UCL Institute of Health Informatics

Contributions of Higher Resolution Observational Evidence from Electronic Health Records to Understand the Causal Relevance of Blood Lipids to Heart Failure and Atrial Fibrillation

Nat Na-Ek

September 2019

A thesis submitted for the degree of Doctor of Philosophy

at University College London

Supervisors: Prof Harry Hemingway & Dr Amitava Banerjee

Declaration

I, Nat Na-Ek, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

28th September 2019

Abstract

Heart failure (HF) and atrial fibrillation (AF) are increasingly prevalent due to aging populations, and both diseases have a big economic and healthcare burden globally. To date, there is no primary prevention specific to healthy populations. Blood lipids (i.e., LDL-C, HDL-C, and TG), which are involved with pathophysiological mechanisms of HF and AF, might play a role in the origin of both diseases. Therefore, the potential causal relevance of blood lipids to HF and AF should be investigated.

Linkage electronic health records (EHRs) provide an opportunity to investigate the association between blood lipids and the incidence of HF and AF, as these records contain large sample sizes (e.g., n>1 million) with a wide range of diseases and biomarkers routinely recorded in clinical practice. Challenges include structuring the data into a research-ready format, accurately defining outcomes, and handling missing data.

The data used in this thesis is from the CALIBER platform, which links routinely collected EHRs from general practices, hospital admission, and the death registries of 3 million patients in England from 1997 to 2016.

In this thesis, I (1) constructed cohorts from EHRs and ensured the validity of the cohorts and (2) examined the association between blood lipids and the incidence of HF and AF using the EHR population-based cohort design. The observed findings were then compared to the results from meta-regression of trials on lipid-lowering drugs and those from a Mendelian randomisation approach, and then I (3) assessed the predictive value of adding blood lipids in the risk prediction of incident HF and AF. Additionally, I developed the model for the prediction of 10-year risk of newly occurring HF and AF.

Taken together, these findings have a valuable implementation. For future research, my findings can be a basis for developing a new drug to fight against HF and AF. For clinical application, my findings can inform clinicians whether blood lipids should be targeted and what levels are needed to protect people from HF and AF. Besides, my results can inform clinicians to monitor their patients for the developing of HF and AF.

Impact Statement

The findings of this thesis provide further insight into the role of blood lipids (i.e., LDL-C, HDL-C, and TG) in the origin of HF and AF. This should be a fundamental research for new drug development or to find a new indication for existing approved drugs to protect people against HF and AF–the two most common cardiac diseases that are becoming more prevalent worldwide.

Regardless of the findings, my study is an example of the triangulation of evidence (i.e., the use of different study approaches that had unrelated and different potential sources of bias to disentangle the same research question) to address the problem and yield more solid and less bias conclusion. In addition, results from my work also points out the research gap in cardiovascular epidemiology in which studies on the prevention of HF and AF in healthy populations are currently lacking, and there is scarce evidence on the risk management for people who are at high risk of HF or AF despite being at low atherosclerotic cardiovascular risk.

The findings of this thesis can be generalised at the national level as my samples were from representative English populations of 3 million individuals, and I plan to publish my work in the peer-reviewed medical journals to raise public awareness of the role of blood lipids in the risk of HF and AF. Moreover, the findings can inform clinicians whether blood lipids should be targeted and, if so, to what extent the levels should be. Also, these could inform clinicians to monitor patients whose lipids are extremely high or low or those who are currently receiving lipid-lowering drugs for the new-onset of HF and AF. Besides, the results of this work could help guide clinicians to give more appropriate interventions (e.g., choice of blood pressure-lowering drugs) to their patients, who carried different risks of HF and AF.

Acknowledgements

My thesis would not be finished without the help and support of the following people. First and most importantly, I would like to express my sincere gratitude to my primary supervisor Professor Harry Hemingway for his continuous support of my PhD study and related research, for his patience, motivation, and immense knowledge given to me until the last minute before my thesis submission. His guidance helped me in all my time studying and writing this thesis. I could not have imagined having a better advisor and mentor for my PhD study. Next, I would like to thank Dr Amitava Banerjee, my subsidiary supervisor, who was always hands-on and supportive throughout my PhD.

My sincere thanks also go to Dr Alireza Moayyeri, Dr Michail Katsoulis, and Dr Laura Pasea for always lending me a hand when I was struggling with anything about my PhD. Without their precious support, my work would not be complete.

Also, I am wholeheartedly thankful for these incredible friends: Dr Piyada Supasa, Dr Wiyada Wongwiwat, Dr Pimphot Seelakate, Dr Jooyin Saejang, Anochao Phetcharat, Georgia Chatzi, Jintana Jankhotkaew, Yuttapong Thawornwattana, Nonthiwat Taesuk, Akkapon Wongkoblap, Papon Muangsanit, Saowalak Changko, and Premkamon Chaipanichkul—for always bringing me joy and happiness throughout my time in London. I am indeed very thankful for each moment we spent together, and I could not have reached this far without the support from you guys.

Last but not least, I would like to thank my parents and my aunts for supporting me mentally and, sometimes, financially for my entire student journey and my life in general. I would like to dedicate all of my work to one particular person—Sudaporn Na-Ek—who is the reason why I decided to pursue my PhD. I just want you to be proud of me, mum.

Statement of Personal Contribution

I would like to confirm that this thesis is my original work. However, there are two parts of my work that were a kind contribution of members of staff at UCL Institute of Health Informatics. First, the summary statistics of the genetic data from UK Biobank were obtained from Dr Michail Katsoulis, which was done under approval application number 12113. Second, the results from the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) Consortium were given by Dr Tom Lumbers.

In addition, the electronic health record (EHR) study of my thesis was a sub-study of the protocol for which approval was granted by the Independent Scientific Advisory Committee of the Medicines and Healthcare products Regulatory Agency (ISAC) and assigned protocol number 12_153RA-RMnAR. This protocol was originally co-developed by Dr Mar Pujades Rodriguez, Dr Marina Daskalopoulou, Dr Anoop Shah, Dr Owen Nicholas, Dr Eleni Rapsomaniki, and Prof Harry Hemingway.

Table of Contents

Declaration 3

Abstract 4

Impact Statement 5

Acknowledgements 6

Statement of Personal Contribution 7

List of Tables 12

List of Figures 14

List of Supplementary Tables 17

List of Supplementary Figures 21

List of Abbreviations 27

Chapter 1 Introduction 29

1.1 Abstract 29

1.2 Introduction 29

1.3 Why blood lipids may be causally relevant in HF and AF ? 35

1.4 Why study HF and AF? 37

1.5 PhD Objectives 40

1.6 Clinical questions 42

Chapter 2 Blood lipids, heart failure and atrial fibrillation: A systematic review and meta-analysis of cohort, trials, and genetic studies 46

2.1 Abstract 46

2.2 Introduction 47

2.3 Methodology 48

2.4 Results 50

2.5 Discussion 56

2.6 Conclusions 59

2.7 Chapter Supplementary 68

Chapter 3 Electronic health record (EHR) cohort: Strengths, limitations, and approach to EHR phenotype validation....118
3.1 Abstract....118
3.2 Introduction to Electronic Health Records (EHRs)....119
3.3 Research platform of national linked EHRs: CALIBER....120
3.4 Approach to data curation in CALIBER platform....123
3.5 Preliminary results and validity of data used in this thesis....129
3.6 Main limitations of CALIBER and further suggestions....135
3.7 Conclusions....138
3.8 Chapter Supplementary....153
Chapter 4 Low-density lipoprotein cholesterol as a cause of heart failure and atrial fibrillation: Cohort, trial, and genetic evidence....166
4.1 Key messages....166
4.2 Abstract....167
4.3 Introduction....168
4.4 Methods....170
4.5 Results....175
4.6 Discussion....178
4.7 Conclusion....186
4.8 Chapter Supplementary....196
Chapter 5 High-density lipoprotein cholesterol as a cause of heart failure and atrial fibrillation: Cohort, trial, and genetic evidence....219
5.1 Key messages....219
5.2 Abstract....220
5.3 Introduction....221
5.4 Methods....223
5.5 Results....229
5.6 Discussion....233

5.7 Conclusion 241
5.8 Chapter Supplementary 251
Chapter 6 Triglycerides as a cause of heart failure and atrial fibrillation: Cohort, trial, and genetic evidence 288
6.1 Key messages 288
6.2 Abstract 289
6.3 Introduction 290
6.4 Methods 292
6.5 Results 296
6.6 Discussion 300
6.7 Conclusion 308
6.8 Chapter Supplementary 317
Chapter 7 Risk prediction of incident heart failure and atrial fibrillation: The Role of blood lipids 339
7.1 Key messages 339
7.2 Abstract 340
7.3 Introduction 341
7.4 Methodology 342
7.5 Results 348
7.6 Discussion 351
7.7 Conclusion 355
7.8 Chapter Supplementary 361
Chapter 8 Conclusions 396
Bibliography 403
Supplementary Appendices 433
Systematic review and meta-analysis (Chapter 2, 4-6) 433
Data sources used in this thesis 441
Codes for HF and AF identified from cases in CALIBER (Chapter 3-7) 445

Codes for subtypes of HF and AF identified from cases in CALIBER (Chapter 3-7) 448

Statistical analysis and multiple imputations (Chapter 4-6) 450

Mediation analysis 454

Static VS Dynamic population design 458

The effect of averaging LDL-C over a year and medication effect on the observational findings 460

The potential effect of collider bias on the observational findings 461

Mendelian randomisation 462

List of Tables

Table 1-1 Clinical recommendations on the prevention of HF in healthy or high-risk populations 43

Table 1-2 Reported risk factors associated with HF and AF 44

Table 2-1 Qualitative summary of cohort studies (n=21) on the association between blood lipids and incident HF and AF 60

Table 2-2 Characteristics of included cohort studies (21 studies) 61

Table 2-3 Summary of cohort, trials, and genetic evidence on the association between blood lipids and incident HF and AF 67

Table 3-1 Characteristics of CALIBER platform 140

Table 3-2 Summary of data sources used to define CALIBER EHR phenotypes in my PhD thesis 142

Table 3-3 Codelists used to define each cardiovascular endpoint and data sources 143

Table 3-4 Codelists used to define blood lipids from primary care data 147

Table 3-5 Validation process of EHR phenotype for MI, HF, and AF 149

Table 3-6 Validity of EHR phenotype for LDL-C 151

Table 3-7 Hazard ratio (HR) point estimates and 95% confidence intervals for selected risk factors and incident MI using the cohort created from CALIBER 152

Table 4-1 Observational cohort: participant characteristics of population-based EHR cohort (n=1,142,656) 187

Table 5-1 Observational cohort: participant characteristics of the population-based EHR cohort (n= 1,338,276) 242

Table 6-1 Observational cohort: participant characteristics of the population-based EHR cohort (n= 1,262,280) 309

Table 7-1 Characteristics of patients at baseline LDL-C measurement 356

Table 7-2 Beta-coefficients and baseline survival at 10 year for the calculation of 10-year risk of HF, AF, and MI from the development cohort of each lipid fraction 357

Table 7-3 Numbers of events, discrimination, and calibration statistics of 10-year risk of HF, AF, and MI in the development and validation cohorts..... 358

Table 7-4 Summary of predictive values of adding blood lipids in the risk prediction of HF, AF, and MI 359

Table 7-5 Comparison of 10-year risk of HF, AF, and MI (n = 446,613)..... 360

Table 8-1 Summary of the contribution of this PhD in comparison to previously published evidence 402

List of Figures

Figure 1-1 Conceptual framework guiding this PhD.....45

Figure 2-1 Meta-analysis of longitudinal cohort studies on the association between blood lipids and incident HF (7 studies) and AF (11 studies).....63

Figure 2-2 Meta-analysis of RCTs on the association between lipid-lowering agents and the risk of HF stratified by class of agents (38 trials).....64

Figure 2-3 Meta-analysis of RCTs on the association between lipid-lowering agents and the risk of AF stratified by class of agents (27 trials).....65

Figure 2-4 Meta-analysis of RCTs on the association between lipid-lowering agents and the risk of MI stratified by class of agents (57 trials). 66

Figure 3-1 Pie charts of 12 CVDs from the total of 3,340,437 patients: First event cases (n=283,953, Top), any event cases (n= 491,480, Bottom) 148

Figure 3-2 Incidence rate of HF (73,795 cases), AF (114,394 cases), and MI (50,691 cases) per 1,000 person-years according to age groups (n=3,340,437) 150

Figure 3-3 Validity of EHR phenotype for HDL-C and TG.....151

Figure 4-1 Observational cohort: The associations of different levels of LDL-C and incident HF (total 6,472,147.27 person-years), AF (total 6,393,476 person-years), and MI (total 6,475,969.07 person-years) among 1,142,656 individuals without diagnosed CVD at baseline over the median follow-up of 5 years (interquartile range: 2-8 years).....189

Figure 4-2 Subgroup analyses for associations between LDL-C and incident HF and AF.....190

Figure 4-3 Competing risk adjustment and subtype analysis.....191

Figure 4-4 RCTs of lipid-lowering agents: Univariable meta-regression of the risk of HF, AF, and MI per 1 mmol/L reduction in LDL-C. 192

Figure 4-5 Genetic (MR) study of associations between 1 SD decrease in LDL-C and the risk of HF, AF, MI, and CHD.....194

Figure 4-6 Comparison of the estimated associations between LDL-C and risk of HF, AF, and MI (or CAD) from cohort (hazard ratio), RCTs (risk ratio), and MR studies (odds ratio). 195

Figure 5-1 Observational cohort: The associations of different levels of HDL-C and incident HF (total 7,607,199 person-years), AF (total 7,513,759 person-years), and MI (total 7,609,784 person-years) among 1,338,276 individuals without diagnosed CVD at baseline over the median follow-up period of 5 years (interquartile range: 2 to 9 years). 244

Figure 5-2 Competing risk adjustment and subtype analysis..... 245

Figure 5-3 RCTs of lipid-lowering agents: Univariable meta-regression of the risk of HF (33 trials: 8,679 events), AF (24 trials: 6,567 events), and MI (52 trials: 27,336 events). 246

Figure 5-4 Genetic (MR) study of associations between genetically determined 1 SD (0.41 mmol/L or 15.85 mg/dL) increased HDL-C and the risk of HF, AF, MI, and CAD..... 248

Figure 5-5 Comparison of the estimated associations between HDL-C and risk of HF, AF, and MI (or CAD) from cohort (hazard ratio), RCTs (risk ratio), and MR study (odds ratio). 250

Figure 6-1 Observational cohort: The association of different levels of TG and incident HF (total 7,543,757 person-years), AF (total 7,449,706 person-years), and MI (total 7,547,422 person-years) among 1,262,280 individuals without diagnosed CVD at baseline over the median follow-up of 5 years (interquartile range: 2-9 years). 311

Figure 6-2 Competing risk adjustment and subtype analysis..... 312

Figure 6-3 RCTs of lipid-lowering agents: Univariable meta-regression of the risk of HF (32 trials: 8,230 events), AF (22 trials: 6,016 events), and MI (49 trials: 26,276 events) per absolute reduction in TG (mmol/L) from control group. 313

Figure 6-4 Genetic (MR) study of associations between genetically determined 1 SD (1 mmol/l) lower TG and the risk of HF, AF, MI (or CAD) 315

Figure 6-5 Comparison of the estimated associations between TG and the risk of HF, AF, and MI (or CAD) from the cohort (hazard ratio), RCTs (risk ratio), and MR study (odds ratio).....316

Figure 8-1 Summary of observational findings of lipids strata with the risk of HF, AF, and MI.....401

List of Supplementary Tables

Table S 2-1 Checklist of items according to PRISMA recommendation 69

Table S 2-2 Terms used and search strategies 71

Table S 2-3 Search filter terms for RCTs and cohort studies..... 72

Table S 2-4 Search results..... 74

Table S 2-5 Characteristics of the included cohort studies (n=9) on incident HF 77

Table S 2-6 Characteristics of the included cohort studies (n=12) on incident AF..... 79

Table S 2-7 Adjusting factors of the individual cohort study (n=21) on HF and AF outcome..... 82

Table S 2-8 Results of individual cohort studies on HF endpoint (n=9)..... 84

Table S 2-9 Results of individual cohort studies on AF endpoint (n=12) 86

Table S 2-10 Summary of RCTs (42 trials) of lipid-lowering agents on HF and AF endpoints..... 88

Table S 2-11 Summary of RCTs (57 trials) of lipid-lowering agents on MI endpoint 91

Table S 2-12 Summary of the quality assessment of included cohort studies (21 studies) based on Newcastle-Ottawa criteria..... 96

Table S 2-13 Sensitivity analysis of meta-analysis of cohort studies on HF (7 studies) and AF (11 studies) outcomes..... 101

Table S 2-14 Summary of genetic studies on the association between lipid traits and the risk of MI and AF 115

Table S 2-15 Summary of GRADE quality of evidence from the meta-analysis of RCTs..... 117

Table S 3-1 Metadata of outcome phenotype..... 156

Table S 3-2 Numbers of new cases with 12 CVDs identified from a cohort of 3,340,437 participants who were free of CVDs at baseline and were followed-

up from 1st Jan 1997 to 30th Jun 2016 with a median follow-up time of 7 years (interquartile range: 3-13 years) 157

Table S 3-3 Incidence rate per 1,000 person-years of 12 CVDs 158

Table S 3-4 Hazard ratio point estimates and 95% confidence interval for selected risk factors and incident AF among 3 cohort studies 160

Table S 3-5 Example of clinical characteristics of populations aged 45-50 years with and without LDL-C measurement at baseline (n=1,496,635) 164

Table S 3-6 Missingness profile of each cohort 165

Table S 4-1 The STROBE and RECORD statement 197

Table S 4-2 Full participant characteristics of the population-based EHR cohorts (n=1,142,656) 202

Table S 4-3 Testing for horizontal pleiotropy 216

Table S 4-4 Power calculation of MR at two-sided alpha of 0.05 216

Table S 4-5 Correlation between instrumental variable effect and exposure effect 216

Table S 4-6 Features comparison of the evidence 217

Table S 5-1 STROBE and RECORD checklist 252

Table S 5-2 Incidence rate of HF and AF by HDL-C levels 259

Table S 5-3 Full participant characteristics of the population-based EHR cohorts (n= 1,338,276) 260

Table S 5-4 Gender-specific characteristics of participants 263

Table S 5-5 Subgroup analysis of categorical HDL-C levels and incident HF in selected characteristics among males 273

Table S 5-6 Subgroup analysis of categorical HDL-C levels and incident HF in selected characteristics among females 274

Table S 5-7 Testing for horizontal pleiotropy 287

Table S 5-8 Power calculation of MR at two-sided alpha of 0.05 287

Table S 5-9 Correlation between instrumental variable effect and exposure effect 287

Table S 6-1 The STROBE and RECORD checklist 318

Table S 6-2 Incidence rate of HF and AF by TG levels..... 325

Table S 6-3 Full participant characteristics of CALIBER population-based EHR cohort (n= 1,262,280) according to TG values at baseline 326

Table S 6-4 Serial adjusted hazard ratio (HR) of the association between TG and incident MI..... 329

Table S 6-5 Testing for horizontal pleiotropy 337

Table S 6-6 Power calculation of MR at two-sided alpha of 0.05..... 337

Table S 6-7 Correlation between instrumental variable effect and exposure effect 337

Table S 7-1 Summary of evidence on the risk prediction score for incident HF, AF, and MI..... 365

Table S 7-2 Characteristics of patients at baseline HDL-C measurement.. 369

Table S 7-3 Characteristics of patients at baseline TG measurement..... 370

Table S 7-4 Incidence rate per 1,000 person-years of HF, AF, and MI according to geographical split cohort..... 371

Table S 7-5 Comparisons of point estimation (hazard ratio) and performance of risk predictive models for MI, HF, and AF with and without LDL-C variable (n=478,703)..... 375

Table S 7-6 Performance statistic between the model with and without LDL-C (reference model) stratified by genders 377

Table S 7-7 Comparisons of point estimation (hazard ratio) and performance of the risk predictive models for MI, HF, and AF with and without HDL-C variable (n=446,613) 379

Table S 7-8 Performance statistic between the model with and without HDL-C (reference model) stratified by genders 381

Table S 7-9 Comparisons of point estimation (hazard ratio) and performance of the risk predictive models for MI, HF, and AF with and without TG variable (n=442,530).....383

Table S 7-10 Performance statistic between the model with and without TG (reference model) stratified by genders 385

List of Supplementary Figures

Figure S 2-1 Study flow diagram of selected cohort studies. 75

Figure S 2-2 Study flow diagram of selected RCTs 75

Figure S 2-3 Study flow diagram of selected genetic studies 76

Figure S 2-4 Risk of bias graph from 38 included RCTs 97

Figure S 2-5 Summary of the risk of bias from 38 included RCTs 98

Figure S 2-6 Meta-analysis of the included cohort studies (n=7) on the association between blood lipids and incident HF 99

Figure S 2-7 Meta-analysis of the included cohort studies (n=11) on the association between blood lipids and incident AF 100

Figure S 2-8 Funnel plots and results from Trim & Fill method in cohort studies of (A) LDL-C (4 studies), (B) HDL-C (6 studies), and (C) TG (4 studies) on HF outcome 102

Figure S 2-9 Funnel plots and results from Trim & Fill method in cohort studies of (A) LDL-C (10 studies), (B) HDL-C (6 studies), and (C) TG (6 studies) on AF outcome 103

Figure S 2-10 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by groups of medications 104

Figure S 2-11 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by types of prevention ... 105

Figure S 2-12 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by types of comparison . 106

Figure S 2-13 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by sizes of trials 107

Figure S 2-14 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by groups of medications 108

Figure S 2-15 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by types of prevention ... 109

Figure S 2-16 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by types of comparison .. 110

Figure S 2-17 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by sizes of trials 111

Figure S 2-18 Meta-analysis of RCTs (57 trials) on the association between lipid-lowering agents and the risk of MI stratified by classes of medications 112

Figure S 2-19 Funnel plots of RCTs on lipid-lowering agents and the risk of HF (38 trials) 113

Figure S 2-20 Funnel plots of RCTs on lipid-lowering agents and the risk of AF (27 trials) 113

Figure S 2-21 Funnel plots of RCTs on lipid-lowering agents and the risk of MI (57 trials) 114

Figure S 3-1 Example of prospective nature of linked data from multiple sources in CALIBER 154

Figure S 3-2 Phenotyping algorithm for HF 154

Figure S 3-3 Phenotyping algorithm for AF 155

Figure S 3-4 Probability of all-cause deaths in 90 days of HF patients 159

Figure S 3-5 Hazard ratio estimates and 95% confidence intervals for selected risk factors and incident AF across EHR sources 160

Figure S 3-6 The risk of ischaemic stroke according to CHA_2DS_2-VASc score based on AF cases identified from AF phenotyping algorithm 161

Figure S 3-7 Example of the interface of CALIBER platform 162

Figure S 3-8 Example of CALIBER phenotyping tools for HF outcome 162

Figure S 3-9 Example of CALIBER EHR phenotyping codes for HF outcome 163

Figure S 3-10 Validation results of HF outcome 163

Figure S 4-1 Venn diagram for incident MI, HF, and AF in 1,142,656 CALIBER cohorts 207

Figure S 4-2 Cumulative incidence of HF and AF by baseline LDL-cholesterol 208

Figure S 4-3 Effect size per LDL-C exposure time in each study design 209

Figure S 4-4 Study flow diagram 210

Figure S 4-5 Schoenfeld residual and proportional hazard plots of HF outcome 211

Figure S 4-6 Schoenfeld residual and proportional hazard plots of AF outcome 212

Figure S 4-7 Subgroup analysis of meta-regression 213

Figure S 4-8 Funnel plots of RCTs on HF (top), AF (middle), and MI (bottom) outcomes 214

Figure S 4-9 Scatter plots of genotype-LDL associations versus genotype-outcome associations 215

Figure S 5-1 Study flow diagram 256

Figure S 5-2 Venn diagrams to illustrate the linkage process of HF (top) AF (middle) and MI (bottom) 257

Figure S 5-3 A Venn diagram for incident MI, HF, and AF in 1,338,276 CALIBER cohorts 258

Figure S 5-4 Gender-specific associations by different levels of HDL-C 266

Figure S 5-5 Subgroup analyses of the associations between HDL-C and incident HF and AF (no gender-specific) 267

Figure S 5-6 Subgroup analyses of the gender-specific associations between HDL-C and incident HF 269

Figure S 5-7 Subgroup analyses of the gender-specific associations between HDL-C and incident AF 271

Figure S 5-8 Schoenfeld residual and proportional hazard plots on HF outcome in males 275

Figure S 5-9 Schoenfeld residual and proportional hazard plots on HF outcome in females 276

Figure S 5-10 Schoenfeld residual and proportional hazard plots on AF outcome in males 277

Figure S 5-11 Schoenfeld residual and proportional hazard plots on AF outcome in females 278

Figure S 5-12 The association between HDL-C levels and all-cause mortality in CALIBER (n=1,338,276) 279

Figure S 5-13 Sensitivity and subgroup analysis of meta-regression of the change in HDL-C levels and the risk of HF and AF 280

Figure S 5-14 Subgroup analysis of meta-regression between change in HDL-C and the risk of MI 281

Figure S 5-15 Funnel plot of RCTs on HF (top), AF (middle), and MI (bottom) outcomes 282

Figure S 5-16 Meta-analysis of trials on HDL-C raising drugs (7 trials) on the risk of HF 283

Figure S 5-17 Meta-analysis of trials on HDL-C raising drugs (5 trials) on the risk of AF 284

Figure S 5-18 Meta-analysis of trials on HDL-C raising drugs (13 trials) on the risk of MI 285

Figure S 5-19 Scatter plots of genotype-HDL-C associations versus genotype-outcome associations 286

Figure S 6-1 Study flow diagram 322

Figure S 6-2 Venn diagram to illustrate linkage process of HF (top) AF (middle) and MI (bottom) 323

Figure S 6-3 Venn diagram for incident MI, HF, and AF in 1,262,280 CALIBER cohorts 324

Figure S 6-4 Subgroup analyses of associations between TG and incident HF, AF, and MI 330

Figure S 6-5 Schoenfeld residual and proportional hazard plots on HF outcome 331

Figure S 6-6 Schoenfeld residual and proportional hazard plots on AF outcome 332

Figure S 6-7 Sensitivity and subgroup analysis of meta-regression of the change in TG levels and the risk of HF (top) and AF (bottom)..... 333

Figure S 6-8 Sensitivity and subgroup analysis of meta-regression of the change in TG levels and the risk of MI..... 334

Figure S 6-9 Funnel plot of RCTs of HF (top), AF (middle), and MI (bottom) outcomes..... 335

Figure S 6-10 Scatter plots of genotype-TG associations versus genotype-outcome associations..... 336

Figure S 7-1 Geographical validation: model discrimination by risk groups and calibration plots (validation LDL-C cohort, n = 103,464)..... 372

Figure S 7-2 Geographical validation: model discrimination by risk groups and calibration plots (validation HDL-C cohort, n = 95,920) 373

Figure S 7-3 Geographical validation: model discrimination by risk groups and calibration plots (validation TG cohort, n = 95,941) 374

Figure S 7-4 Calibration by deciles of MI risk function by genders and by with or without LDL-C in a model..... 387

Figure S 7-5 Calibration by deciles of HF risk function by genders and by with or without LDL-C in a model..... 388

Figure S 7-6 Calibration by deciles of AF risk function by genders and by with or without LDL-C in a model..... 389

Figure S 7-7 Calibration by deciles of MI risk function by genders and by with or without HDL-C in a model..... 390

Figure S 7-8 Calibration by deciles of HF risk function by genders and by with or without HDL-C in a model..... 391

Figure S 7-9 Calibration by deciles of AF risk function by genders and by with or without HDL-C in a model..... 392

Figure S 7-10 Calibration by deciles of MI risk function by genders and by with or without TG in a model 393

Figure S 7-11 Calibration by deciles of HF risk function by genders and by with or without TG in a model 394

Figure S 7-12 Calibration by deciles of AF risk function by genders and by with or without TG in a model 395

List of Abbreviations

AF	Atrial Fibrillation
CAD	Coronary Artery Disease
CALIBER	ClinicAl research using LInked Bespoke studies and Electronic health Records
CHD	Coronary Heart Disease
CPRD	Clinical Practice Research Datalink
CVD	Cardiovascular Disease
EHR	Electronic Health Record
GP	General Practice
HDL-C	High-density Lipoprotein Cholesterol
HES	Hospital Episode Statistics
HF	Heart Failure
LDL-C	Low-density Lipoprotein Cholesterol
MI	Myocardial Infarction
MR	Mendelian Randomisation
ONS	Office for National Statistics
OR	Odds Ratio
RCT	Randomised Controlled Trial
RR	Relative Risk
TG	Triglyceride
HR	Hazards Ratio

CHAPTER 1 INTRODUCTION

1.1 Abstract

Heart failure (HF) and atrial fibrillation (AF) are two of the most common cardiovascular diseases with an upward trend in their incidence and prevalence worldwide. Moreover, there are no curative strategies for HF and AF, and therefore primary preventive approaches are required. The lowering of lipids has played an important role in the primary prevention of myocardial infarction and other cardiovascular diseases.

