity, p=0.10 as cut-off level
- I-squared statistic with 95% CI

Visual depiction of potential heterogeneity:
- Forest plot
- Funnel plot

Meta-analysis:
We will calculate pooled estimates (RR/OR/HR per 1 standard deviation of circulating IL-1RA levels in the respective studies) for all eliglible studies. Effect estimates will be based on models adjusting for age, sex and comparable sets of traditional covariates (demographic, anthropometric, metabolic and lifestyle factors, family history, etc.). Based on effect estimates from the included studies we will calculate a pooled effect estimate for the meta-analysis.

Statistical methods for the calculation of the pooled association estimates:
- Calculation of: number of included studies, total number of participants, association estimate with 95% CI and p-value
- Main analysis: DerSimonian and Laird random effects meta-analysis
- Inverse-variance fixed effect model for comparison
- All statistical analyses will be performed with SAS 9.2 and RevMan 5

Assessment of publication bias:
- Funnel plot
- Egger's regression test of publication bias

Analysis of subgroups or subsets

Sensitivity analyses are not planned.

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Conflicts of interest

None known.