

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) statement¹ 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 We used MEDLINE and Embase as data sources, but also screened cited references in eligible publications and included unpublished data from the MONICA/KORA Augsburg case-cohort study.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 We also asked for further studies which might be eligible for the meta-analysis, which added a further two unpublished studies to the analysis.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 detail¹¹⁻¹³.

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 Rotterdam, 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.

References

1. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6:e1000097.
2. Blankenberg S, Zeller T, Saarela O, et al. Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts: the MONICA, risk, genetics, archiving, and monograph (MORGAM) biomarker project. *Circulation*. 2010;121:2388--2397.
3. Ligthart S, Sedaghat S, Ikram MA, Hofman A, Franco OH, Dehghan A. EN-RAGE: a novel inflammatory marker for incident coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2014;34:2695--2699.
4. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7:177--188.
5. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315:629--634.
6. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014. <http://www.R-project.org/>.
7. van Buuren S, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*. 2011;45:1--67.
8. Schwarzer G. Meta: General package for meta-analysis. 2015. <http://CRAN.R-project.org/package=meta>.
9. Holle R, Happich M, Lowel H, Wichmann HE; MONICA/KORA Study Group. KORA - a research platform for population based health research. *Gesundheitswesen*. 2005;67 Suppl 1:S19--S25.
10. Löwel H, Döring A, Schneider A, Heier M, Thorand B, Meisinger C. The MONICA Augsburg surveys - basis for prospective cohort studies. *Gesundheitswesen*. 2005;67 Suppl 1:S13--S18.
11. Herder C, Baumert J, Thorand B, Koenig W, de Jager W, Meisinger C, Illig T, Martin S, Kolb H. Chemokines as risk factors for type 2 diabetes: Results from the MONICA/KORA Augsburg study, 1984-2002. *Diabetologia*. 2006;49:921--929.
12. Thorand B, Baumert J, Kolb H, Meisinger C, Chambless L, Koenig W, Herder C. Sex differences in the prediction of type 2 diabetes by inflammatory markers: Results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. *Diabetes Care*. 2007;30:854--860.
13. Herder C, Klopp N, Baumert J, Müller M, Khuseyinova N, Meisinger C, Martin S, Illig T, Koenig W, Thorand B. Effect of macrophage migration inhibitory factor (MIF) gene variants and MIF serum concentrations on the risk of type 2 diabetes: Results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. *Diabetologia*. 2008;51:276--284.
14. Vartiainen E, Laatikainen T, Peltonen M, Juolevi A, Männistö S, Sundvall J, Jousilahti P, Salomaa V, Valsta L, Puska P. Thirty-five-year trends in cardiovascular risk factors in Finland. *Int J Epidemiol*. 2010;39:504--518.

15. Yarnell JW. The PRIME study: classical risk factors do not explain the severalfold differences in risk of coronary heart disease between France and Northern Ireland. Prospective epidemiological Study of Myocardial Infarction. *QJM*. 1998;91:667--676.
16. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, Ikram MA, Klaver CC, Nijsten TE, Peeters RP, Stricker BH, Tiemeier HW, Uitterlinden AG, Vernooij MW. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol*. 2015;30:661--708.
17. Löwel H, Lewis M, Hörmann A, Keil U. Case finding, data quality aspects and comparability of myocardial infarction registers: Results of a South German register study. *J Clin Epidemiol*. 1991;44:249--260.
18. Löwel H, Meisinger C, Heier M, Hormann A. The population-based Acute Myocardial Infarction (AMI) registry of the MONICA/KORA study region of Augsburg. *Gesundheitswesen*. 2005;67 Suppl 1:S31--S37.
19. Kolberg JA, Jørgensen T, Gerwien RW, Hamren S, McKenna MP, Moler E, Rowe MW, Urdea MS, Xu XM, Hansen T, Pedersen O, Borch-Johnsen K. Development of a type 2 diabetes risk model from a panel of serum biomarkers from the Inter99 cohort. *Diabetes Care*. 2009;32:1207--1212.
20. Wilsgaard T, Mathiesen EB, Patwardhan A, Rowe MW, Schirmer H, Løchen ML, Sudduth-Klinger J, Hamren S, Bønaa KH, Njølstad I. Clinically significant novel biomarkers for prediction of first ever myocardial infarction: the Tromsø Study. *Circ Cardiovasc Genet*. 2015;8:363--371.
21. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med*. 2011;30:11--21.
22. Chambless LE, Cummiskey CP, Cui G. Several methods to assess improvement in risk prediction models: Extension to survival analysis. *Stat Med*. 2011;30:22--38.
23. Cook NR, Paynter NP, Manson JE, Martin LW, Robinson JG, Wassertheil-Smoller S, Ridker PM. Clinical utility of lipoprotein-associated phospholipase A(2) for cardiovascular disease prediction in a multiethnic cohort of women. *Clin Chem*. 2012;58:1352--1363.
24. Cook NR. [SAS macros to assess performance of risk prediction models with survival outcomes] [accessed 2016-Feb-22]; Available from: <http://ncook.bwh.harvard.edu/sas-macros.html>
25. Wahl S, Boulesteix AL, Zierler A, Thorand B, Avan de Wiel M. Assessment of predictive performance in incomplete data by combining internal validation and multiple imputation. *BMC Med Res Methodol*. 2016;16:144.
26. Jiang B, Zhang XG, Cai TX. Estimating the confidence interval for prediction errors of support vector machine classifiers. *Journal of Machine Learning Research*. 2008;9:521--540.
27. Herder C, Nuotio ML, Shah S, et al. Genetic determinants of circulating interleukin-1 receptor antagonist levels and their association with glycemic traits. *Diabetes*. 2014;63:4343--4359.
28. Antolini L, Nam BH, D'Agostini RB. Inference on correlated discrimination measures in survival analysis: a nonparametric approach. *Communications in Statistics – Theory and Methods*. 2004;33:2117--2135.

29. Leening MJ, Kavousi M, Heeringa J, van Rooij FJ, Verkreest-van Heemst J, Deckers JW, Mattace-Raso FU, Ziere G, Hofman A, Stricker BH, Witteman JC. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. *Eur J Epidemiol.* 2012;27:173--185.

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 datasources: 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 eligible 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.

Review team

- Christian Herder, Maren-Carstensen-Kirberg (Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany)
- Brenda W. C. Bongaerts (Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany)
- Tonia de las Heras Gala, Barbara Thorand, Cornelia Huth (Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany)

Conflicts of interest

None known.