The aim of this chapter is to give an introduction to my PhD research. First, I will briefly overview the definitions of HF and AF and how they are classified. Second, I will describe the current epidemiological burden to demonstrate the importance of these two diseases. Third, current clinical guidelines will be reviewed and recommendations about primary prevention of HF and AF will be compared across the guidelines. Fourth, I will justify the reason why blood lipids are an interesting risk factor for HF and AF that needs examining and why both diseases should be co-investigated. Fifth, the objective of my PhD study along with my research questions that I aim to answer will be elaborated to establish clinical implications of my findings. Lastly, a conceptual framework of my PhD is illustrated at the end of this chapter.

1.2 Introduction

1.2.1 Definition and classification

Heart failure (HF)

According to the European Society of Cardiology, Heart failure (HF) is “a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/ or elevated intracardiac pressures at rest or during stress.”[1]

In other words, rather than being a disease, HF is a group of clinical syndromes caused by heart muscle that pumps insufficient blood into the systemic circulation, leading to signs and symptoms of insufficient blood flow (e.g., fatigue, exercise intolerance) and fluid retention (e.g., peripheral edema, dyspnoea) due to the increased pressure on the heart.

There are three most common classifications of HF used in clinical guidelines, which grade HF based on left ventricular ejection fraction (LVEF), functional classification, and the degree of structural heart change and severity of HF symptoms. The European Society of Cardiology (ESC) groups HF patients into three categories according to their left ventricular ejection fraction (LVEF). These include 1) heart failure with preserved ejection fraction (HFpEF: LVEF ≥ 50%), 2) heart failure with reduced ejection fraction (HFrEF: LVEF < 40%), and 3) heart failure with mid-range ejection fraction (HFmEF: LVEF 40-49%).[1]

The classification of HF based on LVEF is clinically important as it can inform different aetiological mechanisms, comorbidities, and patients' responses to treatments.[2] In addition, HF can also be classified based on functional classification (New York Heart Association [NYHA]), or the degree of structural heart change and severity of HF symptoms (ACCF/AHA stage of HF).[3]

Atrial fibrillation (AF)

AF is the most common heart rhythm disorder. According to the American Heart Association (AHA)/ American College of Cardiology (ACC)/ the Heart Rhythm Society (HRS), AF can be defined as "a supraventricular tachyarrhythmia with uncoordinated atrial activation and consequently ineffective atrial contraction. Characteristics on an electrocardiogram (ECG) include 1) irregular R-R intervals (when atrioventricular [AV] conduction is present), 2) absence of distinct repeating P waves, and 3) irregular atrial activity."[4] In other words, AF is a heart condition where the heart beats irregularly, mostly abnormally fast. This condition can be asymptomatic, but sometimes it can cause tiredness, dizziness, shortness of breath, or ischaemic stroke, the last of which is the major complication of AF.

As with HF, there are also several classifications of AF (i.e., based on AF pattern, AF type, or AF symptom burden). However, this is beyond the scope of this PhD. Details of AF classification can be found elsewhere.[5]

1.2.2 Clinical signs and symptoms and diagnostic criteria of HF and AF

Heart failure (ESC guideline 2016)[1]

Non-acute setting

Symptoms of HF are usually non-specific and do not help discriminate between HF and other diseases. Moreover, signs and symptoms of HF may be difficult to identify and interpret in obese individuals, in the elderly, or in patients with chronic respiratory diseases. Typical symptoms of HF include breathlessness, orthopnoea, paroxysmal nocturnal dyspnoea, reduced exercise tolerance, fatigue, tiredness, increased time to recover after exercise, and ankle swelling. Typical signs of HF include elevated jugular venous pressure, third heart sound (gallop rhythm), and laterally displaced apical impulse.

For the diagnostic of HF, echocardiography is the most useful to establish the diagnosis. According to ESC 2016, patients who had either clinical history (symptoms) or physical examination (signs) suggesting HF should be further testing for natriuretic peptides, if the levels are less than the cut-off threshold (i.e., B-type natriuretic peptide [BNP] < 35 pg/mL or N-terminal pro-B type natriuretic peptide [NT-proBNP] < 125 pg/mL), then HF is unlikely and should consider other diagnosis. In case of abnormal levels or natriuretic peptides testing is not available, echocardiography should be used to confirm HF. If echocardiogram shows normal ventricular and atrial volume and function, then HF is unlikely.

Acute setting

Acute heart failure (AHF) refers to rapid onset or worsening of symptoms and/or signs of HF explained above. AHF is a life-threatening medical condition requiring urgent evaluation and treatment. ESC recommends the test of plasma natriuretic peptides levels in all patients with acute dyspnoea and suspected AHF. The levels less than threshold (i.e., BNP < 100 pg/mL, NT-proBNP < 300 pg/mL, or mid-regional pro A-type natriuretic peptide [MR-

proANP] < 120 pg/mL) should be used to differentiate dyspnoea from non-cardiac causes (recommendation class I-A). Please note that the threshold cut-off for HF diagnosis in acute setting is higher than non-acute setting. Other recommended diagnostic tests are 12-lead ECG, chest-X-ray, cardiac troponins, blood urea nitrogen (BUN), serum creatinine, electrolytes (sodium, potassium), glucose, complete blood count, liver function tests and thyroid stimulating hormone (TSH). Furthermore, echocardiography is also recommended immediately in haemodynamically unstable AHF patients and within 48 hours afterwards.

Atrial fibrillation (ESC guideline 2016)[5]

The definite diagnosis of AF requires rhythm documentation using an electrocardiogram (ECG) showing the typical pattern of AF: Absolutely irregular RR intervals and no discernible, distinct P waves, and an episode lasting for at least 30 second is diagnostic. Since AF is commonly asymptomatic (silent AF), an opportunistic screening for AF is recommended by pulse taking (followed by ECG in those with an irregular pulse) or ECG rhythm strip in patients aged 65 years or above (recommendation class I-B).

1.2.3 Healthcare and economic burden

While the incidence of myocardial infarction (MI) has declined over decades, the number of new cases of heart failure (HF) and atrial fibrillation (AF) has increased year by year and this leads HF and AF to become two globally epidemic cardiovascular diseases.[6] Both HF and AF often share some common risk factors and pathological mechanisms[7,8] and have a poor prognosis, leading to high morbidity and mortality.[9] Due to therapeutic challenges, primary prevention seems to be a promising way to decelerate the epidemic trend of HF and AF.

Heart failure

HF currently affects 23 millions of populations worldwide.[10] In the UK, it has been estimated that the prevalence and incidence of HF increased by approximately 23% and 7%, respectively, from 2002 to 2014.[11] In the US, there were 5.1 million prevalent HF cases in 2006,[3] and the number of new cases has been projected to reach 772,000 in 2040.[12] This global upward trend in

both prevalence and incidence of HF partly reflects an increase in ageing population together with improvement on treatment of hypertension, coronary heart disease, and valvular heart disease, which can effectively prolong patients' survival (but later these patients will end up with HF).

The mortality rate in patients with HF has seen an upward trend. For example, in the US, the mortality rate due to HF rose from 5.8 per 1,000 in 1970 to 16.4 per 1,000 in 1993.[13] Moreover, the 5-year mortality from HF increases by about 50%, which is even worse than that from many cancers,[14] and HF accounts for 7% of all cardiovascular deaths.[3] Furthermore, it has been shown that the 30-day mortality of HF is 10-12% with a 30-day readmission rate of up to one-fourth.[3]

In 2013, HF cost more than $30 billions in the US,[15] and the average cost per HF hospitalisation per patient was $23,077 with an even higher re-admission cost .[3] Therefore, HF still presents a significant health and economic burden.

Atrial fibrillation

AF affected 33.5 million individuals worldwide in 2010 with evidence further suggesting an increase in both prevalence and incidence, especially in ageing society.[16] It is the most common cardiac disease affecting around 1% of the population in the US[17] and its prevalence has been predicted to double to 7.56 million by 2050.[18] A study in England and Wales has shown that the age-standardised prevalence of AF from 1994 to 1998 increased by 22% (male) and 14% (female).[19] The figure has shown an upward trend in the incidence of AF with advancing age. The incidence rate of AF from the Rotterdam study, for instance, is found to have profoundly increased from 1.1 per 1,000 person-years in a population aged 50-59 years to 20.7 per 1,000 person-years in individuals aged 80-84 years.[20]

AF has a high morbidity and mortality rate as a result of frequent hospitalisation and thromboembolic events. This is because AF can increase the risk of ischaemic stroke, HF, dementia, and death around five-fold,[21] three-fold,[22] two-fold,[23] and two-fold,[22] respectively.

In terms of economic burden, treating AF is expensive. In the US, from 2004 to 2006, for example, AF treatment cost around $25 billions per year with a yearly average cost per patient of $8,700.[24]

1.2.4 Current recommendations for the primary prevention

Heart failure

In healthy populations, there are no guideline recommendations for the primary prevention of HF in regard to lipid lowering or any other interventions. Existing recommendations for the primary prevention of HF were made based on high-risk populations (i.e., patients with hypertension, diabetes, metabolic syndrome, or atherosclerotic disease) from which treatment of hypertension and obesity are the most consistent primary preventive recommendations across all of the guidelines (Table 1-1). In addition, there is no current recommendation on using any risk predictive tool to predict the risk of new-onset HF despite that fact that many risk scoring systems have been developed and validated for this purpose (see Chapter 7).

Regarding the relation of blood lipids to the risk of HF, a recommendation has been made based on the effect of reducing low-density lipoprotein cholesterol (LDL-C) on the prevention of coronary heart disease (CHD),[3,25] which is the leading cause of HF, although it does not draw a direct link between blood lipids and HF. In contrast to blood pressure whose value is current recommended to be kept below 130/80 mmHg,[26] no recommendation on targeting blood lipids in order to primarily prevent HF has been made. Although there are several observational studies on the area of blood lipids as the risk of HF, results are ambivalent and inconclusive (see Chapter 2).

Atrial fibrillation

As in HF, there are no guideline recommendations for the primary prevention of AF in healthy population in regard to lipid lowering or any other interventions. Existing recommendations for the primary prevention of AF are scarce and aim at high-risk populations (i.e., people with obesity, hypertension, diabetes, and obstructive sleep apnoea) rather than healthy individuals.[27] There are no specific recommendations for the primary prevention of AF according to the most recently updated guidelines from Europe,[5] America,[4,28] and Canada.[29,30]

However, there is one guideline mentioned blood lipids as a risk factor for AF - It has suggested that high triglyceride (TG) and low HDL-C levels might be associated with an increased risk of AF, while association between LDL-C and the risk of AF was not mentioned.[27] Nonetheless, the evidence for the association of high TG and low HDL-C levels to an increased risk of AF is weak because it was based on only two prospective observational studies.[31,32]

In addition, there is no current suggestion on the use of risk predictive tool for incident AF although many tools have been developed to predict the risk of new-onset AF (see Chapter 7).

Alternatively, there exists upstream therapy, which refers to the use of traditionally non-antiarrhythmic agents that can target the upstream pathological pathways for developing AF (e.g., renin-angiotensin-aldosterone pathway, or oxidative stress and inflammatory pathway) in order to prevent the new occurrence of AF (i.e., primary prevention) or prevent the recurrence of AF (i.e., secondary prevention).[27]

Examples of upstream therapy drugs for AF reported so far include Angiotensin Converting Enzyme Inhibitors (ACEIs), Angiotensin Receptor Blockers (ARBs), aldosterone antagonists, statins, and 3-n polyunsaturated fatty acids (PUFAs). Preliminary results suggested more promising evidence for the primary prevention than the secondary prevention.[33] Nevertheless, most evidence was derived from studies in animal and retrospective analyses. Thus, we cannot prove or disprove the effectiveness of the treatment until results from ongoing prospective randomised controlled trials are revealed. It is worth noting that the search for new indications for old drugs used for the primary prevention of HF raises the possibility that the existing HF drugs might potentially have a role in the prevention of AF as well. This mirrors the intercorrelation between HF and AF, and the shared pathophysiological mechanisms of both diseases.

1.3 Why blood lipids may be causally relevant in HF and AF ?

The following is the rationale behind the examination of the effect of blood lipids on the risk of HF and AF.

There are putative causal pathways between lipids and HF and AF. Blood lipids might be part of some pathological pathways that cause HF and AF. For example, LDL-C and TG might be involved in oxidative stress and an inflammatory pathway,[34] whereas HDL-C is known to have an anti-inflammatory, anti-oxidative, and anti-thrombotic effect.[35]

Figure 1-1 depicted the conceptual framework guiding my analysis on the potential causal relevance of lipids and HF and AF in my PhD. To start with, the causal association between LDL-C and MI is firmly established,[36] and myocardial infarction (MI) is a well-known major cause of both heart failure (HF)[1,3,37] and atrial fibrillation (AF).[4,5,27] For HDL-C, results from clinical trials and Mendelian randomisation suggested no causal relationship between HDL-C and MI.[36] However, the pleiotropic effect of HDL-C (i.e., anti-inflammatory, antioxidative stress) may get involved in the pathophysiological pathway of occurring HF and AF,[33,38,39] and HDL-C may, therefore, potentially be associated with the developing of both diseases. Regarding TG, results from genetic studies indicated its causal relevance to MI.[36] Accordingly, the association between TG and the development of HF and AF, if any, might be mediated by MI.

Other risk factors, such as smoking, body mass index (BMI), hypertension, or diabetes can be related with both lipids (i.e., LDL-C, HDL-C, and TG)[36] and diseases (i.e., MI, HF, and AF),[1,3–5,27,37] and these factors should be taken into account when examining the association between blood lipids and the risk of HF and AF. Apart from examining the association between blood lipids and the risk of HF and AF, it is interesting to further investigate whether the observed association, if any, is independent of MI.

In addition, there are important reasons why adding evidence on causal relevance of lipids and HF and AF is relevant.

1. Blood lipids are targetable and modifiable. Nowadays, we have various groups of lipid-lowering drugs that can modify lipid fractions effectively and some even more specifically. For instance, statins (monotherapy or in combination with ezetimibe and PCSK9 inhibitors) are often prescribed to decrease LDL-C. CETP inhibitors can effectively and specifically double HDL-C levels. In addition, the most recent EU-

approved drug, Volanesorsen (Waylivra®), which inhibits ApoCIII mRNA, can specifically reduce fasting TG levels by 70-75%.[40] Therefore, it is easy for clinicians to implement if any association, or causation, is firmly established. Moreover, the findings might provide off-label indications for some drugs that failed to show the benefit of CHD risk reduction, such as niacins or CETP inhibitors.

2. Measurement of blood lipids is cheap and easy. Moreover, it is available in almost all general practices and hospitals. Therefore, if there is a suggestion that blood lipids should be monitored as a predictor of disease, it is easy to adopt and implement.
3. Measurement of blood lipids is part of a routine clinical check-up, which is performed even in healthy individuals (e.g., as an annual health check-up). This can ensure the adequacy of sample sizes and can minimise selection bias due to selected populations (i.e., if the data is derived from specific subgroups of populations, the findings cannot be generalised to general populations).

1.4 Why study HF and AF?

HF and AF are usually considered separately in clinical practice. By contrast the approach of this PhD is to study both diseases simultaneously. Apart from the fact that HF and AF have become more prevalent and a big burden on a global scale, HF and AF often co-exist (in up to 30 % of patients) and the combination of both diseases leads to a poor prognosis.[41] Both of them share many features, the following are three features that HF and AF share.

1. Both cardiac diseases share common risk factors and they can predispose one another.

HF and AF commonly share the same risk factors (Table 1-2), for example, older age, hypertension, diabetes, and atherosclerosis (especially MI).[42] From an epidemiological point of view, when the association between each disease is examined, it allows us to adjust (or to stratify) the same set of covariates (risk factors) to one another. Also, since both diseases are risk factors for each other, we need to take into account the effect of the intercurrence of each disease. For instance, in the model investigating the association between

exposure and the risk of incident HF, we need to adjust or stratify for the intercurrent AF (i.e., new-onset AF that occurred during the period between exposure and incident HF). In other words, when we want to examine the true association of exposure with HF, we need to minimise the confounding effect due to AF and vice versa.

2. There is a pathophysiological interrelationship between HF and AF.

Both cardiac diseases are associated with pathophysiological conditions that each contribute to the initiation and maintenance of the other. AF precipitates left ventricular dysfunction, which further aggravates HF, via (1) the loss of atrial contraction, (2) the precipitating irregular ventricular rhythm and, (3) rapid ventricular rates. On the other hand, HF results in atrial remodelling due to (1) increased filling pressures, (2) alterations in calcium handling, which leads to increased automaticity together with increased fibrosis and (3) alterations to the electrical properties of the atrial tissue, which lead to the occurrence of AF.[8] Apart from the renin-angiotensin-aldosterone system (RAAS), oxidative stress, and inflammation are among some of the pathological pathways that HF and AF have in common.[1,5]

3. Drugs that are used to treat HF might also impact AF

As mentioned in the previous section, ACEIs have an indication in hypertensive patients at high-risk of HF.[1,3,37] According to the upstream therapy for AF, drugs that alter hemodynamics, fibrosis, and cellular remodeling, which are the upstream pathological pathway of the disease might modify both HF and AF.[33] This is mirrored by positive results from studies suggesting that treatment with statins (in postoperative cardiac surgery)[43] and ACEIs might be beneficial to the prevention of AF.[33] Furthermore, from a pathophysiological stand point, reducing congestion with diuretics in HF patients might additionally be advantageous to the prevention of atrial remodeling which, consequently, delays the onset of AF.[42]

Figure 1-1 shows a simple diagram of the associations between blood lipids and HF and AF. I postulated that MI has a role as the intermediate (or mediator) of the association between LDL-C and TG (not HDL-C) and HF and AF. This is because cumulative evidence has supported the causal

relationship between LDL-C and TG and MI,[36] and MI is a leading cause of HF and AF (see Table 1-2). Therefore, both HF and AF share the same mediator and should be investigated simultaneously.

Since there are many potential confounding factors (i.e., factors that are associated with both blood lipids and HF and AF but are not lining in the biological pathway), using genetic instrument as a proxy of lipids can avoid bias due to these confounders. This is because genetic makeup is mimic a randomisation process according to the Mendel's second laws (i.e., genes for different traits assort independently of one another in the formation of gametes – the law of independent assortment). Therefore, all confounders should be distributed equally amongst groups of exposure. Also, genetic has been determined since conception. Reverse causation is less likely to occur if genetic instrument is used instead of an observed variable.[44]

In order to investigate mediating effect of MI, several methods can be used. The simplest one is the four-step approach suggested by Baron and Kenny.[45] In this approach, four regression analyses are conducted and significance of the coefficients is examined at each step. To investigate the mediating effect of MI on the association between LDL-C and HF, for example, there are four steps as follows: 1) Conducting a regression analysis with LDL-C predicting HF, 2) conducting a regression analysis with LDL-C predicting intercurrent MI, 3) conducting a regression analysis with intercurrent MI predicting HF, and 4) conducting a regression analysis with LDL-C predicting HF controlling for intercurrent MI (a direct effect). The purpose of Steps 1-3 is to establish that zero-order relationships among the variables exist. If one or more of these relationships are nonsignificant, we can conclude that mediation is unlikely. In the Step 4, some forms of mediation can be supported providing that the effect of LDL-C remains significant after controlling for intercurrent MI. If LDL-C is no longer significant when MI is controlled, the finding supports full mediation, whereas if LDL-C is still significant, the finding supports partial mediation.

The method above, however, does not calculate an indirect effect and might miss the true mediating effect due to type II errors.[45] An alternative method is to calculate the indirect effect and test it for significance. There are two methods to achieve this.[46] 1) A method suggested by Judd and Kenny (also

called the difference method) in which the difference between two regression coefficients -- one from the coefficient of exposure controlling for the mediator and another from the coefficient of exposure without controlling form the mediator -- are computed, and 2) a Sobel product of coefficients approach (also called the product method) in which the coefficient of mediator is multiplied by the coefficient of exposure (without controlling for the mediator). Then significant testing can be performed by either bootstrap (nonparametric resampling) or the Monte Carlo method (parametric resampling).

All above approaches are based on single-mediator model. To investigate multiple mediators in more complex model, the structural equation modelling (SEM) can be used.[47] This method can calculate mediating effect in a single analysis instead of testing separate regression analyses. However, since testing the mediating effect is beyond the scope of my thesis (see overall objective in the following section), I will use only the simplest method suggested by Baron and Kenny (Supplementary Appendices).

Apart from testing mediating effect, it is also worth examining the role of MI as an effect modifier (also known as interaction). To achieve this, I can do subgroup analysis with and without intercurrent MI (i.e., MI that occurs after baseline lipid measurement but before incident HF or AF) and compare coefficients of exposure between models using heterogeneity test.

In addition, to test a mediating effect in MR study, there is a method called multi-trait-based conditional and join analysis (mtCOJO), which is useful to estimate the effect of risk factor on disease conditioning on other risk factors. This method requires summary-level data (with LD between genetic variant from a reference sample with individual-level data). Details of mtCOJO and its applications are beyond the scope of this thesis but have been comprehensively described by Zhu *et al*.[48]

1.5 PhD Objectives

1.5.1 Overall objective

The main objective of this PhD research is to examine the associations, the extent of these associations, and the causation between the levels of blood

lipids, including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) and the risk of heart failure (HF) and atrial fibrillation (AF). In achieving this objective, nine clinically relevant questions are expected to be answered too.

1.5.2 Specific objectives

Chapter 1: Introduction

- To introduce the reader to this PhD study and justify why the topic is interesting and what would be the clinical implications of this work.

Chapter 2: Systematic review and meta-analysis

- To synthesise and summarise existing literature as well as to identify and explore the research gap in the area of blood lipids as a risk factor for HF and AF.

Chapter 3: EHR Cohort profile

- To illustrate the data curation process for electronic health records (EHR) from the CALIBER platform.
- To demonstrate the validity of cohorts, covariates, and endpoints used throughout Chapter 4 to 7

Chapter 4: LDL-C and incident HF and AF

- To evaluate the causal relevance of LDL-C to the risk of HF and AF

Chapter 5: HDL-C and incident HF and AF

- To evaluate the causal relevance of HDL-C to the risk of HF and AF

Chapter 6: TG and incident HF and AF

- To evaluate the causal relevance of TG to the risk of HF and AF

Chapter 7: Role of blood lipids in the risk prediction of HF and AF

- To develop and validate the risk prediction model for incident HF and AF
- To assess the role of lipids in the risk prediction of incident HF and AF
- To examine whether people at specific risk of MI will be at the same risk of HF and AF.

Chapter 8: Conclusions

- To summarise the answers to the clinical research questions (see below).

1.6 Clinical questions

Upon completing this PhD, the following set of clinical questions is due to be answered.

1. How commonly are HF and AF firstly diagnosed in comparison to other cardiovascular diseases?
2. Is there clear evidence of any specific lipid-lowering agent being effective in the risk reduction of HF and AF?
3. Is there clear evidence that lipid-modulating strategy is effective in the primary prevention of HF and AF?
4. Are blood lipid levels associated with the incidence of HF and AF?
5. Are blood lipid levels associated with the incidence of both diseases, when studied at lower and higher levels than in previous observational studies or clinical trials?
6. Are blood lipids causally relevant to the risk of HF and AF?
7. Are our findings of MI outcomes, used as a positive control, consistent with those from previous studies?
8. Do the patients who are at low risk of atherosclerotic disease, such as MI, also have a low risk of HF and AF?
9. Do blood lipids add value to the risk prediction of HF and AF?

Table 1-1 Clinical recommendations on the prevention of HF in healthy or high-risk populations

Recommendations	ESC 2016[1] (updated 2019)[49]	AHA 2016[25]	ACCF/AHA 2013[3] (updated 2017)[26]	NHFA CSANZ 2018[37]
For healthy populations (not at high risk)				
Recommendations for risk factor management	○	○	○	○
Assessment of risk of HF with validated risk prediction tools	○	○	○	○
For patients at high risk or ACCF/AHA stage A and B or NYHA class I*				
Smoking cessation	●	○	●	●
Alcohol intake reduction	●	○	●	●
Exercise or regular physical activity	○	●	○	●
Treating obesity	●	●	●	●
Treating hypertension	●	●	●	●
Treating dysglycaemia	●	●	●	○
Treating lipid disorders	○	●	●	○

Note: ● = recommended, ○ = no recommendation *ACCF/AHA Stage of HF: Stage A = At high risk [i.e., people with hypertension, diabetes, atherosclerotic disease, obesity, metabolic syndrome, or people who are using cardiotoxins or have a family history of cardiomyopathy] of HF but without structural heart disease or symptoms of HF, Stage B = Structural heart disease but without signs or symptoms of HF, NYHA functional classification: I = No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF

Abbreviations: ACCF; American College of Cardiology Foundation, ACEIs; Angiotensin-converting enzyme inhibitor, AHA; American Heart Association, ARBs; Angiotensin receptor blockers, ASCVD; Atherosclerotic cardiovascular disease, BBs; Beta-blockers, CAD; Coronary artery disease, CCBs; Calcium channel blockers, ESC; European Society of Cardiology, HF; Heart failure, HTN; Hypertension, LV; Left ventricular, LVEF; Left ventricular ejection fraction, NHFA CSANZ**;** National Heart Foundation of Australia and Cardiac Society of Australia and New Zealand, SGLT2; Sodium-glucose cotransporter 2, T2DM; Type 2 diabetes mellitus

Table 1-2 Reported risk factors associated with HF and AF

Risk factors	Heart failure	Atrial fibrillation
	Published studies	Published studies
Gender	● [50–52]	● [53]
Older age	● [50–52]	● [53]
Smoking	● [50–52]	● [4,5,27,29,30]
Alcohol	● [50–52]	● [4,5,27,29,30]
Obesity	● [50–52]	● [4,5,27,29,30]
Increased C-reactive protein	● [54]	● [4,55]
Hyperthyroidism	● [56]	● [4,5,27,29,30]
Diabetes	● [50–52]	● [4,5,27,29,30]
Hypertension	● [50–52]	● [4,5,27,29,30]
Myocardial infarction	● [50–52]	● [4,5,27,29,30]
Atrial fibrillation	● [50–52]	Not applicable
Heart failure	Not applicable	● [4,5,27,29,30]

Note: ● = reported, ○ = not reported

This informs approach to covariate adjustment in this PhD.

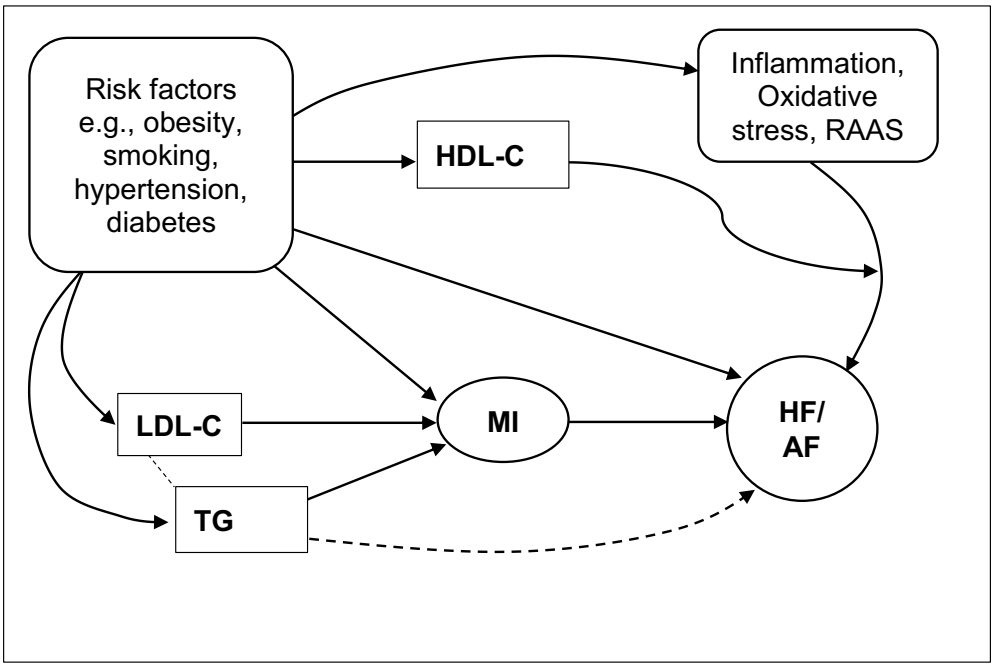


Figure 1-1 Conceptual framework guiding this PhD

Abbreviations: AF; Atrial fibrillation, HDL-C; High-density lipoprotein cholesterol, HF; Heart failure, LDL-C; Low-density lipoprotein cholesterol, MI; Myocardial infarction, RAAS; Renin-angiotensin aldosterone system, TG; Triglyceride

CHAPTER 2 BLOOD LIPIDS, HEART FAILURE AND ATRIAL FIBRILLATION: A SYSTEMATIC REVIEW AND META-ANALYSIS OF COHORT, TRIALS, AND GENETIC STUDIES

2.1 Abstract

Background: Routine measurement of blood lipids and available interventions to target blood lipids might provide a primary prevention strategy for heart failure (HF) and atrial fibrillation (AF). The principal aim of this chapter is to synthesis findings from existing research on the association between blood lipids and the risk of HF and AF, and to identify a research gap in the previous studies.

Methods: Three online databases including MEDLINE, EMBASE, and Cochrane CENTRAL as well as a reference list of relevant studies were sifted through with the last search run on 1 July 2019. We searched for longitudinal cohort, randomised controlled trials (RCT), and Mendelian randomisation (MR). We further meta-analysed cohorts and trials to obtain a hazard ratio (HR) per 1 standard deviation (SD) increase in blood lipids (cohort) and relative risk (RR), comparing active groups with control groups (RCT). We systematically reviewed but did not perform a meta-analysis of genetic studies (MR).

Results: We included 21 cohort studies (490,942 participants: 11,689 and 7,198 of new HF and AF cases, respectively), 42 RCTs (326,112 participants: 8,903 HF and 6,680 AF cases), and 11 MR studies in the systematic review. A meta-analysis of 18 cohort studies showed no association between LDL-C and HF but a weakly inverse one between LDL-C and AF (HR 0.94 [95%CI 0.90-0.98]). We observed an inverse association with both HF (HR 0.84 [0.81-0.88]) and AF (HR 0.96 [0.94-0.99]). Additionally, we detected positive associations between TG and HF (HR 1.19 [1.16-1.22]), whereas none was shown between TG and AF. Moreover, only the association between HDL-C and HF was robust across sensitivity analyses. Following the meta-analysis of

38 RCTs, lipid-regulating agents, especially statins, are found to have been significantly related to a lower risk of HF (RR 0.93 [0.90-0.97]), but not to that of AF (RR 1.00 [0.95, 1.05]). Of the RCTs surveyed, no apparent evidence of publication bias was detected in both outcomes.

Conclusions: Increased HDL-C levels might be associated with a decreased risk of HF in community-dwelling populations, and the use of lipid-regulating agents might be linked to a lower risk of HF. We discovered the following three main gaps: i) to date, no large observational studies have investigated all three lipid fractions in both diseases in the same cohort; ii) no association between change in blood lipids (not drugs) and the risk of HF and AF has been found in the RCTs; and iii) there is a lack of MR research on HF while only one MR study on AF has been published so far.

2.2 Introduction

Heart failure (HF) and atrial fibrillation (AF) have become the major cardiac problems in developed countries. In the UK, for instance, the incidence of MI declined by 33% between 2002 and 2014.[11] Although, during the similar period, the incidence of HF modestly dropped by 7% (adjusted incidence ratio: 0.93, 95%CI 0.91 to 0.99), the incidence among the population aged > 85 years is found to have increased, and a 12% increase in the total number of new cases in the overall population was estimated.[11] The age-adjusted incidence rate per 1,000 person-years for AF increased from 1.11 (95%CI 1.09 to 1.13) in 1998 to 2001 to 1.33 (95%CI 1.31 to 1.35) in 2007-2010. Meanwhile, its prevalence, which had been estimated to be 14.5 cases per 1,000 people in 2010, was projected to be 25.4 cases per 1,000 by 2016.[57]

Primary preventive strategies for HF and AF are currently lacking,[4,58,59] and modifiable risk factors for their primary prevention need to be further identified. These two diseases often share risk factors (e.g., hypertension, smoking, obesity, diabetes, and coronary artery disease) and pathophysiological mechanisms.[7,8] Therefore, it is likely to identify a risk factor that can affect both diseases, and blood lipids are one of the interesting targets for three reasons. Firstly, they are routinely measured in clinical practice. Next, they are targetable by available lipid-lowering agents. For instance, statins (in

monotherapy or in combination with ezetimibe and PCSK9 inhibitors) can be used to decrease LDL-C, while CETP inhibitors can effectively double HDL-C levels. In addition, the most recent EU-approved drug, Volanesorsen (Waylivra®), which inhibits ApoCIII mRNA, can reduce fasting TG levels by 70-75%.[40] Lastly, the causal relevance of LDL-C to MI, which is the major cause of HF and AF, has been firmly established. Therefore, LDL-C and, perhaps, other lipid traits might also be related to HF and AF.[60–62]

In this systematic review and meta-analysis, we aim to synthesise existing literature on the association between blood lipids and the risk of HF and AF. Our specific objectives are as follows:

1) to investigate whether blood lipids (i.e., LDL-C, HDL-C, and TG) are associated with the risk of HF and AF in the community-dwelling people without prior HF and AF.

2) to examine whether and the extent to which lipid-regulating agents affect the risk of HF and AF. In the meantime, we also aim to identify a specific subgroup that might benefit from lipid-regulating agents, in relation to the risk of HF and AF.

3) to explore the genetic evidence for the association between blood lipids and the risk of HF and AF. We chose myocardial infarction (MI) as a positive control so that we can ensure the reproducibility of our findings. Our report has followed the recommendations made by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (see chapter supplementary Table S 2-1).[63,64]

2.3 Methodology

As a part of my PhD, all works in this chapter (i.e., literature searching, data extraction, quality assessment, and data analysis) were done solely by myself under the supervision of my primary and subsidiary supervisor. However, I am aware of the good practice in conducting a systematical review and meta-analysis in which at least two researchers are needed to work independently.

2.3.1 Study eligibility criteria and search strategy

In selecting cohort studies, we searched for longitudinal studies on the association between blood lipids (i.e., LDL-C, HDL-C, and TG) and the incidence of HF and AF among community-dwelling populations who had prior cardiovascular disease (CVD) at enrolment. As to RCTs, at first, we adopted the same eligibility criteria as those used in the Cholesterol Treatment Trialists' (CTT) Collaboration in which only clinical trials with at least 1,000 participants who received a minimum follow-up of one year have been selected. Later, we also included smaller trials (N<1,000) that reported HF or AF outcomes in order to examine the publication bias as funnel plots, and we found that the plots had become more symmetrical (Figure S 2-19 to S 2-21). The search was extensively performed by implementing both medical subject headings (MeSH or thesaurus search) and text-word searches on the three main databases, including Medline (Ovid: 1946 to present), Embase (Ovid: 1974 to present), and Cochrane CENTRAL in the English language with the last search conducted on 1 July 2019. For comparison purposes, we also extracted myocardial infarction (MI) outcomes from RCTs included. We further retrieved additional trials of MI from CTT[65,66] and previous studies.[60,67] Details of the systematic search for genetic studies were provided in the supplementary appendix (e-Method)

2.3.2 Study selection and quality assessment

We used Newcastle-Ottawa Scale (NOS) to assess the quality of included cohort studies, whereas the Cochrane risk of bias tool was employed in included RCTs.

2.3.3 Data synthesis and statistical analysis

The hazard ratio (HR) of incident HF and AF per standard deviation (SD) change in lipid fractions was the primary measure of the exposure effect in longitudinal cohort studies, whereas the relative risk (RR) of HF and AF in an active arm, compared with controlled individuals, was our measurement in RCTs. Regarding MI outcomes, when overall ratios of fatal to nonfatal cases were not reported, the outcomes with higher reported events, which most constituted nonfatal cases, were used in the analysis.

With regard to cohort studies, we initially performed a qualitative review to compare the trend of the associations between blood lipids and incident HF and AF from each included study. In the meta-analysis, we pooled the results based mainly on the fixed-effects model using the inverse variance method. However, if significantly statistical heterogeneity was observed and the source of heterogeneity was unknown, a random-effect model would be used for the pooled estimation.

To uncover publication bias, we created funnel plots with additional Egger's statistics if there were at least ten studies included in the meta-analysis. If potential publication bias was suspected, we further applied Duval and Tweedie's trim and fill method. All analyses in this study have been done using STATA version 15 (IC version, StataCorp). Additional visualisation (i.e., risk of bias and GRADE score) has been done using Review Manager version 5.3 and GRADEpro GDT (accessed online at: https://gradepro.org). A detailed explanation of the methodology employed in this study is provided in e-Method (supplementary appendix).

2.4 Results

2.4.1 Included studies

A total of 21 cohort studies, 38 RCTs, and 11 Mendelian randomisation studies (MR) were included in this review. According to the study flow diagrams (Figure S 2-1 and Figure S 2-2), searching through Medline, Embase, Cochrane Central, and additional reference sources followed by the exclusion of duplications yielded a total of 1,645 and 1,859 articles on cohort studies and RCTs, respectively, which were subsequently screened based on their titles and abstracts. Later, we narrowed the preliminary list of search results down to 63 eligible cohort studies and 54 eligible RCTs whose full-texts were then examined. Of the initially eligible cohort studies, further 42 studies have been excluded due to: i) the absence of reported blood lipids, HF, or AF (11 studies), ii) the presence of prior CVD at baseline (8 studies), iii) the absence of longitudinal design (5 studies), iv) their status as sub-studies (8 studies), and v) their irrelevance to our research questions (10 studies). From the total of 54 RCTs, we further excluded 16 full-texts, since 13 of which did not report HF or

AF outcomes, while 2 of which did not measure blood lipids, and one RCT failed to meet the required minimum follow- up of one year for this study.

2.4.2 Study characteristics

Of 21 cohort studies (involving a total of 490,942 participants) included in this systematic review, ten (11,689 HF cases) reported HF endpoints, while 12 studies (7,198 AF cases) reported AF endpoints (one of them reported both outcomes). Almost all of the included cohort studies have an average follow-up period of over five years, with the exception of three studies.[68–70] Moreover, all of the included studies featured community-dwelling people who had had no prior history of HF or AF at baseline of blood sampling and were well representative of middle-age to elderly people (i.e., mean age > 50), except two studies whose participants were relatively young (i.e., mean age < 40).[71,72] Furthermore, most studies also excluded participants with a history of CVD, even though the prevalence of CVD in those studies was not high (i.e., < 5%).[32,73–75] Table 2-2 illustrates main characteristics of the 21 included cohort studies.

Interestingly, while LDL-C levels were mostly calculated with the Friedewald equation, only two studies stated clearly that they were measured directly.[73,76] Regarding endpoint ascertainment, we found that most of the studies used information from linkage records confirmed by specialists. However, two studies obtained their outcomes from patient self-reporting with a small subset of cases validated.[76,77] Table S 2-5 and Table S 2-6 (see the chapter supplementary) describe characteristics of the included cohort studies. The details of controlling factors in each study are described in Table S 2-7.

Of the 42 RCTs included in this systematic review and meta-analysis, 38 and 27 studies reported HF and AF outcomes, respectively (23 studies reported both outcomes). Among a total of 326,112 participants who were included in the 42 RCTs, there were 8,903 HF cases and 6,680 AF cases. Of the 42 RCTs, one was conducted with the participation of a healthy population, seven enrolled a population with an intermediate to high CV risk, 24 involved an established CVD population, and 10 admitted a mixed population (i.e., those with or without established CVD at baseline). In addition, the included trials cover a wide range of lipid-regulating agents, including statins (n=26), fibrates

(n=4), CETP inhibitors (n=5), PCSK-9 inhibitors (n=4), miscellaneous agents (i.e., omega-3 fatty acid [n=2], and bempedoic acid [n=1]). Table S 2-10 and Table S 2-11 (chapter supplementary) describes characteristics of the included RCTs.

2.4.3 Quality assessment

According to the NOS for cohort studies, the median score of the 21 included studies was 6 with a range between 3 and 9. Moreover, 8 out of the 21 articles (38%) were categorised into the good quality group (i.e., the total score of ≥ 7 with no individual component score of zero). For each subcomponent of the NOS, we noticed that most of the included studies did not report the adequacy of follow-up (or response rate). There were only four cohorts that reported the adequacy of follow-up (i.e., at least 80% of response rate).[32,71,72,78] The representativeness of cohorts (sum score of 7 out of 21), and ascertainment of exposure (sum score of 7 out of 21) were the other two main problems of the included cohorts. The quality assessment of included cohort studies is laid out in detail in Table S 2-12.

Of the included RCTs, we noticed that eight (19%) had a high risk of bias due to incomplete outcome data.[79–86] To ameliorate this problem, most of the studies adopted the intention-to-treat (ITT) approach to the primary analysis. Still, 21% of the included trials had a high risk of other bias, mostly on account of the sources of funding, since the funders of the suspected trials were pharmaceutical companies that were also involved in the data collection and analysis process. Other suspected risk of bias features include incomparable baseline characteristics between groups of samples [79,80,87,88] self-reported outcomes,[89] and the application of inappropriate methods of data analysis and the handling of missingness.[84]

In addition, six trials (14%) had a high risk of bias due to their unblinding of participants or personnel, which might have led to performance bias.[79,80,87,90,91] If patients were aware of the intervention to which they were allocated, for instance, they might have changed their lifestyle, and this could affect their lipid levels at the follow-up visit and consequently might affect the occurrence of HF or AF in the future. A summary of the risk of bias of included trials and

the assessment results for each study can be found in Figure S 2-4 and Figure S 2-5 in the chapter supplementary.

2.4.4 Summary of findings

Evidence from longitudinal observational studies

As shown in Figure 2-1, the associations between blood lipids and incident HF and AF are found to have been inconsistent and inconclusive. Admittedly, there is only one study investigating the association of LDL-C and HDL-C (but not TG) with both HF and AF outcomes.[74] It should be noted that, to date, no studies enlisting large cohorts (e.g., larger than 1 million) to investigate the association of all lipid fractions (i.e., LDL-C, HDL-C, and TG) with the incidence of both HF and AF simultaneously have been conducted.

As illustrated by the forest plot in Figure 2-1, LDL-C was not associated with the risk of HF (HR 1.07 [95%CI 0.91-1.26]) but was inversely associated with the risk of AF (HR 0.94 [95%CI 0.90-0.98]). However, the association of LDL-C with incident AF was inconsistent and became null in all sensitivity analyses (Table S 2-13, supplementary appendix). Meanwhile, the pooled results showed a paradoxical link between HDL-C and the risk of both HF and AF, corresponding HR of 0.84 (95%CI 0.81-0.88) and 0.96 (95%CI 0.94-0.99), respectively. Interestingly, the association of HDL-C with the incidence of HF was robust across all sensitivity analyses, whereas its association with AF was attenuated toward the null. One SD increase in TG was associated with a 19% increase in the risk of HF (HR 1.19 [95%CI 1.16-1.22]) and this direct association was consistent after applying a random-effect model and excluding studies that categorised TG levels. However, further excluding studies that reported different units and measurement effects resulted in a non-significant association between TG and HF. We observed no association between TG and the risk of AF.

As can be seen in Figure 2-1 and Table S 2-13 in the chapter supplementary, most of our meta-analysis results from the included cohort studies showed a high degree of statistical heterogeneity (i.e., $I^2 > 75\%$ or p-value < 0.1), with the exception of the pooled results of HDL-C and HF and AF whose heterogeneity was relatively low (i.e., $I^2 < 50\%$). As far as publication bias (i.e.,

funnel plot and Egger's p-value [see Figure S 2-7 and Figure S 2-8 in supplementary chapter]) is concerned, we found no apparent evidence of publication bias (i.e. symmetrical shape of funnel plot and corresponding Egger's P-value > 0.05 if more than 10 studies were included). Further, the use of Trim and Fill method to adjust for potential publication bias did not change our conclusion.

Evidence from randomised controlled trials (RCTs)

As shown in Figure 2-2 and Figure 2-3, it is found that the use of a lipid-regulating agent, compared with placebo or usual care, might have been associated with the lower risk of HF (RR 0.93 [95%CI 0.90 to 0.97]). However, we found no link between the use of a lipid-regulating agent and the risk of AF (RR 1.00 [95%CI 0.95 to 1.05]). A further subgroup analysis has revealed that the lower risk of HF was observed in some groups of medications (i.e., statins and fibrates), as well as in secondary prevention and a mixed population. Further addition of small studies (N<1,000) did not alter our conclusion (see Figure S 2-9 to Figure S 2-17). Moreover, no significant heterogeneity was detected among the included trials (i.e., I^2 < 25%), and no difference was found between subgroups (P-value for heterogeneity > 0.05). Further investigation into potential publication bias by the visualisation of funnel plots (Figure S 2-19 to Figure S 2-21) did not reveal any apparent asymmetric shape and P-value from the Egger's test > 0.05. Publication bias was, therefore, unlikely to be a major concern. In addition, a summary of the GRADE quality of evidence is provided in Table S 2-15.

For comparison purposes, the meta-analysis of 57 trials showed that lipid-lowering drugs can decrease the risk of MI by 18% (95%CI 15%, 22%). This beneficial effect was separately found in statins, fibrates, PCSK-9 inhibitors, and other groups, but not in niacin and CETP inhibitors (Figure 2-4). However, we discovered a high degree of heterogeneity (I^2 = 64.1%, P-value < 0.001) and potential publication bias (Egger's P-value = 0.013, Figure S 2-21), which might collectively weaken the reliability of our findings.

We provide a summary of characteristics of the included genetic studies in Table S 2-14. Among all of the MR studies, we found only one on blood lipids

and AF (AFGen consortium),[92] whereas no genetic evidence for blood lipids and HF outcomes has been reported thus far.

Putting all evidence together

A summary of evidence is provided in Table 2-3. As robustly substantiated by the cohort studies[93,94] and meta-regression of trials for lipid-lowering drugs[60,67], LDL-C is found to have been directly associated with the risk of MI and the genetic studies further supported the causal relation between the two. (Table S 2-14). However, the observational research findings from the HF and AF outcomes were ambivalent and inconclusive. To date, there has been no existing research linking LDL-C to the risk of HF, while only one genetic study showed no causal relevance of LDL-C to the risk of AF.[92]

Based on the cohort studies, HDL-C is found to have had a strongly inverse association with the risk of MI[95], whereas its relation to the risk of HF and AF was less clear. However, as evidenced by the meta-regression of 51 trials for lipid-lowering agents, no association between HDL-C and the risk of CHD was found.[96] In contrast to the direct association between LDL-C and the risk of MI pointed out above, evidence linking HDL-C to the risk of MI in the genetic studies was ambivalent. Although most of the genetic studies showed a lack of association between a genetically determined HDL-C change and the risk of MI (or CHD), [97] two of them indicated otherwise (Table S 2-14). In addition, one genetic study suggested no causal association between HDL-C and the risk of AF.[92] Notably, none of the previous studies drew a direct link between change in HDL-C levels and the risk of HF and AF.

With regard to the associations between TG and the risk of HF, AF, and MI, again, the evidence was less clear. Based on the observational studies, a positive relation between TG levels and MI outcomes was found, which gradually became attenuated towards the null upon multivariable risk adjustment.[95] Furthermore, most of the genetic studies collectively suggested that TG might be causally related to the risk of MI (or CHD),[98] despite that one of the genetic studies did not show any association between the two (Table S 2-14).

2.5 Discussion

2.5.1 Summary of evidence and comparison with previous literature

In this systematic review and meta-analysis, we included 21 longitudinal studies involving 490,942 individuals (11,698 HF cases and 7,198 AF cases), 38 RCTs with 510,043 participants (8,737 HF cases and 6,591 AF cases), and 11 MR studies. To date, we have found no observational study that investigated all three lipid fractions together (i.e., LDL-C, HDL-C, and TG) simultaneously in both HF and AF outcomes. Additionally, we are the first to conduct a meta-analysis of major RCTs that examines the effects of various classes of lipid-regulating agents (not just statins) on the risk of both diseases. We found a robust inverse association between HDL-C and the incidence of HF. The opposite, but less robust, direction of the association was also found between TG and the risk of AF. The inverse associations between LDL-C and HDL-C and incident AF were attenuated towards the null in sensitivity analyses. No other associations have been found between lipid fractions and the incidence of both diseases.

In the meta-analysis of RCTs, the use of lipid-regulating agents, especially statins and mixed populations were associated with a lower risk of HF. No association has been found between the use of lipid-regulating agents and the risk of AF. Our findings are consistent with the previous meta-analysis of the relation between statins and HF which found that statins might modestly reduce the risk of non-fatal HF hospitalisation by around 10% (RR 0.90, 95%CI 0.84-0.97)[66], whereas no benefit of statins has been found for AF.[65] Additionally, no apparent evidence of publication bias has been spotted across all of the findings, except one of the MI outcomes. However, pooled results suggesting relations between LDL-C and HF and AF showed a significantly high degree of heterogeneity. We observed that the exclusion of studies with different reported effect sizes, units, and categories of lipid fractions minimised heterogeneity in the overall association with LDL-C, suggesting these were the potential source of heterogeneity in our study.

Regarding MI outcomes, our meta-analysis of RCTs (Figure 2-4) was partially consistent with the previous network meta-analysis of 67 non-statin trials,

which showed that a combined use of statins and PCSK-9 inhibitors can decrease the risk of non-fatal MI by 18% (95%CI 7%, 28%), whereas a combined use of statins and niacin or CETP inhibitors neither affected either CHD death nor non-fatal MI.[99] The discrepancy might be a result of our inclusion criteria according to which only trials involving sample sizes larger than 1,000 were included, while sample sizes were not taken into account in previous studies. The asymmetrical shape of our funnel plot thus suggested further trials should be added. However, limiting a trial size is less likely to affect our findings of HF and AF outcomes since we also found that most small trials tend not to present these outcomes in their reports.

Genetic findings for LDL-C and MI were the most consistent, whereas caution is needed when interpreting results from genetic studies of other lipid traits. For instance, we found mixed results (mostly null) of genetic studies on HDL-C and the risk of MI. We noticed that one of the studies focusing on a CETP locus showed an inverse association with the risk of MI.[100] However, a recent genetic study suggested that this association might be mediated through a non HDL-C pathway.[101] Furthermore, it is questionable whether the genetic association between TG and MI is due to pleiotropy, because genes affecting TG levels also regulate other lipid traits, such as LDL-C, HDL-C, and Lipoprotein(a) that might play a causal role in the incidence of MI.[102] Moreover, in the most recent genetic study, LPL (representing TG) and LDLR (representing LDL-C) genetic scores produced the similar magnitude of the association per unit change in apolipoprotein B (ApoB)-containing particles with the risk of CHD. The magnitude of the association in turn suggested that the causal association found in the genetic study of TG might be related to ApoB particles, rather than TG content itself (both TG and LDL-C are carried through blood circulation by ApoB particles).[103]

2.5.2 Strengths

To date, the current study provides the first systematic review and meta-analysis that comprehensively investigated the association of three blood lipids (i.e., LDL-C, HDL-C, and TG) with the risk of the two most common, but less studied, cardiac diseases (i.e., HF and AF) using MI as a control. Our study drew on two types of study designs ranked first and second in the

hierarchy of quality evidence: randomised controlled trials and prospective observational studies. Furthermore, we applied different sensitivity methods to ensure the robustness and validity of our findings from both cohort studies and RCTs. In addition, we also included a systematic review of human genetic studies to provide more insight into our research question - whether blood lipids are causally related to the risk of HF and AF.

2.5.3 Limitations

Study and outcome level limitations

First, although we applied a random-effect model to partially account for between-study variability and yield more conservative results (i.e., less precise estimation), this method did not neutralise the issue of heterogeneity. After several sensitivity analyses were performed, only a few results lessened the degree of heterogeneity, which implies that the inconsistent reporting of the results accounts for the small degree of between-study heterogeneity. A potential source of heterogeneity might come differences in participants' characteristics (e.g. CVD vs CVD-free at baseline), or differences in the method for ascertaining lipid levels in different studies (e.g. calculation vs direct measurement). It is shown that the Friedewald equation was prone to underestimate LDL-C levels if TG ≥ 150 mg/dL (≥ 1.7 mmol/L), which caused increased inaccuracy (can be up to 59% underestimation) as TG levels increased.[104] This might lead to misclassification bias. The results of meta-analysis with significant heterogeneity, therefore, must be interpreted and generalised with cautions. Second, despite the fact that our results did not show apparent evidence of publication bias, a small number of included studies (i.e., n<10) might have underpowered statistics. However, the comprehensive and systematic search strategy employed should lessen the likelihood of publication bias to occur in our findings.

Review level limitations

Our search was restricted to only publications in the English language released between 2000 and 2019. However, searching through more than one major database with additional screening of a reference list of included articles for relevant studies might partially offset this limitation. Furthermore, since most

RCTs did not prespecify HF and AF as their primary outcomes of interest, we directly contacted the corresponding authors of each trial to enquire the exact numbers of HF and AF cases in their trials. However, out of 45 researchers contacted, only 16 (36%) responded to our enquiries and only one offered us a positive response (response (i.e., numbers of prevalent and incident cases of HF and AF were given).[105] As a result, we might have underestimated the number of HF and AF cases reported in the included trials, and we cannot differentiate incidents from prevalent cases.

2.6 Conclusions

Evidence gathered from a summary of longitudinal studies suggests a negative association between HDL-C and incident HF. However, other associations were weak, with a significantly high degree of heterogeneity. Additionally, a relatively small sample size might underpower estimated effect sizes in many cohort studies. We discovered three major gaps in this area of research: i) a lack of observational studies that investigate all three lipid fractions in both diseases simultaneously, ii) the absence of direct association between blood lipids and the risk of HF and AF drawn from RCTs, and iii) a lack of MR studies on blood lipids and HF outcomes, and the availability of only one studies on AF outcomes. Therefore, more research is required in order to provide further insight into the relationship between blood lipids and the risk of HF and AF. Future observational studies need a huge sample size to overcome the limitations of the existing findings. And RCTs that directly investigation the role of lipid levels *per se* would also be a most welcome addition.

Table 2-1 Qualitative summary of cohort studies (n=21) on the association between blood lipids and incident HF and AF

Study, published year[Ref]	Total N	Events (%)		LDL-C		HDL-C		TG	
		HF	AF	HF	AF	HF	AF	HF	AF
Wang, 2010[106]	1,032	303 (29%)	NA	NA	NA	↓	NA	↔	NA
Barkas, 2017[107]	1,223	NA	34 (3%)	NA	↓	NA	↓	NA	↔
Ingelsson,2005[108]	2,321	259 (11%)	NA	↔	NA	↓*	NA	↔	NA
B-D, 2009[72]	2,637	26 (1%)	NA	↔	NA	↔	NA	NA	NA
Sciacqua, 2015[68]	3,549	NA	546 (15%)	NA	↑	NA	NA	NA	NA
He, 2017[109]	3,557	452 (13%)	NA	↔	NA	↓	NA	NA	NA
Knuiman, 2014[75]	4,267	NA	343 (8%)	NA	↔	NA	↔	NA	↔
Smith[$], 2010[74]	5,187	112 (2%)	284 (6%)	NA	↔	NA	NA	NA	NA
Ebong, 2013[78]	5,688	152 (3%)	NA	NA	NA	↓	NA	↑	NA
Velagaleti,2009[71]	6,860	680 (10%)	NA	NA	NA	↓	NA	NA	NA
Alonso[$], 2014[31]	7,142	NA	480 (7%)	NA	↔	NA	↓	NA	↑
Kodani, 2019[110]	10,430	NA	133 (1%)	NA	↔	NA	NA	NA	↔
Dhingra, 2008[77]	10,813	222 (2%)	NA	NA	NA	↔[A]	NA	NA	NA
Lopez[$], 2012[32]	13,969	NA	1,433 (10%)	NA	↓	NA	↔	NA	↔
Kim, 2018[111]	21,981	NA	168 (1%)	NA	NA	NA	↔	NA	↔
Mora, 2002[76]	23,738	NA	795 (3%)	NA	↓	NA	↔[B]	NA	↔
Watanabe,2011[69]	28,449	NA	265 (1%)	NA	↓	NA	↓/↔[C]	NA	↔
Mourtzinis[$],2018[70]	51,020	NA	2,389 (5%)	NA	↓	NA	↔	NA	↔
Holme[$], 2009[112]	84,740	5,890 (7%)	NA	↑	NA	↓	NA	↑	NA
Li, 2018[73]	88,785	NA	328 (0.4%)	NA	↓	NA	↔	NA	↔
Varbo[$], 2018[113]	113,554	3,593 (3%)	NA	↔	NA	NA	NA	↑	NA
This PhD	> 1 m	> 25,000	> 46,000	Chapter 4		Chapter 5		Chapter 6	

Note: of studies was on the basis of studies' sample size. [$]Studies that clearly stated the use of linkage electronic health records (EHRs) for outcome ascertainment (n=6), [A] Results from male only, [B] Results from female only, [C] ↔ in male but ↓ in female

Symbol	Interpretation
↓	Inverse (indirect, negative) association (i.e., lower lipids, higher risk of disease)
↔	Null association
↑	Direct (positive) association (i.e., lower lipids, lower risk of disease)
NA	No available results (not conducted)

Abbreviations: AF; Atrial fibrillation, HDL-C; high-density lipoprotein cholesterol, HF; Heart failure, LDL-C; low-density lipoprotein cholesterol, NA; Not available, N; Number, TG; Triglyceride.

Table 2-2 Characteristics of included cohort studies (21 studies)

Study, published year[Ref]	Contemporary		Baseline blood lipids (mmol/L)*			EHR Study?	Adjustment for intercurrent MI or CVD?	Reported HF and AF in the same study?
	Start to end year	Follow-up (year)	LDL-C	HDL-C	TG			
Wang, 2010[106]	1986	20.7	Not report	1.32 ± 0.36	1.75 ± 1.83	○	●	○
Barkas, 2017[107]	1999-2015	6.0	168 (132, 198)	52 (44, 62)	131 (94, 189)	○	○	○
Ingelsson, 2005[108]	1970-2001	28.8	5.3 ± 1.3	1.3 (1.1, 1.6)	1.7 (1.3, 2.2)	○	●	○
B-D, 2009[72]	1985	20.0	109.5 ± 32.2	54.6 ± 13.3	Not report	○	○	○
Sciacqua, 2015[68]	1998-2011	3.4	125.8 ± 34.1	48.6 ± 12.9	Not report	○	○	○
He, 2017[109]	2003-2012	6.3	101.7 (35.3)	47.4 (15.7)	Not report	○	○	○
Knuiman, 2014[75]	1994-2010	15.0	3.6 (1.0)	1.4 (0.4)	1.3 (0.9)	○	○	○
Smith[$], 2010[74]	1991-2007	13.8	4.2 ± 1.0	1.4 ± 0.4	Not report	●	○	●
Ebong, 2013[78]	2000-2012	8.5	Not report	51.2 ± 15.0	109 (81.0)	○	●	○
Velagaleti, 2009[71]	1948-1991	26.0	Not report	52 ± 16	Not report	○	●	○
Alonso[$], 2014[31]	1999-2010	11.9	120 (31)	51 (15)	126 (66)	●	●	○
Kodani, 2019[110]	2008-2015	6.9	126.7 ± 30.1	63.7 ± 16.7	115.8 ± 78.8	○	●	○
Dhingra, 2008[77]	1982-1996	6.0	Not report	10.0 to 141.7	Not report	○	●	○
Lopez[$], 2012[32]	1987-2007	18.7	Not report	Not report	Not report	●	●	○
Kim, 2018[111]	2003-2016	8.7	121.7 ± 29.9	48.0 ± 11.1	124.1 ± 73.2	○	○	○
Mora, 2002[76]	2004	16.4	3.1 (2.6, 3.7)	1.3 (1.1, 1.6)	1.3 (0.9, 1.9)	○	○	○
Watanabe,2011[69]	1996-2005	4.5	128 ± 33	62 ± 16	104 ± 71	○	○	○
Mourtzinis[$],2018[70]	2001-2008	3.5	3.2 ± 0.9	1.4 ± 0.4	1.6 ± 0.9	●	○	○
Holme[$], 2009[112]	1985-2002	11.8	3.8 ± 1.0	1.4 ± 0.4	1.5 ± 0.8	●	●	○
Li, 2018[73]	2006-2015	7.1	90.7 ± 35.4	59.7 ± 15.5	147.8 ± 119.9	○	●	○
Varbo[$], 2018[113]	1991-2014	6.0	3.2 (2.6, 3.8)	1.6 (1.2, 1.9)	1.4 (1.0, 2.0)	●	●	○
This PhD	1997-2016	5.0	3.4 ± 1.0	1.4 ± 0.4	1.3 (0.9, 1.9)	●	●	●

Note: *mean ± SD or median (interquartile range)

Symbol	Interpretation
●	Yes
○	No

Abbreviations: AF; Atrial fibrillation, CVD; Cardiovascular disease, EHR; Electronic health record, HDL-C; High-density lipoprotein cholesterol, HF; Heart failure, LDL-C; Low-density lipoprotein cholesterol, MI; Myocardial infarction, TG; Triglyceride

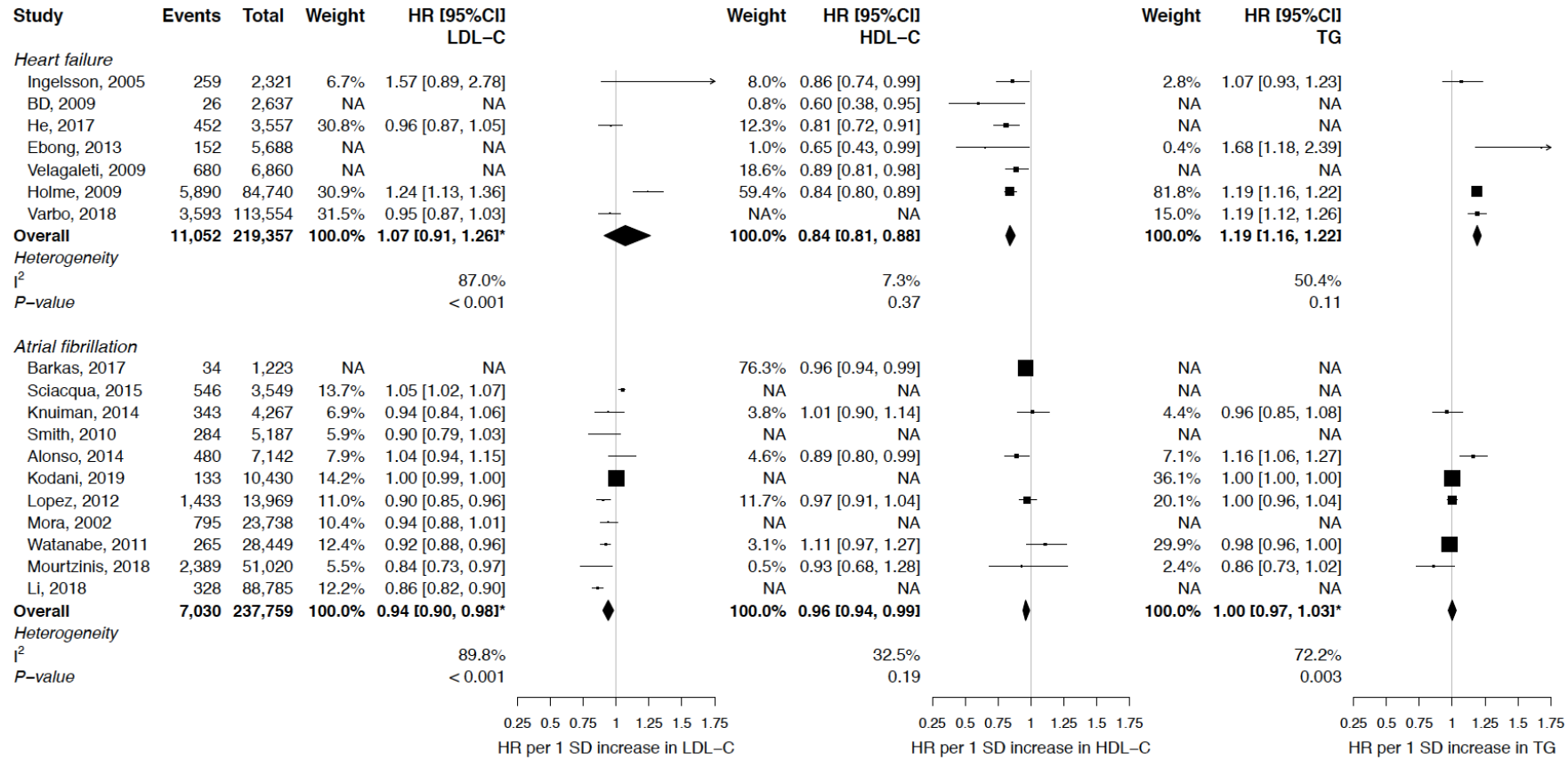


Figure 2-1 Meta-analysis of longitudinal cohort studies on the association between blood lipids and incident HF (7 studies) and AF (11 studies)

Note: Inverse variance weighted method, *Pooled estimate was based on the random-effect model.

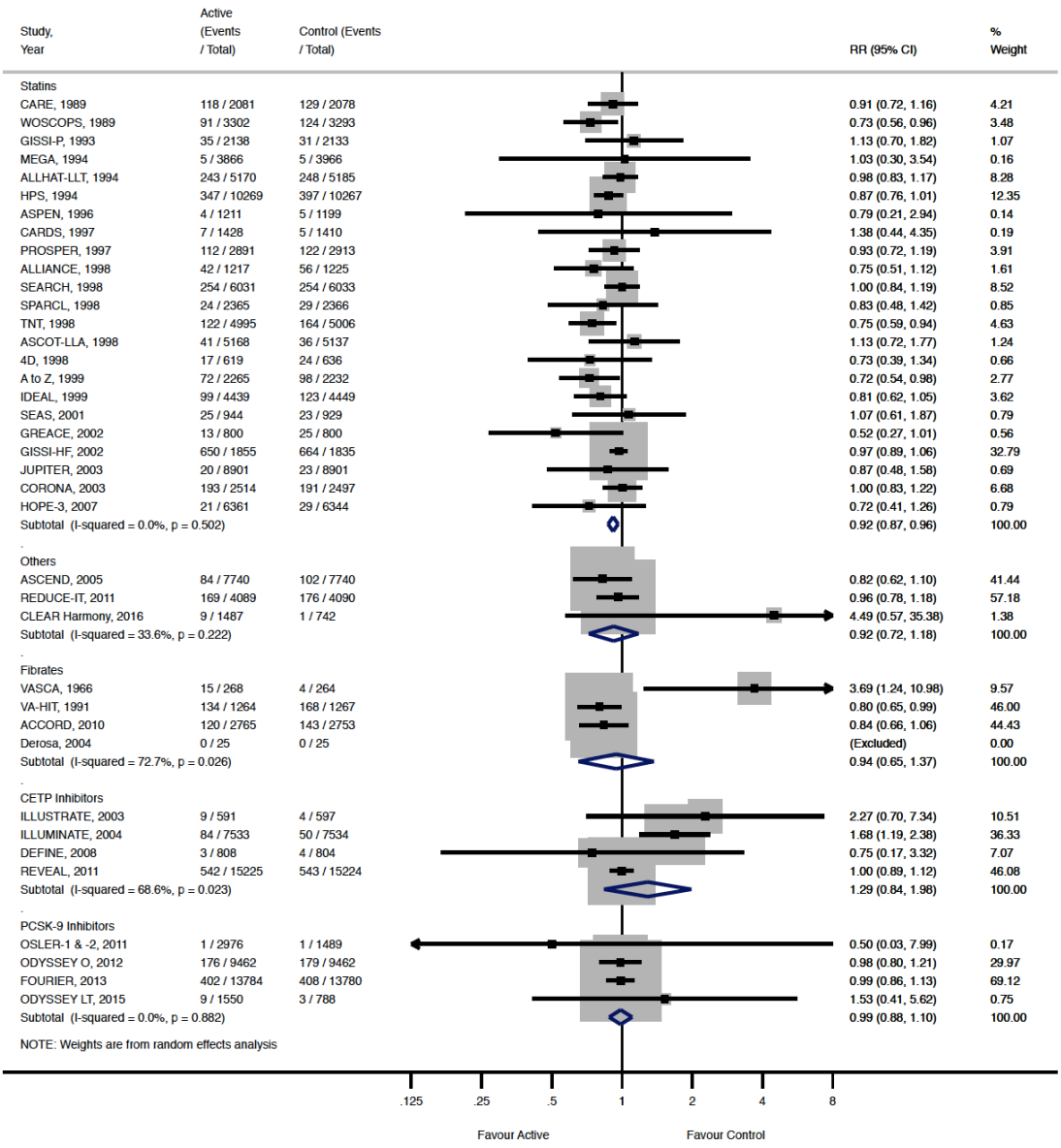


Figure 2-2 Meta-analysis of RCTs on the association between lipid-lowering agents and the risk of HF stratified by class of agents (38 trials).

Note: Overall results were from the random-effect model.

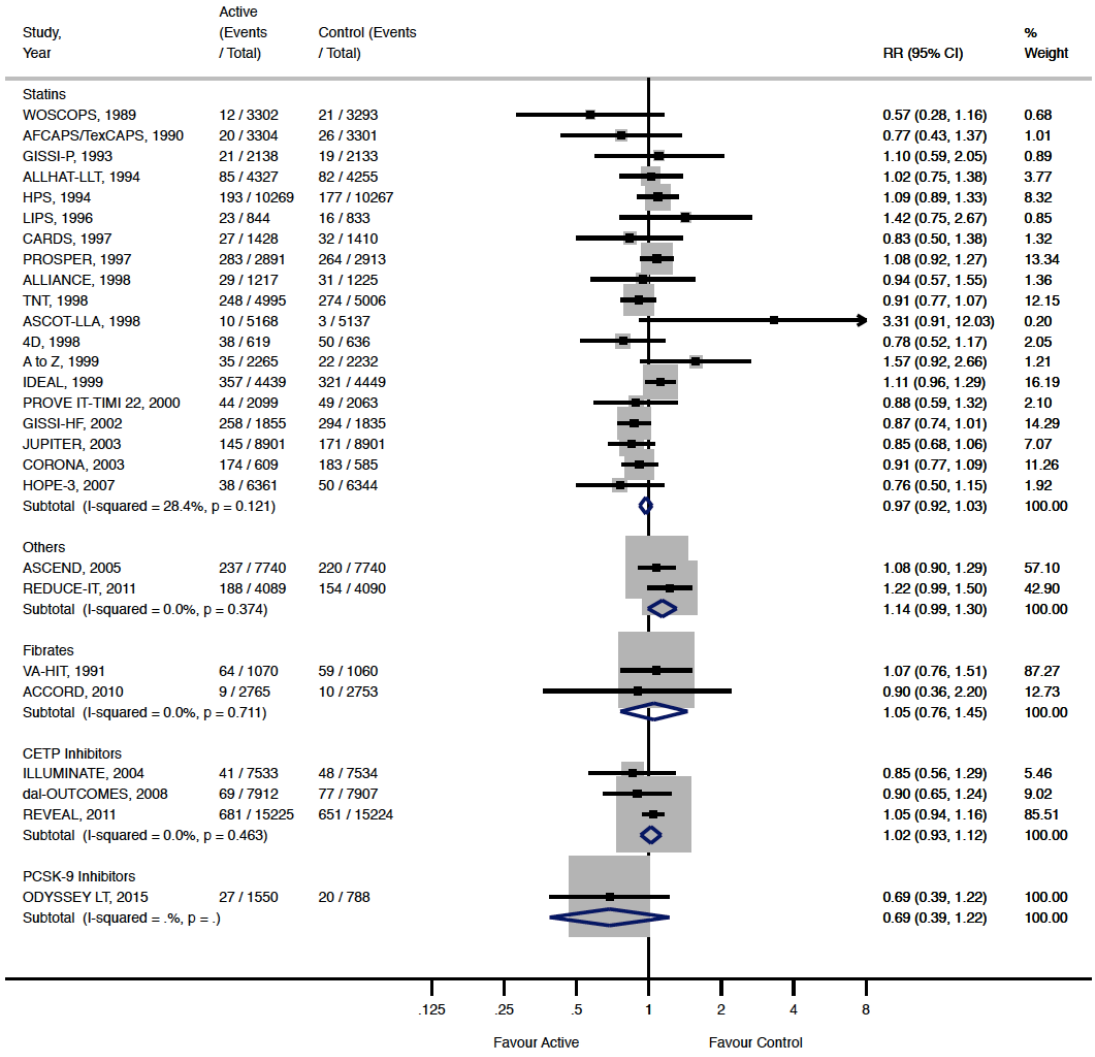


Figure 2-3 Meta-analysis of RCTs on the association between lipid-lowering agents and the risk of AF stratified by class of agents (27 trials).

Note: Overall results were from the fixed-effect model.

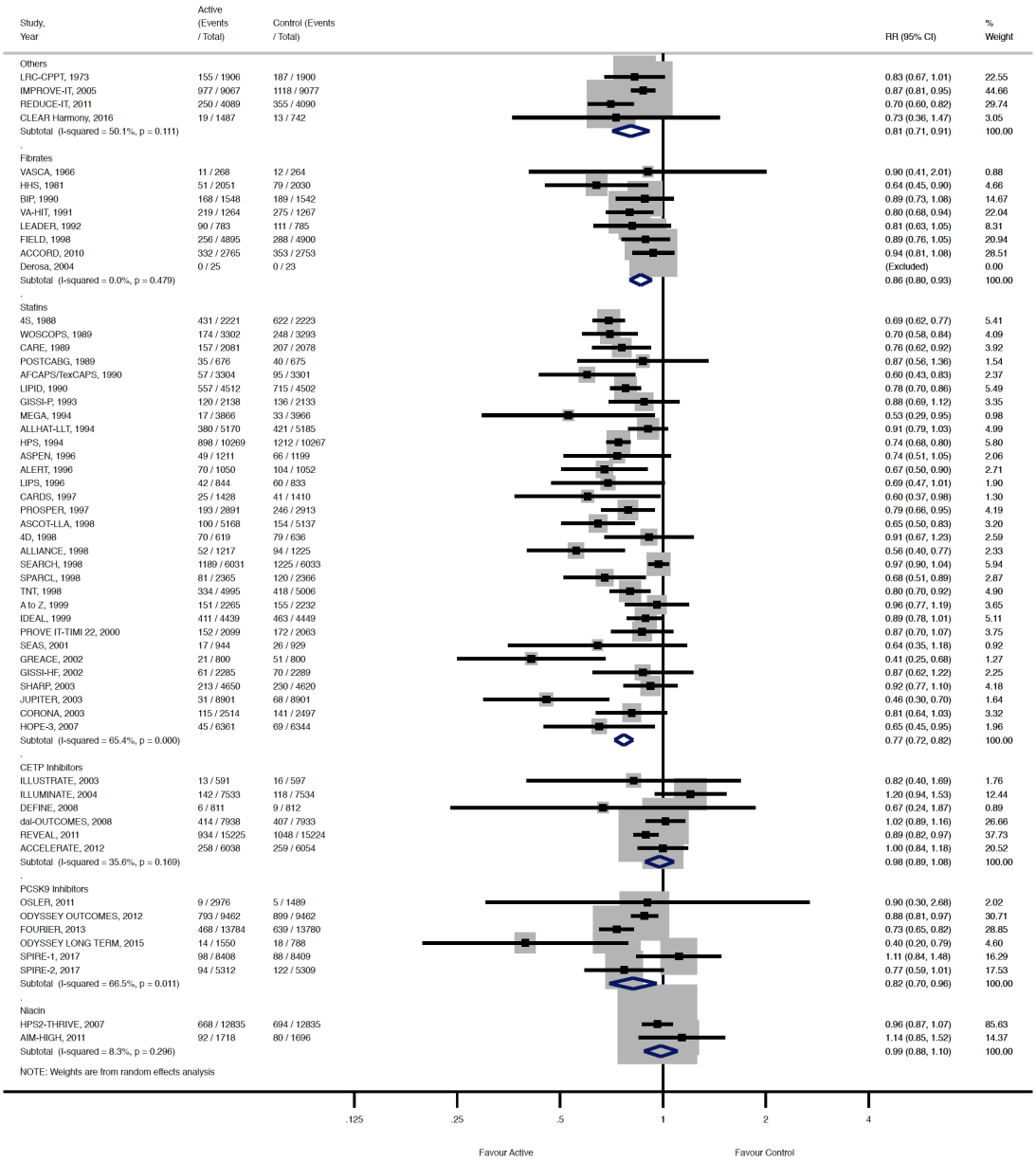


Figure 2-4 Meta-analysis of RCTs on the association between lipid-lowering agents and the risk of MI stratified by class of agents (57 trials).

Note: Overall results were from the random-effect model.

Table 2-3 Summary of cohort, trials, and genetic evidence on the association between blood lipids and incident HF and AF

Evidence	HF	AF	MI
LDL-C			
Cohort evidence	↔ ↑	↓ ↔ ↑	↑
Trials evidence (meta-regression of trials)	0	0	↑
Genetic evidence (MR)	0	↔	↑
HDL-C			
Cohort evidence	↓ ↔	↓ ↔	↓
Trials evidence (meta-regression of trials)	0	0	↔
Genetic evidence (MR)	0	↔	↓ ↔
TG			
Cohort evidence	↔ ↑	↔ ↑	↔
Trials evidence (meta-regression of trials)	0	0	0
Genetic evidence (MR)	0	↔	↔ ↑
Trials evidence of lipid-lowering drugs (active VS control)			
Statins	+	↔	+
PCSK-9 Inhibitors	↔	↔	+
CETP Inhibitors	↔	↔	↔
Niacin	0	0	↔
Fibrates	↔	↔	+
Others*	↔	↔	+

Note: ** Others included trials on ezetimibe (IMPROVE-IT), cholestyramine (LRC-CPPT), omega-3 fatty acid (ASCEND, REDUCE-IT), and Bempedoic acid (CLEAR Harmony).

Symbol	Interpretation
↓	Inverse (indirect, negative) association (i.e., lower lipids, higher risk of disease)
↔	Null association
↑	Direct (positive) association (i.e., lower lipids, lower risk of disease)
U	U shaped association (i.e., lower and higher lipids, higher risk of disease)
0	No previous evidence
↔ ↑	Conflicting evidence denoted by two or more symbols
+	Favour lipid-lowering drugs (i.e., lipid-lowering drugs decrease the risk of disease)
-	Favour control (i.e., lipid-lowering drugs increase the risk of disease)

Abbreviations: AF; atrial fibrillation, HDL-C; high-density lipoprotein cholesterol, HF; heart failure, LDL-C; low-density lipoprotein cholesterol, MI; myocardial infarction, NA; Not available, TG; Triglyceride.

2.7 Chapter Supplementary

Table S 2-1 Checklist of items according to PRISMA recommendation

Section/topic	Item No.	Checklist item	Reported on Page No.
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	Page 46
Abstract			
Structured summary	2	Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, interventions, study appraisal and synthesis methods, results, limitations, conclusions and implications of key findings, systematic review registration number	Page 46-47
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	Page 47-48
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	Page 48
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (such as web address), and, if available, provide registration information including registration number	Not applicable
Eligibility criteria	6	Specify study characteristics (such as PICOS, length of follow-up) and report characteristics (such as years considered, language, publication status) used as criteria for eligibility, giving rationale	Page 49
Information sources	7	Describe all information sources (such as databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	Page 49-50
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	Supplementary appendices page 433-434
Study selection	9	State the process for selecting studies (that is, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	Not applicable
Data collection process	10	Describe method of data extraction from reports (such as piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	Not applicable
Data items	11	List and define all variables for which data were sought (such as PICOS, funding sources) and any assumptions and simplifications made	Page 49-50 Supplementary appendices page 434-436
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	Page 49 Supplementary appendices page 437-439

Section/topic	Item No.	Checklist item	Reported on Page No.
Summary measures	13	State the principal summary measures (such as risk ratio, difference in means).	Page 49-50
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (such as I^2) for each meta-analysis	Page 49-50 Supplementary appendices page 439-441
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (such as publication bias, selective reporting within studies)	Page 49-50
Additional analyses	16	Describe methods of additional analyses (such as sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified	Page 49-50
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	Page 50-51, Figure S 2-1 to S 2-3
Study characteristics	18	For each study, present characteristics for which data were extracted (such as study size, PICOS, follow-up period) and provide the citations	Page 51-52, Table S 2-5 to S 2-6, S 2-10 to S 2-11, and S 2-14
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12).	Page 52-53, Table S 2-12, Figure S 2-4 to 2-5
Results of individual studies	20	For all outcomes considered (benefits or harms), present for each study (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot	Page 53-55 Figure 2-1 to 2-4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	Page 53-55
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)	Table S 2-12, Fig S 2-4 to -5
Additional analysis	23	Give results of additional analyses, if done (such as sensitivity or subgroup analyses, meta-regression [see item 16])	Table S 2-13, Figure S 2-10 to S 2-18
Discussion			
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (such as health care providers, users, and policy makers)	Page 56-57
Limitations	25	Discuss limitations at study and outcome level (such as risk of bias), and at review level (such as incomplete retrieval of identified research, reporting bias)	Page 58-59
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	Page 59
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (such as supply of data) and role of funders for the systematic review	Not applicable

Table S 2-2 Terms used and search strategies

No.	Searching terms
1.	exp Cholesterol, LDL/
2.	(LDL Cholesterol or Low Density Lipoprotein Cholesterol or (Cholesterol adj1 beta-Lipoprotein) or beta Lipoprotein Cholesterol or (LDL adj1 Cholesteryl Linoleate)).tw.
3.	1. or 2.
4.	exp Cholesterol, HDL/
5.	(High Density Lipoprotein Cholesterol or (alpha-Lipoprotein adj1 Cholesterol) or alpha Lipoprotein Cholesterol or (HDL* adj1 Cholesterol)).tw.
6.	4. or 5.
7.	exp Triglycerides/
8.	Triglyceride* or Triacyglycerol*.tw.
9.	7. or 8.
10.	3. or 6. or 9.
11.	exp Heart Failure/
12.	(((heart or cardiac or myocardia) adj failure) or (heart adj1 decompensation) or ((right-sided or (right adj sided) or left-sided or (left adj sided)) adj1 heart failure) or (congestive adj1 heart failure)).tw.
13.	11. or 12.
14.	exp Atrial Fibrillation/
15.	((atrial or auricular) adj1 fibrillation*).tw.
16.	14. or 15.
17.	13. or 16.
18.	10. and 17.
19.	limit 18 to (humans and yr="2000 - 2019" and English language)

Table S 2-3 Search filter terms for RCTs and cohort studies

No.	Randomised control trials		Observational (cohort) studies	
	Medline	Embase	Medline	Embase
1	Randomized Controlled Trials as Topic/	Clinical Trial/	Epidemiologic studies/	Clinical study/
2	randomized controlled trial/	Randomized Controlled Trial/	exp cohort studies/	Family study/
3	Random Allocation/	controlled clinical trial/	(cohort adj (study or studies)).tw.	Longitudinal study/
4	Double Blind Method/	multicenter study/	Cohort analy$.tw.	Retrospective study/
5	Single Blind Method/	Phase 3 clinical trial/	(Follow up adj (study or studies)).tw.	Prospective study/
6	clinical trial/	Phase 4 clinical trial/	(observational adj (study or studies)).tw.	Randomized controlled trial/
7	clinical trial, phase i.pt	exp RANDOMIZATION/	Longitudinal.tw.	5 not 6
8	clinical trial, phase ii.pt	Single Blind Procedure/	Retrospective.tw.	Cohort analysis/
9	clinical trial, phase iii.pt	Double Blind Procedure/	Or/1-8	(Cohort adj (study or studies)).mp.
10	clinical trial, phase iv.pt	Crossover Procedure/	exp case control studies/	(follow up adj (study or studies)).tw.
11	controlled clinical trial.pt	PLACEBO/	Case control.tw.	(observational adj (study or studies)).tw.
12	randomized controlled trial.pt	randomi?ed controlled trial$.tw.	Cross sectional.tw.	(epidemiologic$ adj (study or studies)).tw.
13	multicenter study.pt	rct.tw.	Cross-sectional studies/	Or/1-4,7-12
14	clinical trial.pt	(random$ adj2 allocat$).tw.	Or/10-13	exp case control study/
15	exp Clinical Trials as topic/	single blind$.tw.	9 not 14	(Case control adj (study or studies)).tw.
16	or/1-15	double blind$.tw.	-	(cross sectional adj (study or studies)).tw.
17	(clinical adj trial$).tw	((treble or triple) adj blind$).tw.	-	Or/14-16
18	((singl$ or doubl$ or treb$ or tripl$) adj (blind$3 or mask$3)).tw	placebo$.tw.	-	13 not 17
19	PLACEBOS/	Prospective Study/	-	-
20	placebo$.tw	or/1-19	-	-
21	randomly allocated.tw	Case Study/	-	-
22	(allocated adj2 random$).tw	case report.tw.	-	-

No.	Randomised control trials		Observational (cohort) studies	
	Medline	Embase	Medline	Embase
23	or/17-22	abstract report/ or letter/	-	-
24	16 or 23	Conference proceeding.pt.	-	-
25	case report.tw	Conference abstract.pt.	-	-
26	letter/	Editorial.pt.	-	-
27	historical article/	Letter.pt.	-	-
28	or/25-27	Note.pt.	-	-
29	24 not 28	or/21-28	-	-
30	-	20 not 29	-	-

Adapted from https://www.sign.ac.uk/search-filters.html

Table S 2-4 Search results

No.*	Number of literatures retrieved from each Database		
	Medline[$]	Embase**	Cochrane Central
1.	26,123	95,247	4,556
2.	44,278	60,584	Non-applicable
3.	55,293	109,292	Non-applicable
4.	27,310	98,459	3,621
5.	51,470	70,280	Non-applicable
6.	61,665	117,068	Non-applicable
7.	74,487	183,317	6,161
8.	138,528	151,281	Non-applicable
9.	139,792	224,176	Non-applicable
10.	182,966	294,732	9,410
11.	114,152	463,879	8,292
12.	162,896	266,616	Non-applicable
13.	197,406	507,984	Non-applicable
14.	50,627	56,268	3,999
15.	64,377	113,676	Non-applicable
16.	74,870	127,756	Non-applicable
17.	261,778	605,062	12,066
18.	1,604	7,947	31
19.	1,042	6,622	27
20.[#]	308	1,753	Non-applicable
21.[##]	321	1,496	Non-applicable

*No. corresponds with no. of search terms used in Table S2

[$]Ovide Medline 1946 to June Week 4 2019

**Embase 1974 to 2019 July 02

[#]The step after applying the search filter terms for RCTs (Table S2)

[##]The step after applying the search filter terms for observational cohort studies (Table S2)

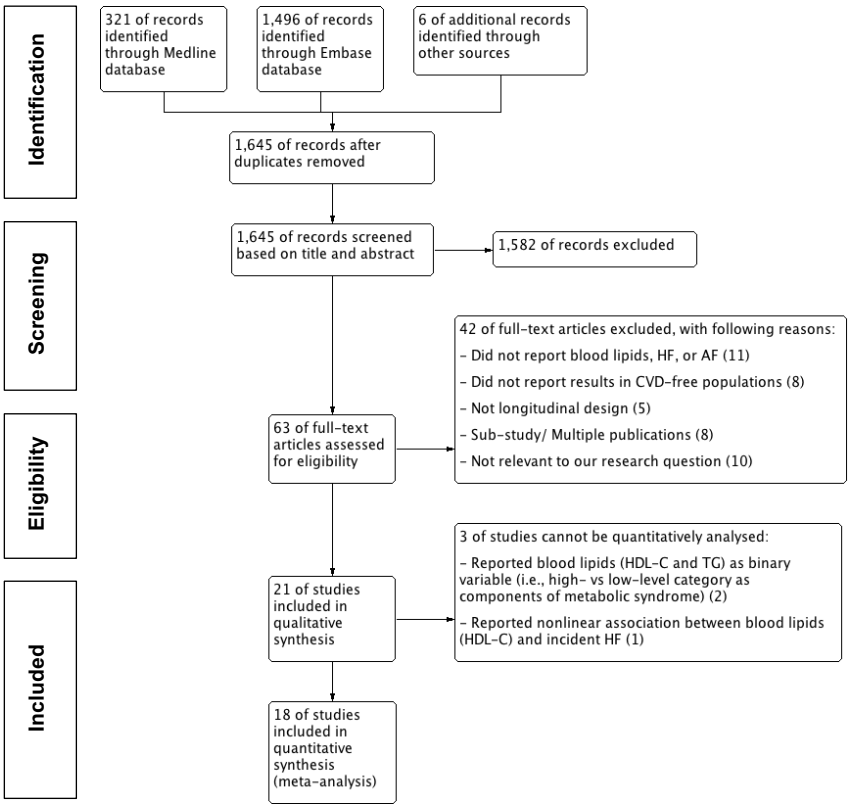


Figure S 2-1 Study flow diagram of selected cohort studies.

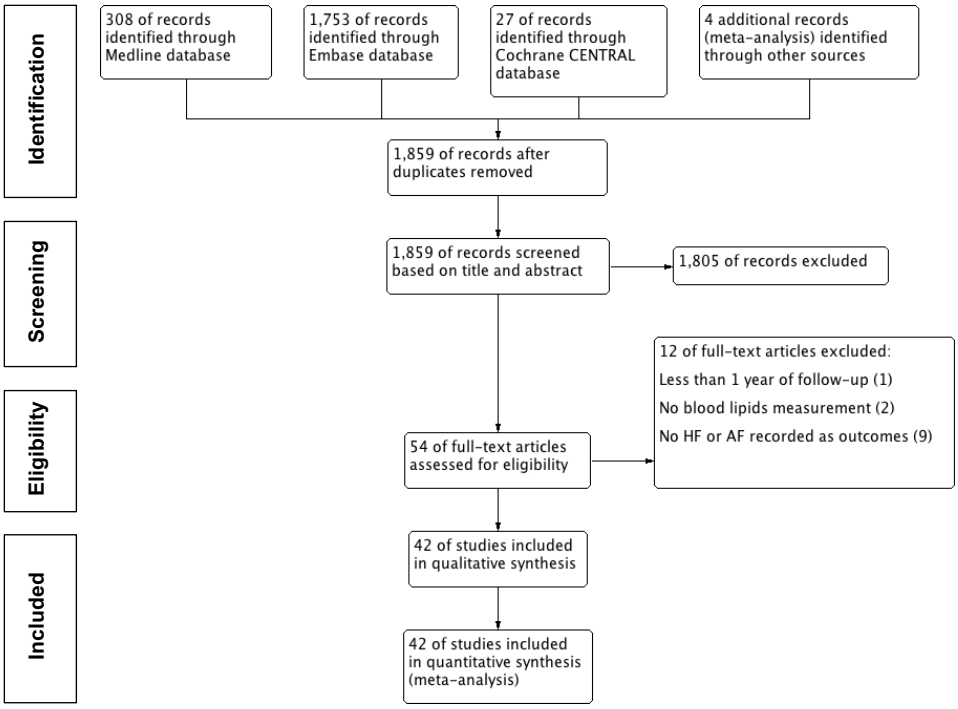


Figure S 2-2 Study flow diagram of selected RCTs

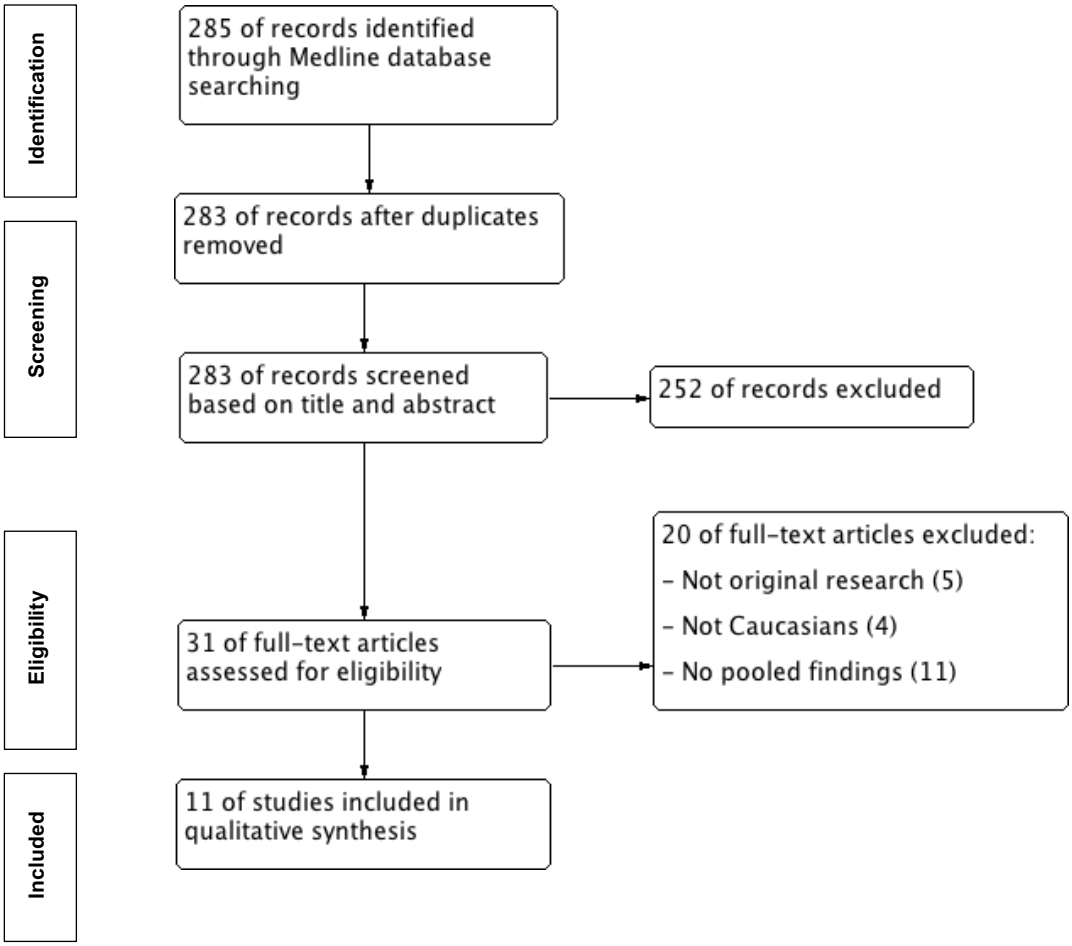


Figure S 2-3 Study flow diagram of selected genetic studies

Table S 2-5 Characteristics of the included cohort studies (n=9) on incident HF

Source	Setting	Number of participants	HF events (%)	Mean age (years)	% Male	Inclusion criteria	Lipid ascertainment	HF ascertainment	F/U time (years)
Wang, 2000[106]	Community, Finland	1,032	303 (29%)	69 ± 3	38%	Subjects born between 1912 and 1921 were randomly selected from the population register including all inhabitants of Kuopio. All subjects with HF at baseline were excluded.	Not mentioned	Based on specialist (diagnostic and etiological examination).	Median 20.7 (19.8, 21.4)
Ingelsson, 2005[108]	Community, Sweden	2,321	259 (11%)	50	100%	All 50-year-old men living in Uppsala in 1970 to 1974 were invited for regular health examinations, in-hospital data was updated using national registers. Excluding valvular heart disease at baseline.	Not mentioned	Based on two blinded baseline physicians according to hospital discharge register of HF	Median 28.8 (0.04, 31.7)
Bibbins-Domingo, 2009[72]	Community, Various countries	2,637	26 (1%)	25	45%	Study began in 1985-6 with 5115 black and white men and women age 18–30 recruited from Birmingham, AL, Chicago, IL, Minneapolis, MN, and Oakland, CA.	LDL-C was calculated by Friedewald equation.	Yearly telephone interview and request record if HF suspected	20
He, 2017[109]	Community, 8 centres USA	3,557	452 (13%)	54-60	50-60%	CKD patients age between 21-74 years with an eGFR between 20 and 70 mL/min per 1.73 m^2. Without haemodialysis, kidney transplant, HF at baseline	Not mentioned	Codes relevant to heart failure resulted in retrieval of medical records by study personnel for centralized adjudicated review	Median 6.3
Ebong, 2013[78]	Community, 6 regions USA	5,688	152 (3%)	61-70	47%	Men and women of Caucasian (38%), African American (28%), Hispanic (22%), and Chinese origin (12%), aged 45 to 84 years (2000–2002) and without known clinical CVD at baseline.	Lipid measures were obtained on fasting samples	Hospital records were abstracted and reviewed by paired physicians for independent end point classification and assignment of incidence dates	Median 8.5 (IQR 0.97)

Source	Setting	Number of participants	HF events (%)	Mean age (years)	% Male	Inclusion criteria	Lipid ascertainment	HF ascertainment	F/U time (years)
Velagaleti, 2009[71]	Community	6,860	680 (10%)	44 ± 15	55%	Original cohorts were originally enrolled in 1948 and offspring cohorts were enrolled in 1971 who had lipid measurement at baseline without HF, MI, valve disease, or on lipid-lowering therapy.	Based on standard laboratory procedure.	All suspected CVD events were reviewed by a committee of 3 physicians according to established criteria on medical records.	Mean 26
Dhingra, 2008[77]	Community	10,813	222 (2%)	68-69	100%	Healthy male physicians without prior history of CVD, cancer, liver disease, or renal dysfunction were recruited	Measurement of TC and HDL-C was done according to the standard lipid research clinic Standards.	Self-reports from physician-participants on the annual questionnaire. Validation was drawn from subset.	Mean 6
Holme*, 2009[112]	Community, Sweden	84,740	5,890 (7%)	NA	NA	Subjects mainly from the greater Stockholm area who underwent blood sampling during health check-ups in outpatient clinics between 1985 and 1996. AMI, stroke, or HF including cardiomyopathy prior to blood sampling were excluded	LDL-C and HDL-C levels were calculated and the validations have been reported.	All hospital discharges and deaths due to HF (ICD-7) were extracted from the hospital discharge register and the cause-of-death register.	Mean 11.8
Varbo*, 2018[113]	Community, Denmark	113,554	3,593 (3%)	58-60	45%	Individuals were randomly selected from the national Danish Civil Registration System.	LDL-C was calculated by Friedewald equation.	Information on HF (ICD) was collected by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry and Death Registry.	Median 6 (range: 0-11) and 19 (range: 0-23)

Abbreviations: F/U; follow-up, HDL-C; high-density lipoprotein cholesterol, HF; heart failure, ICD; international classification of disease, IQR; Interquartile range, LDL-C; low-density lipoprotein cholesterol, NA; not available

* represent studies that clearly stated the use of linkage electronic health records (EHRs) for outcome ascertainment.

Table S 2-6 Characteristics of the included cohort studies (n=12) on incident AF

Source	Setting	Number of participants	AF events (%)	Mean age (years)	% Male	Inclusion criteria	Lipid ascertainment	AF ascertainment	F/U time (years)
Barkas, 2017[107]	Community, Greece	1,223	34 (3%)	48-71	47%	Dyslipidemic adults followed-up for ⩾3 years (from 1999 to 2015) in the outpatient lipid clinic of the University Hospital of Ioannina in Greece were included. Excluding for acute coronary syndrome, hypo/hyper thyroidism, valvular heart disease, neoplasia, infection, liver disease	LDL-C was calculated using the Friedewald formula.	Reported at the most recent visit (⩾3 years after the baseline visit) and diagnosed based on physical findings or self-report or medications confirmed by ECG	Median 6 (IQR: 4–10)
Sciacqua, 2015[68]	Community, Italy	3,549	546 (15%)	60.7 ± 10.6	52%	Enrolled (1998-2011) 3,549 outpatients, 1,829 men and 1,720 women, aged 60.7 ± 10.6 years consisting of outpatients with CV risk factors referred to tertiary care setting for CV and metabolic screening program.	All lipids were measured by enzymatic methods	AF diagnosis was made by standard ECG, hospital discharge diagnoses, and by the all-clinical documentation provided by the patients or presence in the general practitioner files.	Mean 3.4 ± 1.8
Knuiman, 2014[75]	Community, Australia	4,267	343 (8%)	52 ± 15	44%	A total of 4,843 participated in the survey (1994/95: response rate 57 %), after restricting to age 25–84 this became 4,465. Mean age 52 years, < 3% of prior CVD at baseline.	Not mentioned	Incident AF events were defined as a hospital admission with a primary or other diagnosis of atrial fibrillation/flutter (ICD-9).	15
Smith*, 2010[74]	Community, Sweden	5,187	284 (6%)	58	41%	Individuals with a baseline examination between 1991 and 1994 were randomly selected to participate in a study of cardiovascular risk factors of whom 5,543 underwent blood sampling under standardized fasting conditions. 2% had history of MI.	Not mentioned	End points were ascertained by linkage of Swedish personal identification numbers to the national Swedish registers (Swedish Hospital Discharge Register, Swedish Cause of Death Register)	Median 13.8
Alonso*, 2014[31]	Community, 6 communities in the US	7,142	480 (7%)	MESA: 58 FHS: 62	47%	Population from community who were CVD-free at baseline during 2000-2002 (MESA) and 1995-1998 (FHS). Study excluded participants with prevalent AF at baseline, those taking lipid-lowering medications.	LDL-C was calculated using the Friedewald formula.	Incident AF were identified through event surveillance, Medicare, inpatient Medicare claims data. All hospitalizations are identified every 9 to 12 months during follow-up (MESA). ECG obtained from outpatient clinic visit adjudicated by cardiologists (FHS).	MESA: Mean 8.2 FHS: Mean 11.9

Source	Setting	Number of participants	AF events (%)	Mean age (years)	% Male	Inclusion criteria	Lipid ascertainment	AF ascertainment	F/U time (years)
Kodani, 2019[110]	Community, Japan	10,430	133 (1%)	64.9 ± 7.1	42%	All participants (general population) were aged 40–74 years at the time of entry because the special health checkups are open for subjects aged ⩾40 years, and individuals aged ⩾75 years are not eligible for this insurance.	Not mentioned	AF was diagnosed directly by physicians or based on automatic analysis of 12-lead ECG in each clinic or hospital, regardless of the electrocardiograph model and vendor.	Mean 6.9
Lopez*, 2012[32]	Community, 4 communities in the US	13,969	1,433 (10%)	54	46%	Participants at baseline (1987–1989) included 15,792 men and women aged 45– 64, recruited from four communities in the US (Washington County, MD; suburbs of Minneapolis, MN; Jackson, MS; Forsyth County, NC). Had history of CHD, stroke, HF at baseline	LDL-C was calculated using the Friedewald formula.	AF diagnoses were ascertained by three different sources in the ARIC study: electrocardiograms (ECG) performed at study visits, hospital discharge codes, and death certificates (annual follow-up)	Median 18.7
Kim, 2018[111]	Community, Korea	21,981	168 (1%)	46	100%	Most of the individuals who participated in the health examination were employees of heavy industries and most of them were men (n=24,800, 97%); Women and AF/AFL at initial check-up were excluded.	Not mentioned	AF and AFL were diagnosed from the 12-lead ECG recorded at a follow-up visit (annual or biennial).	Mean 8.7
Mora, 2002[76]	Community, US	23,738	795 (3%)	53-59	0%	Participants were drawn from the Women's Health Study: they were apparently healthy female healthcare professionals, aged ⩾45 years, and free of prior cardiovascular disease and cancer.	All standard lipids were directly measured	Participants were asked to report diagnoses of AF at baseline, 48 months, and annually thereafter. Women who reported an event were sent a questionnaire beginning in 2006 to collect additional information.	Median 16.4
Watanabe, 2011[69]	Community, Japan	28,449	265 (1%)	59 ± 11	34%	Based on voluntary annual health examinations in the Niigata prefecture of Japan, which were available to residents aged ⩾20 years. The population of the prefecture is approximately 2,400,000, and approximately 250,000 residents (approximately 10%) receive the examinations at Niigata Health Foundation every year.	LDL-C was calculated using the Friedewald formula.	AF was diagnosed from a 12-lead ECG recorded at an annual follow-up visit. The ECG diagnoses were made by physicians, and any abnormalities were confirmed by cardiologists.	Mean 4.5 ± 2.7
Mourtzinis*, 2018[70]	Community, Sweden	51,020	2,389 (5%)	64 ± 12	45%	A cohort of 74,751 hypertensive patients ⩾30 years old (mean age of 64 years) attending primary health care during 2001 to 2008 in 1 of 48 primary health care	Not mentioned	The primary endpoint of AF diagnosis was recorded through linkage to both the participating health care centers' medical	Mean 3.5

Source	Setting	Number of participants	AF events (%)	Mean age (years)	% Male	Inclusion criteria	Lipid ascertainment	AF ascertainment	F/U time (years)
						centers in a rural and an urban region of Sweden.		records and the Swedish National Patient Register.	
Li, 2018[73]	Community, China	88,785	328 (0.4%)	51 ± 12	79%	Study population, which included 101 510 men and women (mean age 51 years), completed structured questionnaires by interviews and underwent clinical examination across 11 subsidiary hospitals responsible for healthcare of this community. <2% had history of MI or stroke at baseline.	HDL-C and LDL-C were measured by direct test method	AF diagnoses were ascertained based on 12-lead ECG at biennial follow-up visits.	Mean 7.1

Abbreviations: AF/AFL; atrial fibrillation/atrial flutter, CHD; coronary heart disease, ECG; electrocardiogram, FSH; Framingham Heart Study, HF; heart failure, MESA; Multi-Ethnic Study of Atherosclerosis

* represent studies that clearly stated the use of linkage electronic health records (EHRs) for outcome ascertainment.

Table S 2-7 Adjusting factors of the individual cohort study (n=21) on HF and AF outcome

Source	Main controlling factors							Adjusting for interim CVD	Additional adjusting factors
	Gender	Age	BMI or obesity	Smoking status	HTN or blood pressure	T2DM or blood glucose	DLP or blood lipids		
Wang, 2010[106]	✓	✓	✗	✓	✓	✓	✓	✓	Physical activity, Alcohol consumption
Barkas, 2017[107]	✗	✓	✗	✗	✓	✓	✓	✗	Antihypertensive medications, antiplatelet, CKD
Ingelsson, 2005[108]	✓	✓	✓	✓	✓	✓	✓	✓	Prior AMI, ECG-LVH
B-D, 2009[72]	✓	✓	✓	✓	✓	✓	✓	✗	LVH
Sciacqua, 2015[68]	✓	✓	✓	✓	✓	✓	✓	✗	CHA_2DS_2-VASc
He, 2017[109]	✓	✓	✓	✓	✓	✓	✓	✗	CKD markers, novel inflammatory markers
Knuiman, 2014[75]	✓	✓	✓	✗	✓	✗	✗	✗	-
Smith, 2010[74]	✓	✓	✓	✓	✓	✓	✓	✗	History of MI, lnMR-proANP, lnCRP
Ebong, 2013[78]	✓	✓	✓	✓	✓	✓	✗	✓	Ethnicity, education, centre, exercise, ECG-LVH, albuminuria, IL-6
Velagaleti, 2009[71]	✓	✓	✓	✓	✓	✓	✗	✓	-
Alonso, 2014[31]	✓	✓	✓	✓	✓	✓	✓	✓	Incident heart failure
Kodani, 2019[110]	✓	✓	✓	✓	✓	✓	✓	✓	-
Dhingra, 2008[77]	✓	✓	✓	✓	✓	✓	✓	✓	Alcohol use, exercise days
Lopez, 2012[32]	✓	✓	✓	✓	✓	✓	✓	✓	Incident stroke, incident HF
Kim, 2018[111]	✓	✓	✓	✓	✓	✓	✓	✗	Alcohol consumption, components of metabolic syndrome
Mora, 2002[76]	✓	✓	✓	✓	✓	✓	✓	✗	Income, education, menopausal status, inflammatory/endothelial function markers.
Watanabe,2011[69]	✓	✓	✓	✗	✓	✓	✓	✗	-
Mourtzinis,2018[70]	✓	✓	✓	✓	✓	✓	✓	✗	HF, IHD, cerebrovascular disease, heart valvular disease, CKD, thyroid disorder, COPD, obstructive sleep apnea, alcohol abuse
Holme, 2009[112]	✓	✓	✗	✗	✓	✓	✓	✓	Haptoglobin, uric acid

Source	Main controlling factors							Adjusting for interim CVD	Additional adjusting factors
	Gender	Age	BMI or obesity	Smoking status	HTN or blood pressure	T2DM or blood glucose	DLP or blood lipids		
Li, 2018[73]	✓	✓	✓	✓	✓	✓	✓	✓	Education, income, height, physical activity, CRP, serum uric acid, cerebral infarction
Varbo, 2018[113]	✓	✓	✓	✓	✓	✓	✓	✓	Alcohol intake, atrial fibrillation

Abbreviations: AMI; acute myocardial infarction, CHA_2DS_2-VASc; congestive heart failure; hypertension-age >75 years (doubled)-type 2 diabetes-previous stroke, transient ischemic attack (TIA), or thromboembolism (doubled)-vascular disease-age 65–75 years-and gender, CKD; chronic kidney disease, COPD; chronic obstructive pulmonary disease, CRP; C-reactive protein, CVD; cardiovascular disease, DLP; dyslipidaemia, ECG-LVH; electrocardiogram-left ventricular hypertrophy, HF; heart failure, HTN; hypertension, IHD; ischaemic heart disease, IL-6; interleukin-6, MR-proANP; midregional pro-atrial natriuretic peptide, T2DM; type 2 diabetes mellitus,

Table S 2-8 Results of individual cohort studies on HF endpoint (n=9)

Study	Reported unit of effect size	Reported effect size (95%CI)*		
		LDL-C	HDL-C	TG
Wang, 2010	Hazard ratio (Dichotomised lipid fractions) HDL-C < 1.03 mmol/L (female < 1.29 mmol/L) TG ≥ 1.7 mmol/L	Not measured	1.55 (1.15, 2.10)	1.29 (0.95, 1.75)
Ingelsson, 2005	Hazard ratio per 1 SD increase 1 SD LDL-C = 1.3 mmol/L 1 SD HDL-C = not reported 1 SD TG = not reported	1.57 (0.89, 2.80)	0.86 (0.74, 0.99)	1.07 (0.93, 1.22)
Bibbins-Domingo, 2009	Hazard ratio per 1 SD increase 1 SD HDL-C = 13.3 mg/dL	Measured but not reported	0.60 (0.40, 1.00)	Not measured
He, 2017	Hazard ratio per 1 SD increase 1 SD LDL-C = 35.5 mg/dL 1 SD HDL-C = 15.6 mg/dL	0.96 (0.87, 1.05)	0.81 (0.72, 0.91)	Not measured
Ebong, 2013	Hazard ratio per 1 SD increase 1 SD HDL-C = 12.17 mg/dL 1 SD log TG = 0.58	Not measured	0.65 (0.43, 0.99)	1.68 (1.18, 2.38)
Velagaleti, 2009	Hazard ratio per 1 SD increase 1 SD HDL-C = 16 mg/dL	Not measured	0.89 (0.81, 0.98)	Not measured
Dhingra, 2008	Hazard ratio (Quartiles of HDL-C)	Not measured	Quartile 1: 1.00 (Reference) Quartile 2: 0.80 (0.58, 1.18) Quartile 3: 0.70 (0.46, 1.07) Quartile 4: 1.13 (0.76, 1.69) P-trend = 0.78	Not measured
Holme, 2009	Hazard ratio per 1 SD increase 1 SD LDL-C = 0.99 mmol/L (male), 1.06 mmol/L (female) 1 SD HDL-C = 0.41 mmol/L (male), 0.42 mmol/L (female) 1 SD TG = 0.82 mmol/L (male), 0.66 mmol/L (female)	Male: 1.30 (1.26, 1.33) Female: 1.18 (1.13, 1.23)	Male: 0.82 (0.79, 0.84) Female: 0.87 (0.83, 0.90)	Male: 1.19 (1.15, 1.23) Female: 1.20 (1.15, 1.24)
Varbo, 2018	Hazard ratio per 1 mmol/L increase	0.95 (0.88, 1.04)	Not measured	1.19 (1.12, 1.26)

Note: Reported effect size was taken from multivariable adjusted model with additional adjusting for intercurrent CVD (if applicable). To convert lipid fraction from mmol/L to mg/dL, multiplied by the factor of 38.67, 38.67, and 88.57 for LDL-C, HDL-C, and TG, respectively.

Table S 2-9 Results of individual cohort studies on AF endpoint (n=12)

Study	Reported unit of effect size	Reported effect size (95%CI)*		
		LDL-C	HDL-C	TG
Barkas, 2017	Odds ratio per 1 mg/dL increase	Measured but not reported	0.96 (0.93, 0.98)	Measured but not reported
Sciacqua, 2015	Hazard ratio per 10 mg/dL increase	1.046 (1.021, 1.071)	Not measured	Not measured
Knuiman, 2014	Hazard ratio per 1 SD increase 1 SD LDL-C = 0.97 mmol/L 1 SD HDL-C = 0.39 mmol/L 1 SD log TG = 0.55	0.94 (0.84, 1.06)	1.01 (0.90, 1.14)	0.96 (0.85, 1.08)
Smith, 2010	Hazard ratio per 1 SD increase 1 SD LDL-C = 1.0 mmol/L	0.90 (0.78, 1.02)	Not measured	Not measured
Alonso, 2014	Hazard ratio per 1 SD increase 1 SD LDL-C = 35 mg/dL 1 SD HDL-C = 15 mg/dL 1 SD TG = 65 mg/dL	1.04 (0.94, 1.15)	0.89 (0.80, 0.99)	1.16 (1.06, 1.27)
Kodani, 2019	Hazard ratio per 1 mg/dL increase	0.995 (0.988, 1.002)	Not measured	1.001 (0.999, 1.002)
Lopez, 2012	Hazard ratio per 1 SD increase 1 SD LDL-C = not reported 1 SD HDL-C = not reported 1 SD TG = not reported	0.90 (0.85, 0.96)	0.97 (0.91, 1.04)	1.00 (0.96, 1.04)

Study	Reported unit of effect size	Reported effect size (95%CI)*		
		LDL-C	HDL-C	TG
Kim, 2018	Hazard ratio (Dichotomised lipid fractions) - Reduced HDL-C (<40 mg/dL or drug treatment for lowering HDL-C). - Raised TG (⩾150 mg/dL or drug treatment for high TG)	Not measured	1.06 (0.74, 1.53)	0.71 (0.49, 1.02)
Mora, 2002	Hazard ratio (Quintiles of lipids)	Quintile 1: 1.00 (Reference) Quintile 2: 0.84 (0.66, 1.06) Quintile 3: 0.86 (0.69, 1.09) Quintile 4: 0.86 (0.68, 1.09) Quintile 5: 0.72 (0.56, 0.92) P-trend = 0.02	Quintile 1: 1.00 (Reference) Quintile 2: 0.98 (0.76, 1.25) Quintile 3: 1.02 (0.79, 1.32) Quintile 4: 1.02 (0.79, 1.32) Quintile 5: 1.07 (0.83, 1.39) P-trend = 0.49	Quintile 1: 1.00 (Reference) Quintile 2: 0.96 (0.72, 1.27) Quintile 3: (1.01 (0.77, 1.33) Quintile 4: 0.94 (0.72, 1.23) Quintile 5: 0.83 (0.63, 1.09) P-trend = 0.08
Watanabe, 2011	Hazard ratio per 10 mg/dL increase in LDL-C and TG but per 10 mg/dL decrease in HDL-C	0.92 (0.88, 0.96)	Overall: 1.11 (0.97, 1.26) Male: 0.99 (1.10, 0.89) Female: 1.28 (1.50, 1.08)	0.98 (0.96, 1.00)
Mourtzinis, 2018	Hazard ratio per 1 mmol/L increase	0.84 (0.73, 0.97)	Overall: 0.93 (0.68, 1.28) Male: 1.08 (0.68, 1.69) Female: 0.80 (0.51, 1.26)	0.86 (0.73, 1.02)
Li, 2018	Hazard ratio (Quartiles of lipids)	Quartile 1: 1.00 (Reference) Quartile 2: 0.86 (0.64, 1.16) Quartile 3: 0.79 (0.58, 1.08) Quartile 4: 0.59 (0.43, 0.83) P-trend = 0.002	Quartile 1: 1.00 (Reference) Quartile 2: 1.10 (0.79, 1.52) Quartile 3: 1.13 (0.81, 1.56) Quartile 4: 0.99 (0.71, 1.38) P-trend = 0.958	Quartile 1: 1.00 (Reference) Quartile 2: 0.76 (0.53, 1.05) Quartile 3: 0.84 (0.60, 1.16) Quartile 4: 0.87 (0.62, 1.22) P-trend = 0.674

Note: Reported effect size was taken from multivariable adjusted model with additional adjusting for intercurrent CVD (if applicable). To convert lipid fraction from mmol/L to mg/dL, multiplied by the factor of 38.67, 38.67, and 88.57 for LDL-C, HDL-C, and TG, respectively.

Table S 2-10 Summary of RCTs (42 trials) of lipid-lowering agents on HF and AF endpoints

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			HF hospitalisation or death		Reported AF or cardiac dysrhythmia	
						LDL-C	HDL-C	TG	Active	Control	Active	Control
Healthy individuals												
1. AFCAPS/ TexCAPS[114]	1990	Healthy, age 58 years	Lovastatin (3,304)	Placebo (3,301)	5.2	-1.06	0.03	-0.23	NR	NR	20	26
Intermediate to high risk individuals (without documented angina or MI)												
2. MEGA[91]	1994	Hypercholesterolaemia, age 59 years	Pravastatin (3,866)	Usual care (3,966)	5.3	-0.50	0.08	-0.16	5	5	NR	NR
3. ASPEN[115]	1996	Type 2 DM, age 62 years	Atorvastatin (1,211)	Placebo (1,199)	4.0	-0.88	0.03	-0.21	4	5	NR	NR
4. CARD[116]	1997	Type 2 DM, age 62 years	Atorvastatin (1,428)	Placebo (1,410)	3.9	-1.01	0.03	-0.29	7	5	27	32
5. SEAS[117]	2001	Aortic valve stenosis, age 68 years	Simvastatin + Ezetimibe (944)	Placebo (929)	4.4	-1.79	NR	NR	25	23	NR	NR
6. JUPITER[118]	2003	↑ CRP, age 66 years	Rosuvastatin (8,901)	Placebo (8,901)	1.9	-1.40	0.00	-0.21	20	23	145	171
7. ASCEND[89]	2005	Type 2 DM, age 64 years	Omega-3 FA (7,740)	Placebo (7,740)	7.4	NR	0.03	NR	84	102	237	220
8. HOPE-3[119]	2007	≥ 1 CHD risk, age 66 years	Rosuvastatin (6,361)	Placebo (6,344)	5.6	-0.72	NR	-0.24	21	29	38	50
Mixed population												
9. WOSCOPS[120,121]	1989	Men, SA 5%, age 56 years	Pravastatin (3,302)	Placebo (3,293)	4.9	-1.29	0.06	-0.22	91	123	12	21
10. ALLHAT-LLT[79]	1994	Hypertension with 15% history of CVD, age 67 years	Pravastatin (5,170)	Usual care (5,185)	4.8	-0.44	0.09	-0.02	243	248	85/ 4,327*	82/ 4,255*
11. HPS[122]	1994	MI 41%, age 64 years	Simvastatin (10,269)	Placebo (10,267)	5.0	-0.68	0.03	-0.30	347	397	193	177
12. PROSPER[123]	1997	CVD 44%, age 76 years	Pravastatin (2,891)	Placebo (2,913)	3.2	-1.30	0.06	-0.18	112	122	283	264
13. ASCOT-LLA[124]	1998	Hypertension with 10% storke, age 64 years	Atorvastatin (5,168)	Placebo (5,137)	3.3	-0.95	0.02	-0.20	41	36	10	3

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			HF hospitalisation or death		Reported AF or cardiac dysrhythmia	
						LDL-C	HDL-C	TG	Active	Control	Active	Control
14. 4D[125]	1998	DM + HD, age 66 years	Atorvastatin (619)	Placebo (636)	3.9	-0.57	NR	NR	17	24	38	50
15. DEFINE[81]	2008	CHD 55%, age 63 years	Anacetrapib (811)	Placebo (812)	1.5	-0.72	1.48	-0.19	3/ 808*	4/ 804*	NR	NR
16. ACCORD[126]	2010 #	DM, CHD 37%, age 62 years	Fenofibrate + Simvastatin (2,765)	Placebo + Simvastatin (2,753)	4.7	0.00	0.01	-0.28	120	143	9	10
17. OSLER-1 & -2[84]	2011	CAD 20%, age 58 years	Evolocumab (2,976)	Usual care (1,489)	1.0	-1.89	0.08	-0.16	1	1	NR	NR
18. ODYSSEY LONG TERM[83]	2015 #	CAD 69%, age 61 years	Alirocumab (1,550)	Placebo (788)	1.5	-1.83	0.05	-0.29	9	3	27	20
CVD population												
19. VASCA[127]	1966	Men with cerebral infarction, age 50-59 years	Clofibrate (268)	Placebo (264)	1.8	NR	NR	-0.20	15	4	NR	NR
20. CARE[86]	1989	MI, age 59 years	Pravastatin (2,081)	Placebo (2,078)	5.0	-0.85	0.05	-0.25	118	129	NR	NR
21. VA-HIT[128,129]	1991	CHD, age 64 years	Gemfibrozil (1,264)	Placebo (1,267)	5.1	0.00	0.05	-0.58	134	168	64 / 1,070	59 / 1,060
22. GISSI-P[87]	1993	MI, age 60 years	Pravastatin (2,138)	Usual care (2,133)	2.0	-0.47	NR	NR	35	31	21	19
23. LIPS[130]	1996	Angina & PCI, age 60 years	Fluvastatin (844)	Placebo (833)	3.9	-0.78	0.03	0.00	NR	NR	23	16
24. ALLIANCE[80]	1998	CHD, age 62 years	Atorvastatin (1,217)	Usual care (1,225)	4.5	-0.39	-0.03	-0.15	42	56	29	31
25. SEARCH[85]	1998	MI, age 65 years	Simvastatin 80 mg (6,031)	Simvastatin 20 mg (6,033)	6.7	-0.34	0.02	-0.15	254	254	NR	NR
26. SPARCL[131]	1998	Stroke or TIA, age 63 years	Atorvastatin (2,365)	Placebo (2,366)	4.9	-1.44	0.03	-0.38	24	29	NR	NR
27. TNT[132]	1998	CHD, age 61 years	Atorvastatin 80 mg (4,995)	Atorvastatin 10 mg (5,006)	4.9	-0.52	0.00	-0.15	122	164	248	274
28. A to Z[133]	1999	ACS, age 61 years	Simvastatin 80 mg (2,265)	Simvastatin 20 mg (2,232)	2.0	-0.39	0.00	-0.14	72	98	35	22
29. IDEAL[90]	1999	MI, age 62 years	Atorvastatin 80 mg (4,439)	Simvastatin 20 mg (4,449)	4.8	-0.51	-0.01	-0.21	99	123	357	321
30. PROVE IT-TIMI 22[88]	2000	ACS, age 59 years	Atorvastatin 80 mg (2,099)	Pravastatin 40 mg (2,063)	2.0	-0.85	-0.04	NR	NR	NR	44	49
31. GREACE[134]	2002 #	MI, age 58.5 years	Atorvastatin (800)	Usual care (800)	3.0	-1.86	0.05	-0.50	13	25	NR	NR

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			HF hospitalisation or death		Reported AF or cardiac dysrhythmia	
						LDL-C	HDL-C	TG	Active	Control	Active	Control
32. GISSI-HF[105]	2002	CHF, age 67 years	Rosuvastatin (1,855)	Placebo (1,835)	3.7	-0.75	0.00	-0.20	650	664	258	294
33. CORONA[82]	2003	CHF, age 73 years	Rosuvastatin (2,514)	Placebo (2,497)	2.7	-1.60	0.08	-0.45	193	191	174/ 609**	183/ 585**
34. ILLUSTRATE[135]	2003	Underwent cardiac catheterisation, age 57 years	Torcetrapib + Atorvastatin (591)	Atorvastatin (597)	2.0	-0.51	1.57	-0.07	9	4	NR	NR
35. Derosa[136]	2004 #	CHD, age 60 years	Fenofibrate + Fluvastatin (25)	Fluvastatin (25)	1.0	-0.50	0.20	-0.30	0	0	NR	NR
36. ILLUMINATE[137]	2004	Previous CVD, age 61.3 years	Torcetrapib + Atorvastatin (7,533)	Atorvastatin (7,534)	1.0	-0.58	0.87	-0.12	84	50	41	48
37. dal-OUTCOMES[138]	2008	ACS, age 61 years	Dalcetrapib (7,938)	Placebo (7,933)	2.6	0.00	0.31	-0.05	NR	NR	69/ 7,912*	77/ 7,907*
38. REDUCE-IT[139]	2011	CVD, age 64 years	Icosapent ethyl (4,089)	Placebo (4,090)	4.9	-0.17	-0.04	-0.38	169	176	188	154
39. REVEAL[140]	2011	CHD, age 67 years	Anacetrapib (15,225)	Placebo (15,224)	4.1	-0.67	1.11	-0.11	542	543	681	651
40. ODYSSEY OUTCOMES[141]	2012	Prior ACS, age 59 years	Alirocumab (9,462)	Placebo (9,462)	2.8	-0.96	0.00	-0.15	176	179	NR	NR
41. FOURIER[142]	2013	ASCVD, age 62.5 years	Evolocumab (13,784)	Placebo (13,780)	2.2	-1.45	0.09	-0.22	402	408	NR	NR
42. CLEAR Harmony[143]	2016	ASCVD, age 66 years	Bempedoic acid(1,487)	Placebo (742)	1.0	-0.46	-0.07	-0.06	9	1	NR	NR

Abbreviations: See Table S 2-11

Table S 2-11 Summary of RCTs (57 trials) of lipid-lowering agents on MI endpoint

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			Fatal or nonfatal MI	
						LDL-C	HDL-C	TG	Active	Control
Healthy individuals										
1. LRC-CPPT[144]	1973	Healthy men, age 47.8 years	Cholestyramine (1,906)	Placebo (1,900)	7.4	-0.59	0.03	0.10	155	187
2. HHS[145]	1981	Healthy men, age 48 years	Gemfibrozil (2,051)	Placebo (2,030)	5.0	-0.46	0.11	-0.71	51	79
3. AFCAPS/ TexCAPS[114]	1990	Healthy, age 58 years	Lovastatin (3,304)	Placebo (3,301)	5.2	-1.06	0.03	-0.23	57	95
Intermediate to high risk individuals (without documented angina or MI)										
4. LEADER[146]	1992	Lower extremity arterial disease, age 69 years	Bezafibrate (783)	Placebo (785)	4.6	-0.30	0.00	-0.40	90	111
5. MEGA[91]	1994	Hypercholesterolaemia, age 59 years	Pravastatin (3,866)	Usual care (3,966)	5.3	-0.50	0.08	-0.16	17	33
6. ASPEN[115]	1996	Type 2 DM, age 62 years	Atorvastatin (1,211)	Placebo (1,199)	4.0	-0.88	0.03	-0.21	49	66
7. CARDS[116]	1997	Type 2 DM, age 62 years	Atorvastatin (1,428)	Placebo (1,410)	3.9	-1.01	0.03	-0.29	NR	NR
8. SEAS[117]	2001	Aortic valve stenosis, age 68 years	Simvastatin + Ezetimibe (944)	Placebo (929)	4.4	-1.79	NR	NR	NR	NR
9. SHARP[147]	2003	CKD, age 62 years	Simvastatin + Ezetimibe (4,650)	Placebo (4,620)	4.9	-0.77	NR	NR	213	230
10. JUPITER[118]	2003	↑ CRP, age 66 years	Rosuvastatin (8,901)	Placebo (8,901)	1.9	-1.40	0.00	-0.21	31	68
11. HOPE-3[119]	2007	≥ 1 CHD risk, age 66 years	Rosuvastatin (6,361)	Placebo (6,344)	5.6	-0.72	NR	-0.24	45	69
Mixed population										
12. WOSCOPS[121]	1989	Men, SA 5%, age 56 years	Pravastatin (3,302)	Placebo (3,293)	4.9	-1.29	0.06	-0.22	174	248
13. ALLHAT-LLT[79]	1994	Hypertension, age 67 years	Pravastatin (5,170)	Usual care (5,185)	4.8	-0.44	0.09	-0.02	380	421
14. HPS[122]	1994	MI 41%, age 64 years	Simvastatin (10,269)	Placebo (10,267)	5.0	-0.68	0.03	-0.30	898	1,212
15. ALERT[148]	1996	Renal transplant, MI 33%, age 50 years	Fluvastatin (1,050)	Placebo (1,052)	5.4	-1.00	NR	NR	70	104

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			Fatal or nonfatal MI	
						LDL-C	HDL-C	TG	Active	Control
16. PROSPER[123]	1997	CVD 44%, age 76 years	Pravastatin (2,891)	Placebo (2,913)	3.2	-1.30	0.06	-0.18	193	246
17. ASCOT-LLA[124]	1998	Hypertension, age 64 years	Atorvastatin (5,168)	Placebo (5,137)	3.3	-0.95	0.02	-0.20	100	154
18. 4D[125]	1998	DM + HD, MI 18%, age 66 years	Atorvastatin (619)	Placebo (636)	3.9	-0.57	NR	NR	NR	NR
19. FIELD[149]	1998	DM, MI 5%, age 62.2 years	Fenofibrate (4,895)	Placebo (4,900)	5.0	-0.17	0.00	-0.41	256	288
20. DEFINE[81]	2008	CHD 55%, age 63 years	Anacetrapib (811)	Placebo (812)	1.5	-0.72	1.48	-0.19	NR	NR
21. ACCORD[126]	2010[#]	DM, CHD 37%, age 62 years	Fenofibrate + Simvastatin (2,765)	Placebo + Simvastatin (2,753)	4.7	0.00	0.01	-0.28	332	353
22. OSLER-1&-2[84]	2011	CAD 20%, age 58 years	Evolocumab (2,976)	Usual care (1,489)	0.9	-1.89	0.08	-0.16	NR	NR
23. ODYSSEY LONG TERM[83]	2015[#]	CAD 69%, age 61 years	Alirocumab (1,550)	Placebo (788)	1.5	-1.83	0.05	-0.29	NR	NR
<u>CVD population</u>										
20. VASCA[127]	1966	Men with cerebral infarction age 50-59years	Clofibrate (268)	Placebo (264)	1.8	NR	NR	-0.20	11	12
24. 4S[150]	1988	MI, age 58.5 years	Simvastatin (2,221)	Placebo (2,223)	5.4	-1.75	0.07	-0.28	431	622
25. CARE[86]	1989	MI, age 59 years	Pravastatin (2,081)	Placebo (2,078)	5.0	-0.85	0.05	-0.25	157	207
26. POST CABG[151]	1989	MI, age 62 years	Lovastatin 40-80 mg/day (676)	Lovastatin 2.5-5 mg/day (675)	4.3	-0.99	0.00	-0.23	35	40
27. LIPID[152]	1990	MI, age 62 years	Pravastatin (4,512)	Placebo (4,502)	6.1	-0.97	0.05	-0.17	557	715
28. BIP[153]	1990	MI, age 60.1 years	Bezafibrate (1,548)	Placebo (1,542)	6.2	-0.22	0.13	-0.41	168	189
29. VA-HIT[129]	1991	CHD, age 64 years	Gemfibrozil (1,264)	Placebo (1,267)	5.1	0.00	0.05	-0.58	219	275
30. GISSI-P[87]	1993	MI, age 60 years	Pravastatin (2,138)	Usual care (2,133)	2.0	-0.47	NR	NR	120	136
31. LIPS[130]	1996	Angina & PCI, age 60 years	Fluvastatin (844)	Placebo (833)	3.9	-0.78	0.03	0.00	42	60

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			Fatal or nonfatal MI	
						LDL-C	HDL-C	TG	Active	Control
32. ALLIANCE[80]	1998	CHD, age 62 years	Atorvastatin (1,217)	Usual care (1,225)	4.5	-0.39	-0.03	-0.15	NR	NR
33. SEARCH[85]	1998	MI, age 65 years	Simvastatin 80 mg (6,031)	Simvastatin 20 mg (6,033)	6.7	-0.34	0.02	-0.15	1,189	1,225
34. SPARCL[131]	1998	Stroke or TIA, age 63 years	Atorvastatin (2,365)	Placebo (2,366)	4.9	-1.44	0.03	-0.38	81	120
35. TNT[132]	1998	CHD, age 61 years	Atorvastatin 80 mg (4,995)	Atorvastatin 10 mg (5,006)	4.9	-0.52	0.00	-0.15	334	418
36. A to Z[133]	1999	ACS, age 61 years	Simvastatin 80 mg (2,265)	Simvastatin 20 mg (2,232)	2.0	-0.39	0.00	-0.14	NR	NR
37. IDEAL[90]	1999	MI, age 62 years	Atorvastatin 80 mg (4,439)	Simvastatin 20 mg (4,449)	4.8	-0.51	-0.01	-0.21	411	463
38. PROVE IT-TIMI 22[88]	2000	ACS, age 59 years	Atorvastatin 80 mg (2,099)	Pravastatin 40 mg (2,063)	2.0	-0.85	-0.04	NR	152	172
39. GREACE[134]	2002[#]	MI, age 58.5 years	Atorvastatin (800)	Usual care (800)	3.0	-1.86	0.05	-0.50	NR	NR
40. GISSI-HF[105]	2002	CHF, age 67 years	Rosuvastatin (2,285)	Placebo (2,289)	3.7	-0.75	0.00	-0.20	61	70
41. CORONA[82]	2003	CHF, age 73 years	Rosuvastatin (2,514)	Placebo (2,497)	2.7	-1.60	0.08	-0.45	NR	NR
38. ILLUSTRATE[135]	2003	Underwent cardiac catheterisation, age 57 years	Torcetrapib + Atorvastatin (591)	Atorvastatin (597)	2.0	-0.51	1.57	-0.07	NR	NR
39. Derosa[136]	2004[#]	CHD, T2DM, age 60 years	Fluvastatin + fenofibrate (25)	Fluvastatin (23)	1.0	-0.50	0.20	-0.30	0	0
40. ILLUMINATE[137]	2004	CVD, age 61.3 years	Torcetrapib + Atorvastatin (7,533)	Atorvastatin (7,534)	1.0	-0.58	0.87	-0.12	NR	NR
42. IMPROVE-IT[154]	2005	ACS, age 63.6 years	Simvastatin + Ezetimibe (9,067)	Simvastatin (9,077)	6.0	-0.42	0.02	-0.19	977	1,118
43. HPS2-THRIVE[155]	2007	CVD, age 64.9 years	ER-Niacin + Laropriprant (12,835)	Placebo (12,835)	3.9	-0.26	0.16	-0.37	668	694

Trials[Ref]	Start year	Population condition	Active (N)	Control (N)	Follow up (years)	Absolute change of blood lipids between groups[##] (mmol/L)			Fatal or nonfatal MI	
						LDL-C	HDL-C	TG	Active	Control
44. dal-OUTCOMES[138]	2008	ACS, age 61 years	Dalcetrapib (7,938)	Placebo (7,933)	2.6	0.00	0.31	-0.05	NR	NR
45. REDUCE-IT[139]	2011	CVD, age 64 years	Icosapent ethyl (4,089)	Placebo (4,090)	4.9	-0.17	-0.04	-0.38	250	355
46. AIM-HIGH[156]	2011[#]	CVD, age 64 years	ER-Niacin + Simvastatin/ ezetimibe (1,718)	Simvastatin/ ezetimibe (1,696)	3.0	-0.08	0.13	-0.36	NR	NR
47. REVEAL[140]	2011	CHD, age 67 years	Anacetrapib (15,225)	Placebo (15,224)	4.1	-0.67	1.11	-0.11	934	1,048
48. ACCELERATE[157]	2012	CAD, age 65 years	Evacetrapib (6,038)	Placebo (6,054)	2.2	-0.75	1.51	-0.09	NR	NR
49. ODYSSEY OUTCOMES[141]	2012	Prior ACS, age 59 years	Alirocumab (9,462)	Placebo (9,462)	2.8	-0.96	0.00	-0.15	793	899
50. FOURIER[142]	2013	ASCVD, age 62.5 years	Evolocumab (13,784)	Placebo (13,780)	2.2	-1.45	0.09	-0.22	NR	NR
51. CLEAR HARMONY[143]	2016	ASCVD, age 66 years	Bempedoic acid (1,487)	Placebo (742)	1.0	-0.46	-0.07	-0.06	NR	NR
52. SPIRE-1[158]	2017[#]	CVD, age 62.9 years	Bococizumab (8,408)	Placebo (8,409)	0.6	-1.24	0.05	-0.15	NR	NR
53. SPIRE-2[158]	2017[#]	CVD, age 62.9 years	Bococizumab (5,312)	Placebo (5,309)	1.0	-1.48	0.07	-0.14	NR	NR

Abbreviations: ACCELERATE; Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibi- tion with Evacetrapib in Patients at a High Risk for Vascular Outcomes, **ACCORD**; Action to Control Cardiovascular Risk in Diabetes, **AFCAPS/TexCAPS**; Air Force/Texas Coronary Atherosclerosis Prevention Study, **AIM-HIGH**; Athero-thrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyc- erides: Impact on Global Health Out- comes, **ALERT**; Assessment of LEscol in Renal Transplantation, **ALLIANCE**; Aggressive Lipid-Lowering Initiation Abates New Cardiac Events, **ALLHAT-LLT**; Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial - Lipid Lowering Trial component, **ASCOT-LLA**; Anglo-Scandinavian Cardiac Outcomes Trial - Lipid Lowering Arm, **ASPEN**; Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in Non-Insulin-Dependent Diabetes Mellitus, **BIP**; Bezafibrate Infarction Prevention, **CARD**; Collaborative Atorvastatin Diabetes Study, **CARE**; Cholesterol and Recurrent Events Trial, **CLEAR HARMONY**; Cholesterol Lowering via Bempedoic Acid, an ACL-Inhibiting Regimen Harmony trial, **CORONA**; Controlled Rosuvastatin Multinational Trial in Heart Failure, **DEFINE**; Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib, **FIELD**; Fenofibrate Intervention and Event Lowering in Diabetes, **FOURIER**;

Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk, **GISSI-P**; Gruppo Italiano per lo Studio della Sopravvivenza nell'Insuffi cienza cardiaca - Prevenzione, **GISSI-HF**; Gruppo Italiano per lo Studio della Sopravvivenza nell'Insuffi cienza cardiaca - Heart Failure, **GREACE**; the Greek Atorvastatin and Coronary- heart-disease Evaluation, **HHS**; Helsinki Heart Study, **HOPE-3**; Heart Outcomes Prevention Evaluation-3, **HPS**; Heart Protection Study, **IDEAL**; Incremental Decrease in End Points Through Aggressive Lipid Lowering, **IMPROVE-IT**; Improved Reduction of Outcomes: Vytorin Efficacy International Trial, **ILLUMINATE**; Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events, **ILLUSTRATE**; Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation, **JUPITER**; Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin, **LEADER**; Lower Extremity Arterial Disease Event Reduction, **LIPID**; Long-Term Intervention with Pravastatin In Ischaemic Disease, **LIPS**; Lescol Intervention Prevention Study, **LRC-CPPT**; Lipid Research Clinics Coronary Primary Prevention Trial, **HPS2-THRIVE**; Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events, **MEGA**; Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese, **ODYSSEY LONG TERM**; Long-term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy, **OSLER**; Open-Label Study of Long-Term Evaluation against LDL Cholesterol, **POST CABG**; Post Coronary Artery Bypass Graft, **PROSPER**; Prospective Study of Pravastatin in the Elderly at Risk, **PROVE IT-TIMI 22**; Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22, **REDUCE-IT**; Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial, **REVEAL**; Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification, **SEARCH**; Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine, **SEAS**; Simvastatin and Ezetimibe in Aortic Stenosis, **SHARP**; Study of Heart and Renal Protection, **SPARCL**; Stroke Prevention by Aggressive Reduction in Cholesterol Levels, **SPIRE**; Studies of PCSK9 Inhibition and the Reduction of Vascular Events, **TNT**; Treating to New Targets, **VA-HIT**; Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study, **VASCA**; Veterans Administration Cooperative Study of Atherosclerosis, **WOSCOP**; West of Scotland Coronary Prevention Study, **4D**; Die Deutsche Diabetes Dialyse. **4S**; Scandinavian Simvastatin Survival Study, ACS; acute coronary syndromes, ASCVD; atherosclerotic cardiovascular disease, CAD; coronary artery disease, CHD; coronary heart disease, CRP; C-reactive protein, CHF; chronic heart failure, CKD; chronic kidney disease, CVD; cardiovascular disease, DM; diabetes mellitus, ER; extended release, HD; haemodialysis, LDL-C; low density lipoprotein-cholesterol, MI; myocardial infarction, NR; not reported, PCI; percutaneous coronary intervention, SA; stable angina, TIA; transient ischaemic attack.

[##]Calculated from (change of lipids from baseline of active group) minus change of lipids from baseline in control group

Table S 2-12 Summary of the quality assessment of included cohort studies (21 studies) based on Newcastle-Ottawa criteria

Author, Year	Selection (4)				Compar-ability (2)		Outcome (3)			Total Score (9)
	Representativeness	Selection of non-exposed cohort	Ascertainment of exposure	Outcome of interest not present at baseline	Controlled for main factors	Controlled for additional factors	Assessment of outcome	Length of follow-up	Adequacy of follow-up	
Wang, 2010[106]	1	1	0	1	1	1	1	1	0	7
Barkas, 2017[107]	0	1	0	1	0	0	1	1	0	4
Ingelsson, 2005[108]	0	1	0	0	1	1	1	1	0	5
Bibbins-Domingo, 2009[72]	0	1	0	0	1	0	1	1	1	5
Sciacqua, 2015[68]	1	1	1	0	1	1	1	1	0	7
He, 2017[109]	0	1	0	1	1	1	1	1	0	6
Knuiman, 2014[75]	1	1	0	1	0	0	1	1	0	5
Smith, 2010[74]	1	1	0	1	1	1	1	1	0	7
Ebong, 2013[78]	1	1	1	1	1	1	1	1	1	9
Velagaleti, 2009[71]	0	1	1	1	1	1	1	1	1	8
Alonso, 2014[31]	1	1	0	1	1	1	1	1	0	7
Kodani, 2019[110]	0	1	0	1	1	1	1	1	0	6
Dhingra, 2008[77]	0	1	1	1	1	1	0	1	0	6
Lopez, 2012[32]	0	1	0	1	1	1	1	1	1	7
Kim, 2018[111]	0	1	0	1	1	0	1	1	0	5

Author, Year	Selection (4)				Compar-ability (2)		Outcome (3)			Total Score (9)
	Representativeness	Selection of non-exposed cohort	Ascertainment of exposure	Outcome of interest not present at baseline	Controlled for main factors	Controlled for additional factors	Assessment of outcome	Length of follow-up	Adequacy of follow-up	
Mora, 2002[76]	0	1	1	1	1	1	0	1	0	6
Watanabe,2011[69]	0	1	0	1	0	0	1	0	0	3
Mourtzinis,2018[70]	0	1	0	1	1	0	1	0	0	4
Holme, 2009[112]	0	1	0	1	0	1	1	1	0	5
Li, 2018[73]	1	1	1	1	1	1	1	1	0	**8**
Varbo, 2018[113]	0	1	0	0	1	1	1	1	0	5
Sum score (21)	7	21	6	17	17	15	19	19	4	-

Note: A study with total score of ≥ 7 with no individual component score of 0 will be considered as “good quality” (embolden figures).

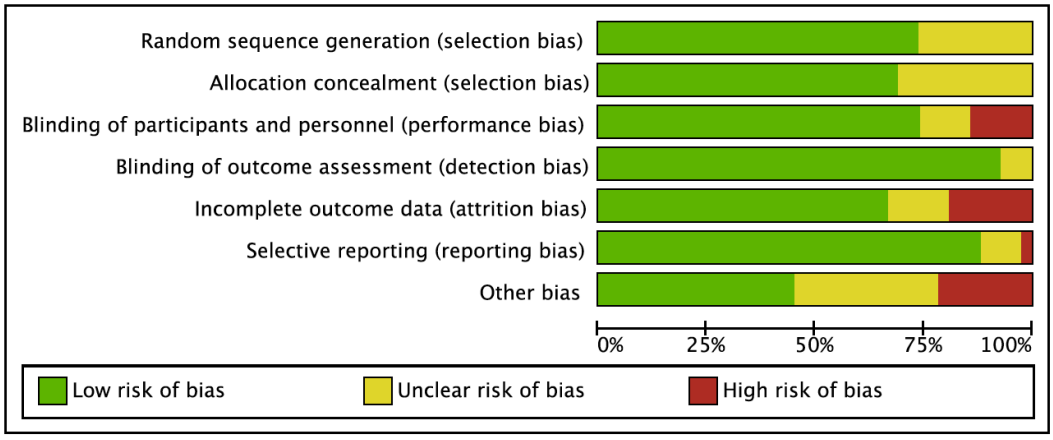


Figure S 2-4 Risk of bias graph from 38 included RCTs

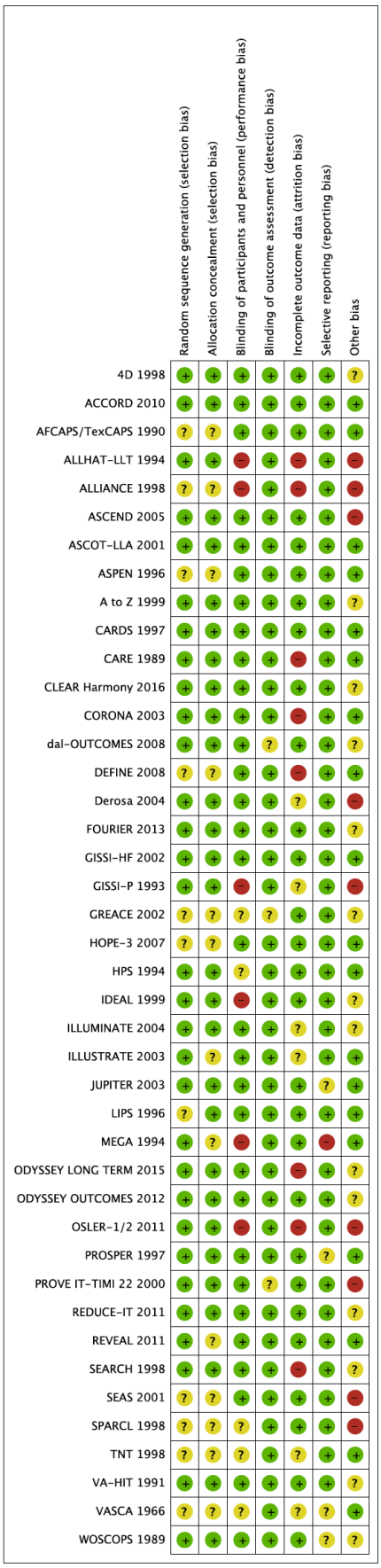


Figure S 2-5 Summary of the risk of bias from 38 included RCTs

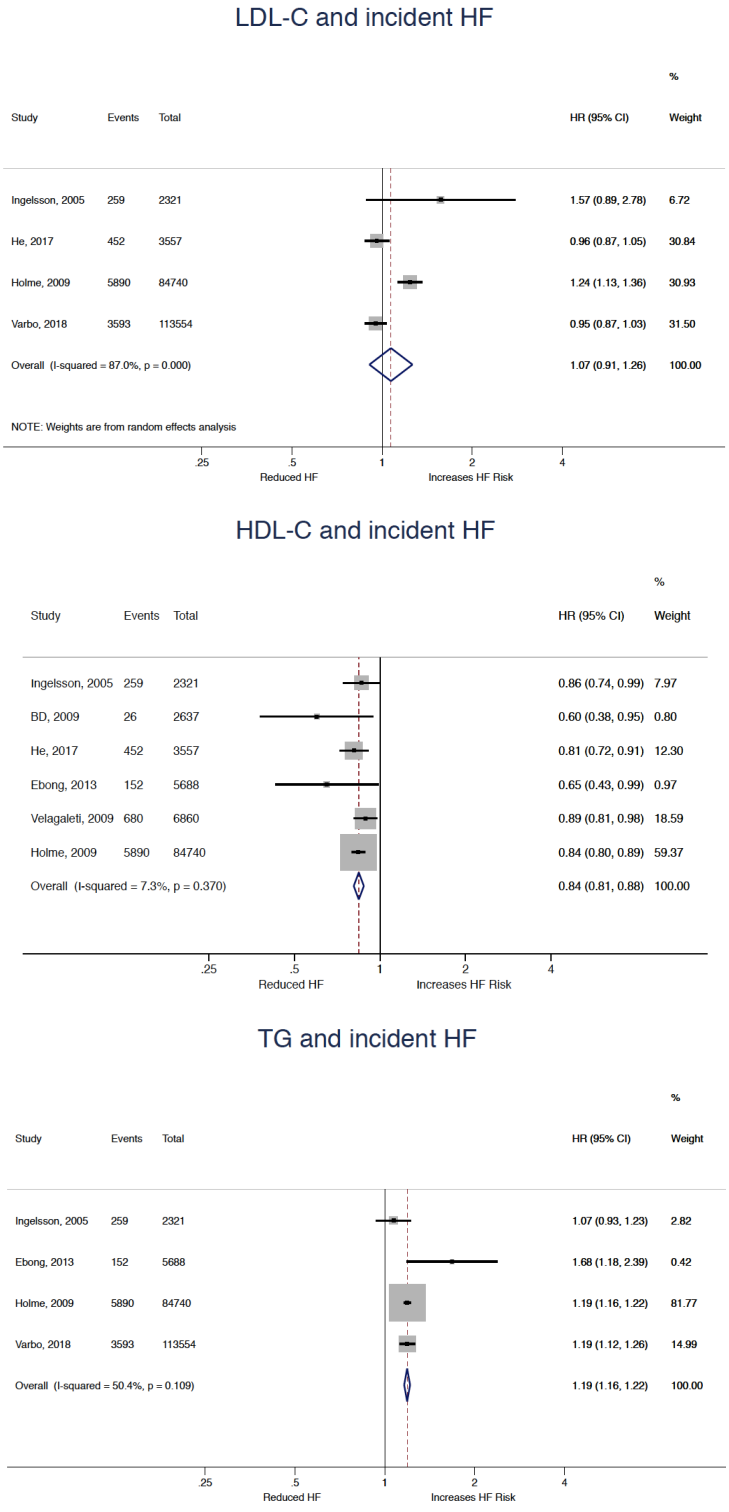


Figure S 2-6 Meta-analysis of the included cohort studies (n=7) on the association between blood lipids and incident HF

Note: Hazard ratio (HR) per 1 SD increase in lipid fractions

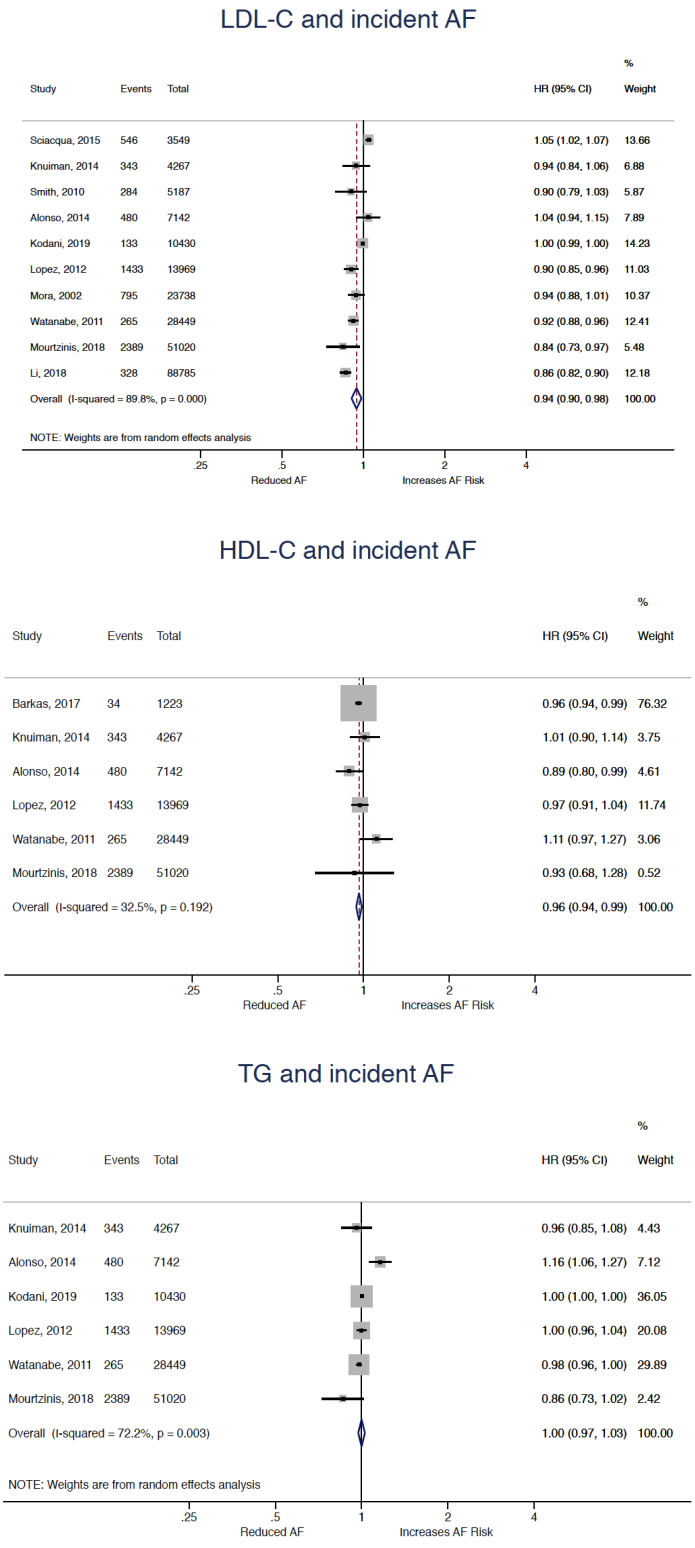


Figure S 2-7 Meta-analysis of the included cohort studies (n=11) on the association between blood lipids and incident AF

Note: Hazard ratio (HR) per 1 SD increase in lipid fractions

Table S 2-13 Sensitivity analysis of meta-analysis of cohort studies on HF (7 studies) and AF (11 studies) outcomes

Lipid fractions	Hazard ratio (95%CI) per 1 SD increase in lipid fractions, number of included studies (n) and heterogeneity parameters (I^2 and $P_{heterogeneity}$)				
	Main results	Sensitivity 1		Sensitivity 2	Sensitivity 3
		Fixed-effect	Random-effect		
Heart failure					
LDL-C	1.07 (0.91, 1.26)	1.04 (0.99, 1.09)	Main results	0.96 (0.89, 1.05)	1.13 (0.72, 1.77)
	n = 4 I^2 = 87.0 % $P_{heterogeneity}$ <0.001	n = 4 I^2 = 87.0 % $P_{heterogeneity}$ <0.001		n = 3 I^2 = 30.8 % $P_{heterogeneity}$ = 0.24	n = 2 I^2 = 63.7 % $P_{heterogeneity}$ =0.097
HDL-C	**0.84 (0.81, 0.88)**	Main results	**0.84 (0.80, 0.88)**	**0.84 (0.77, 0.91)**	**0.84 (0.77, 0.91)**
	n = 6 I^2 = 7.3 % $P_{heterogeneity}$ = 0.37		n = 6 I^2 = 7.3 % $P_{heterogeneity}$ = 0.37	n = 5 I^2 = 25.4 % $P_{heterogeneity}$ = 0.25	n = 5 I^2 = 25.4 % $P_{heterogeneity}$ = 0.25
TG	**1.19 (1.16, 1.22)**	Main results	**1.18 (1.12, 1.25)**	**1.20 (1.04, 1.38)**	1.30 (0.84, 2.02)
	n = 4 I^2 = 50.4 % $P_{heterogeneity}$ = 0.11		n = 4 I^2 = 50.4 % $P_{heterogeneity}$ = 0.11	n = 3 I^2 = 66.5 % $P_{heterogeneity}$ = 0.05	n = 2 I^2 = 81.9 % $P_{heterogeneity}$ =0.019
Atrial fibrillation					
LDL-C	**0.94 (0.90, 0.98)**	0.99 (0.99, 1.00)	Main results	0.96 (0.92, 1.00)	0.94 (0.88, 1.01)
	n = 10 I^2 = 89.8 % $P_{heterogeneity}$ <0.001	n = 10 I^2 = 89.8 % $P_{heterogeneity}$ <0.001		n = 8 I^2 = 85.7 % $P_{heterogeneity}$ <0.001	n = 4 I^2 = 50.8 % $P_{heterogeneity}$ = 0.11
HDL-C	**0.96 (0.94, 0.99)**	Main results	0.97 (0.93, 1.01)	0.97 (0.93, 1.01)	0.96 (0.90, 1.02)
	n = 6 I^2 = 32.5 % $P_{heterogeneity}$ = 0.19		n = 6 I^2 = 32.5 % $P_{heterogeneity}$ = 0.19	n = 6 I^2 = 32.5 % $P_{heterogeneity}$ = 0.19	n = 3 I^2 = 26.9 % $P_{heterogeneity}$ = 0.25
TG	1.00 (0.97, 1.03)	1.00 (1.00, 1.00)	Main results	1.00 (0.97, 1.03)	1.04 (0.94, 1.15)
	n = 6 I^2 = 72.2 % $P_{heterogeneity}$ =0.003	n = 6 I^2 = 72.2 % $P_{heterogeneity}$ =0.003		n = 6 I^2 = 72.2 % $P_{heterogeneity}$ =0.003	n = 3 I^2 = 79.4 % $P_{heterogeneity}$ =0.008

Note: Embolden figure represents statistically significant value.

Sensitivity 1: Varying the model used for pooled estimation (i.e., fixed- vs random effect model). Sensitivity 2: Excluding studies that did not report their effect size as per continuous change in lipid fractions. The pooled estimation was based on a random-effect model. Sensitivity 3: Sensitivity 2 with additional excluding studies that did not use the unit of change in lipid fractions as per standard deviation (SD) change or those that did not report the effect size as hazard ratio (HR). The pooled estimation was based on a random-effect model.

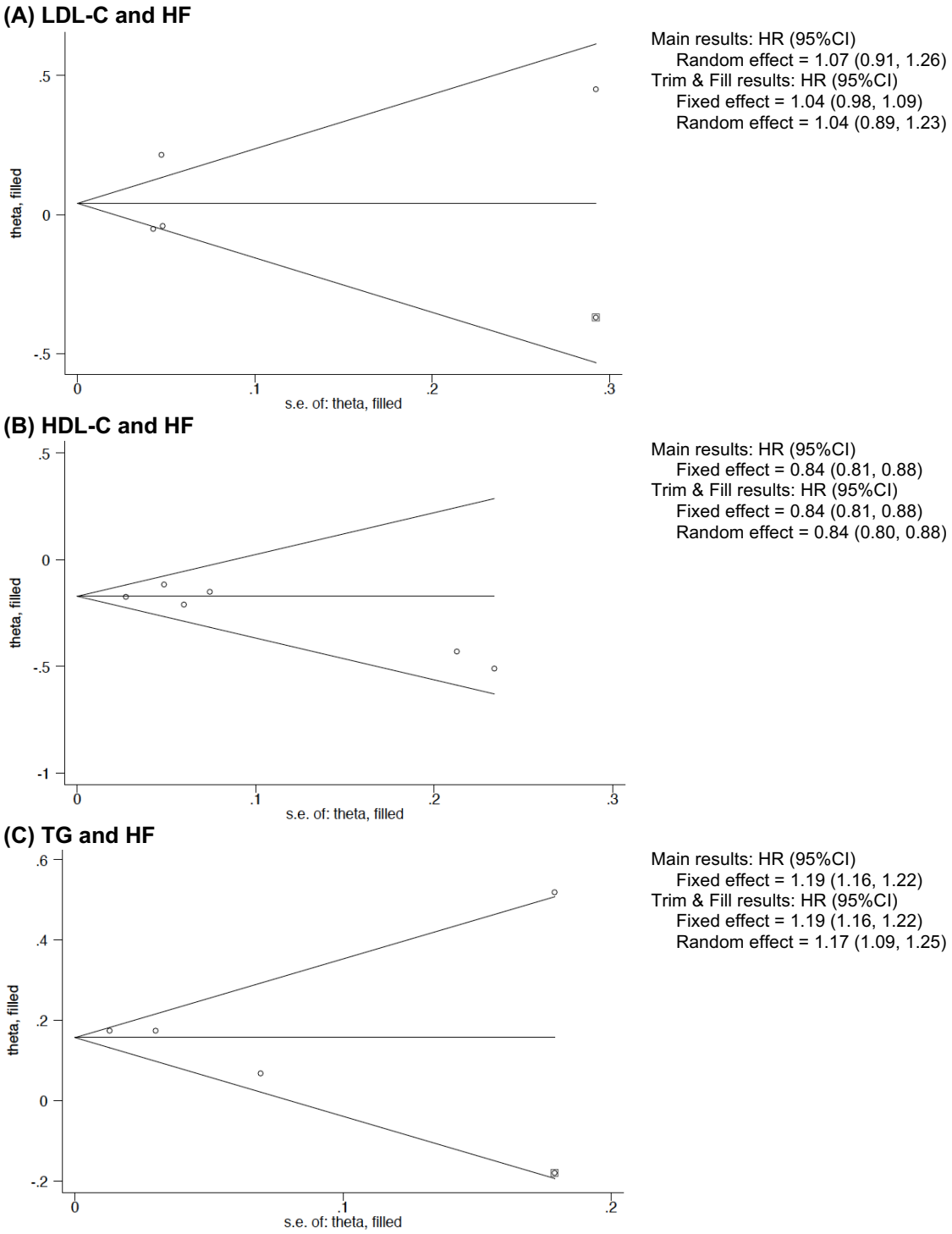


Figure S 2-8 Funnel plots and results from Trim & Fill method in cohort studies of (A) LDL-C (4 studies), (B) HDL-C (6 studies), and (C) TG (4 studies) on HF outcome

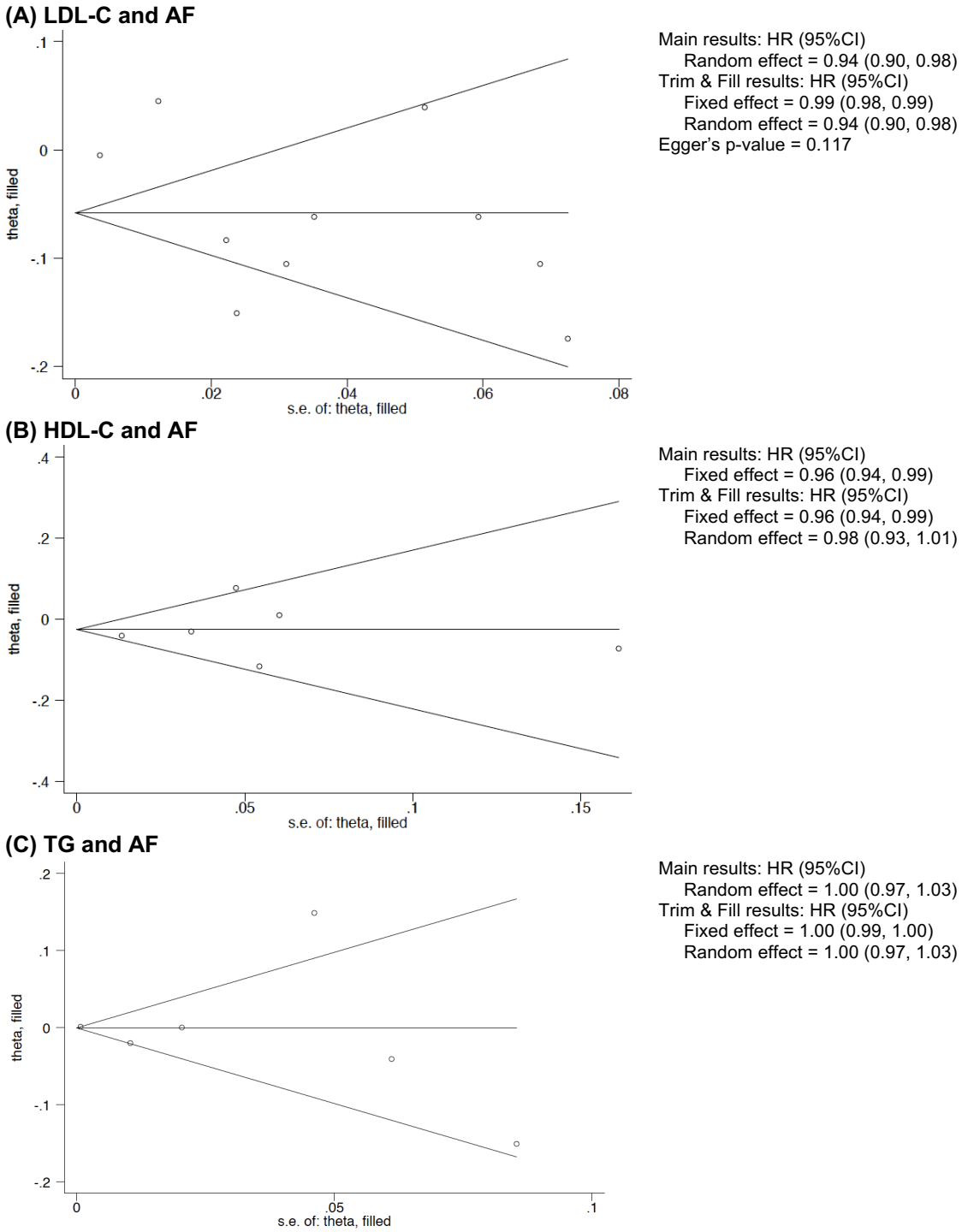


Figure S 2-9 Funnel plots and results from Trim & Fill method in cohort studies of (A) LDL-C (10 studies), (B) HDL-C (6 studies), and (C) TG (6 studies) on AF outcome

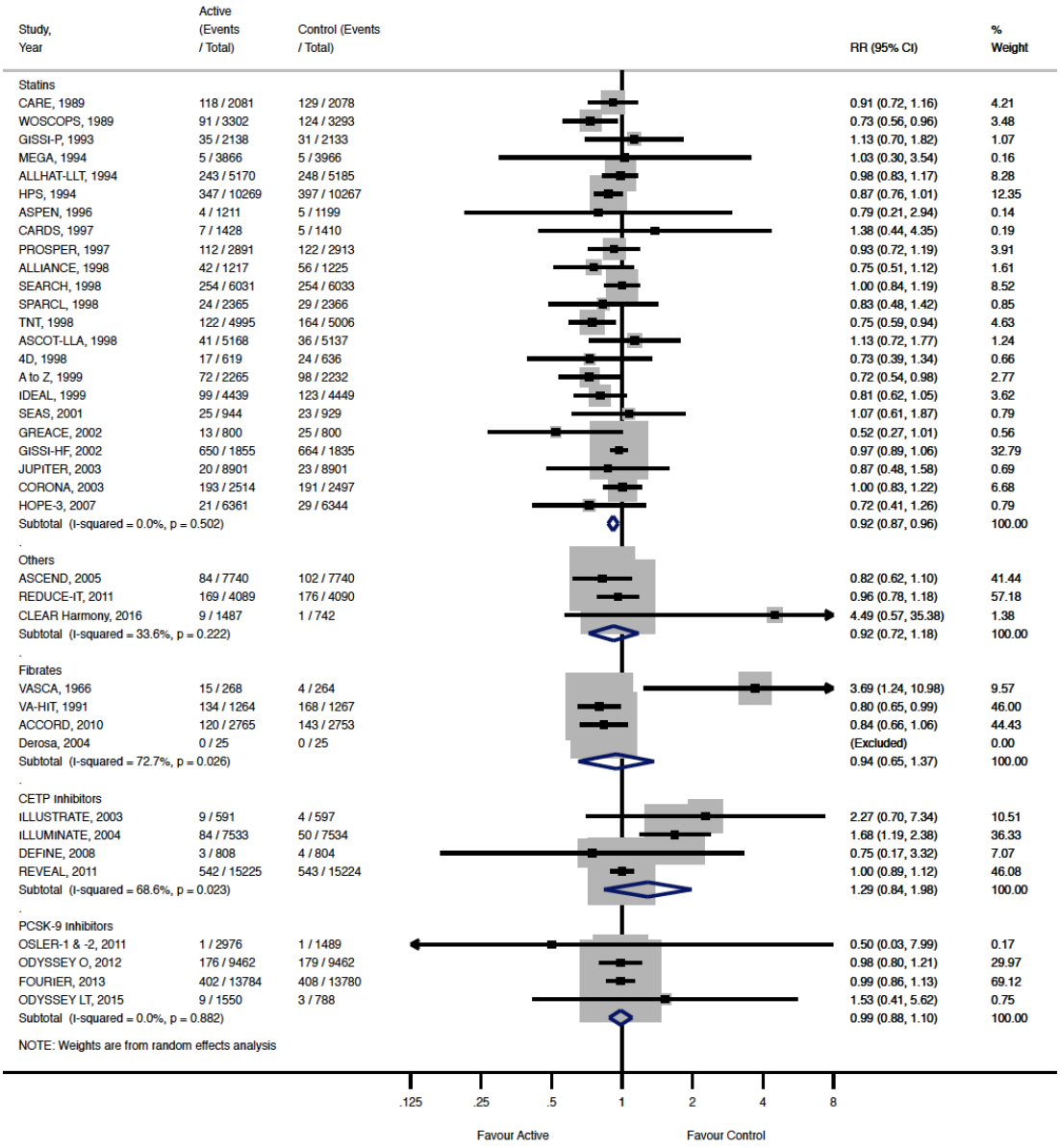


Figure S 2-10 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by groups of medications

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

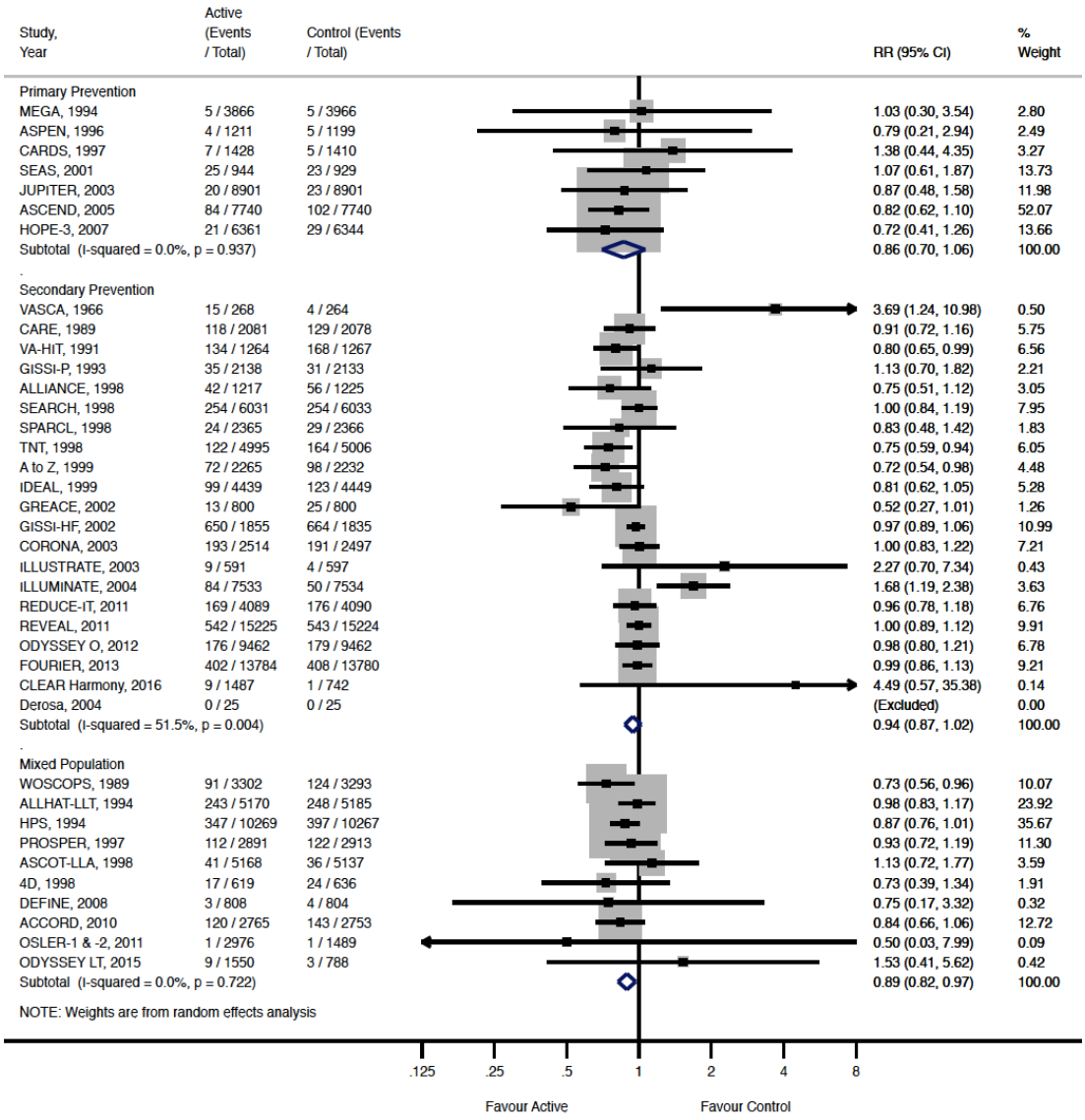


Figure S 2-11 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by types of prevention

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

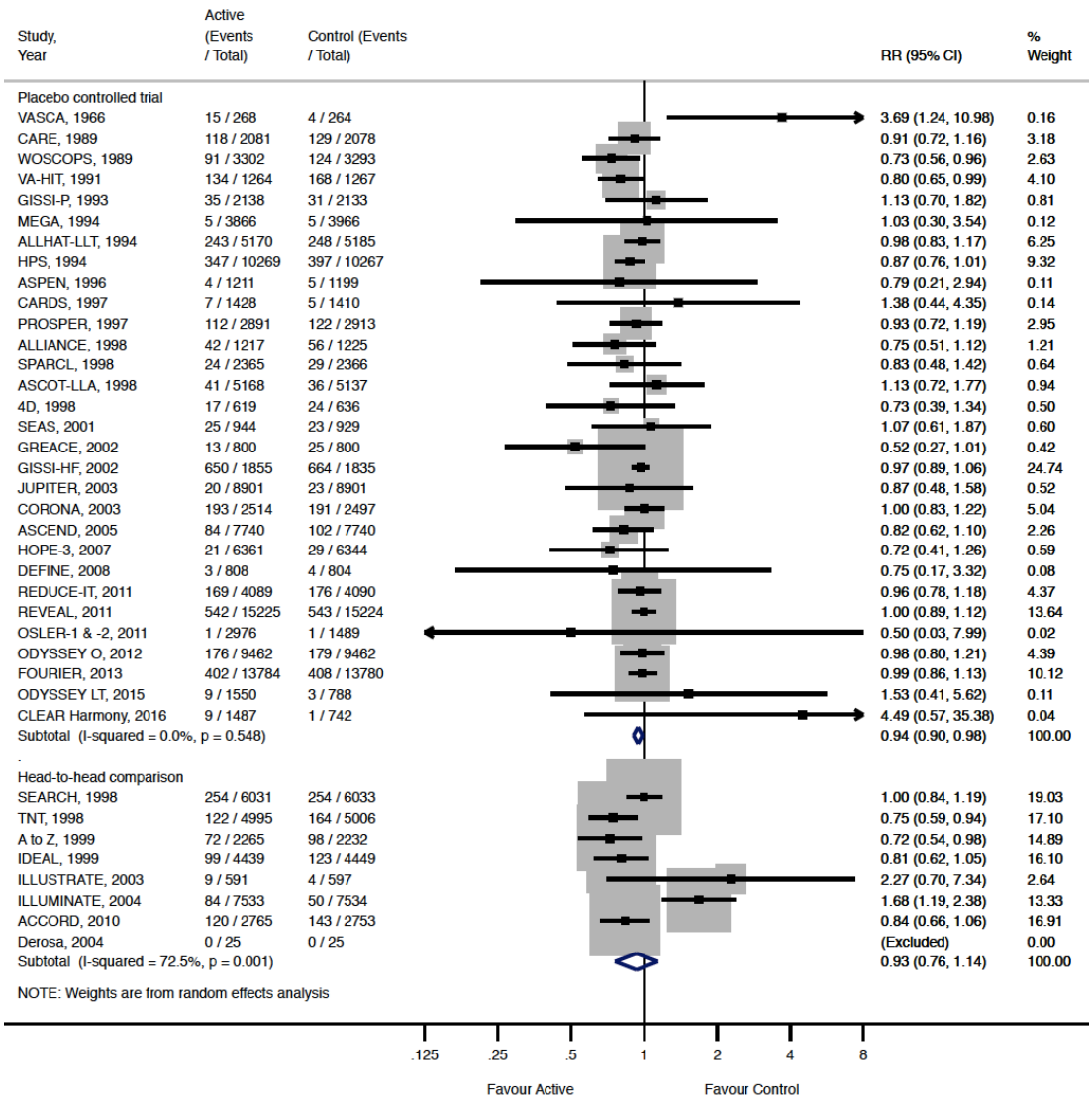


Figure S 2-12 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by types of comparison

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

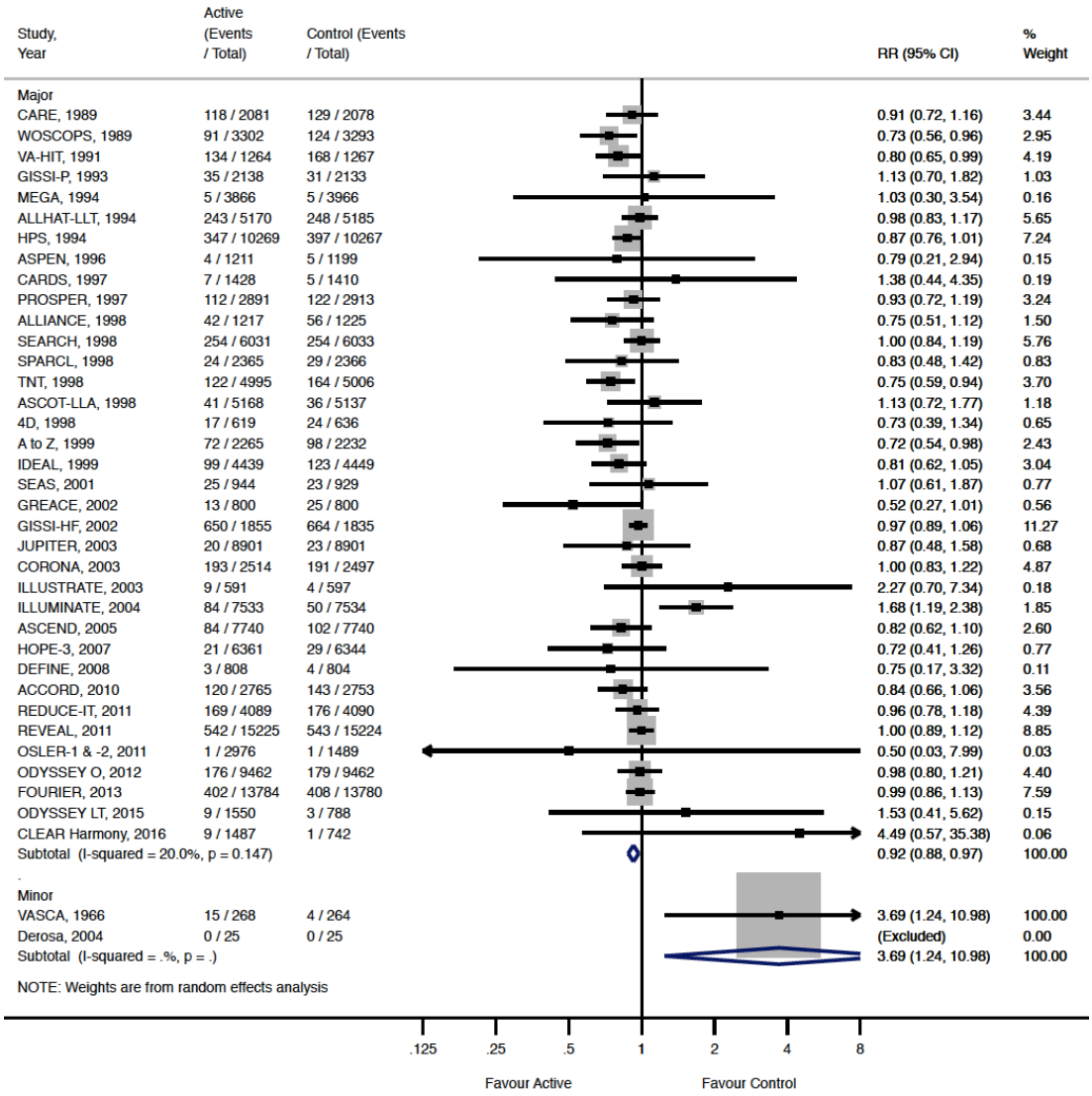


Figure S 2-13 Meta-analysis of RCTs (38 trials) on the association between lipid-lowering agents and the risk of HF stratified by sizes of trials

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

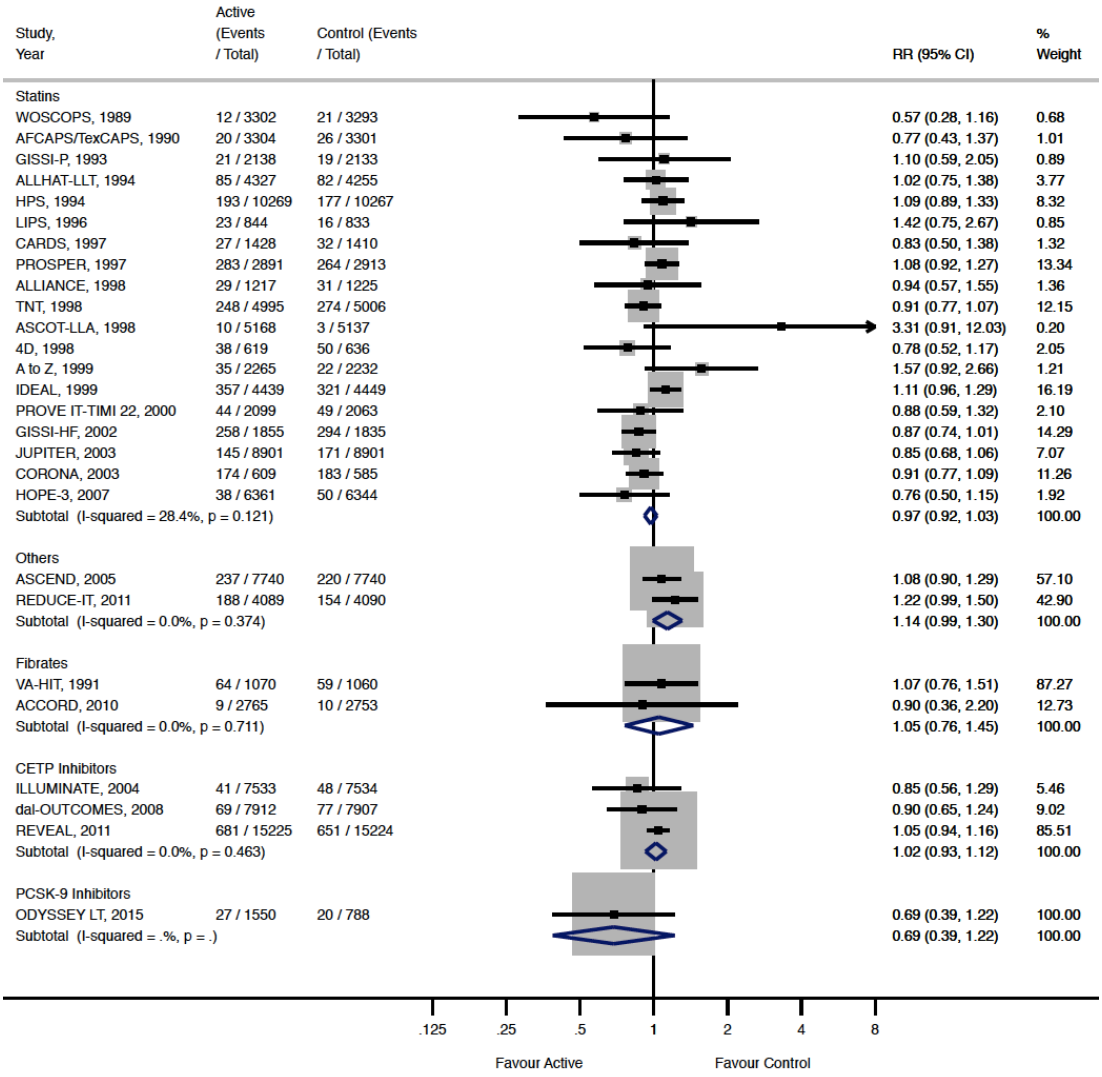


Figure S 2-14 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by groups of medications

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

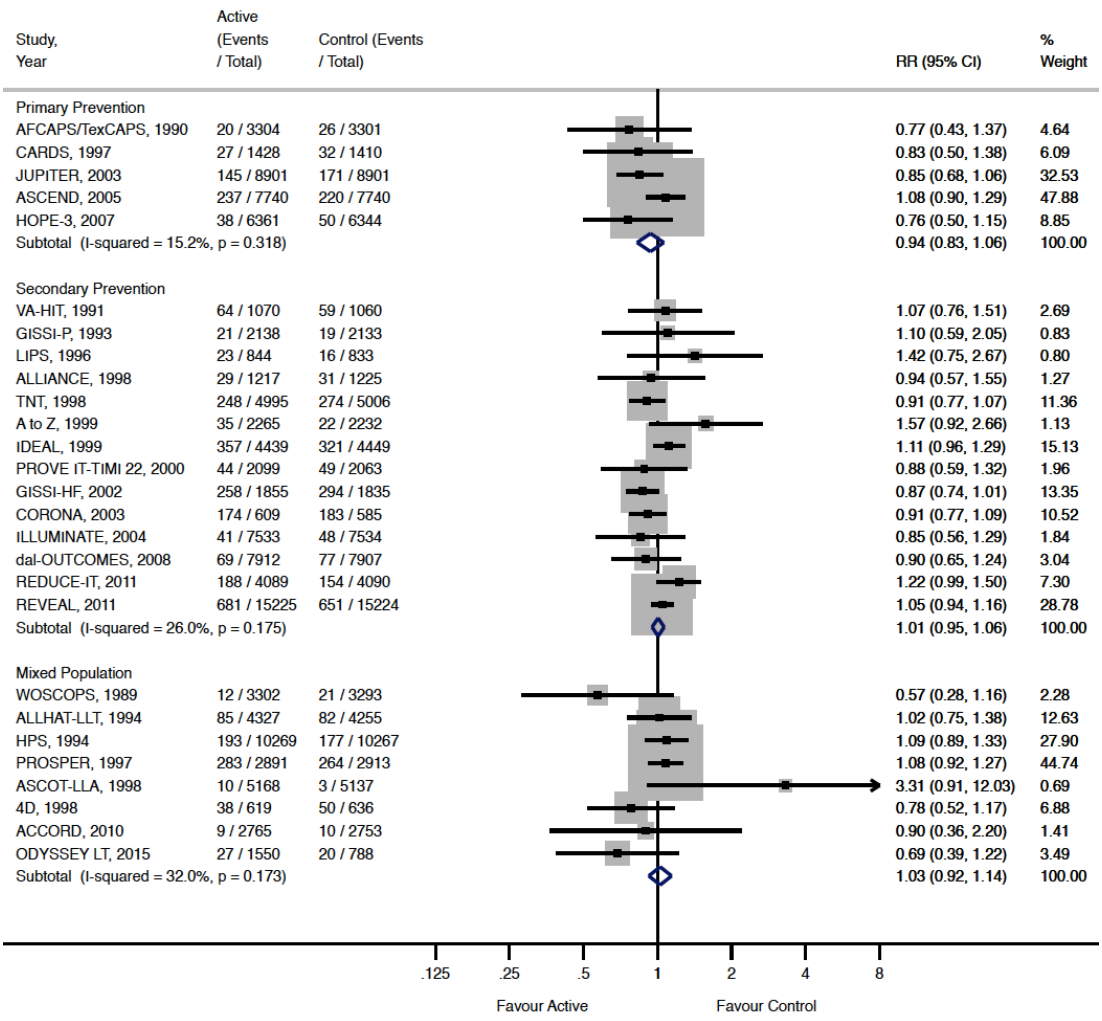


Figure S 2-15 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by types of prevention

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

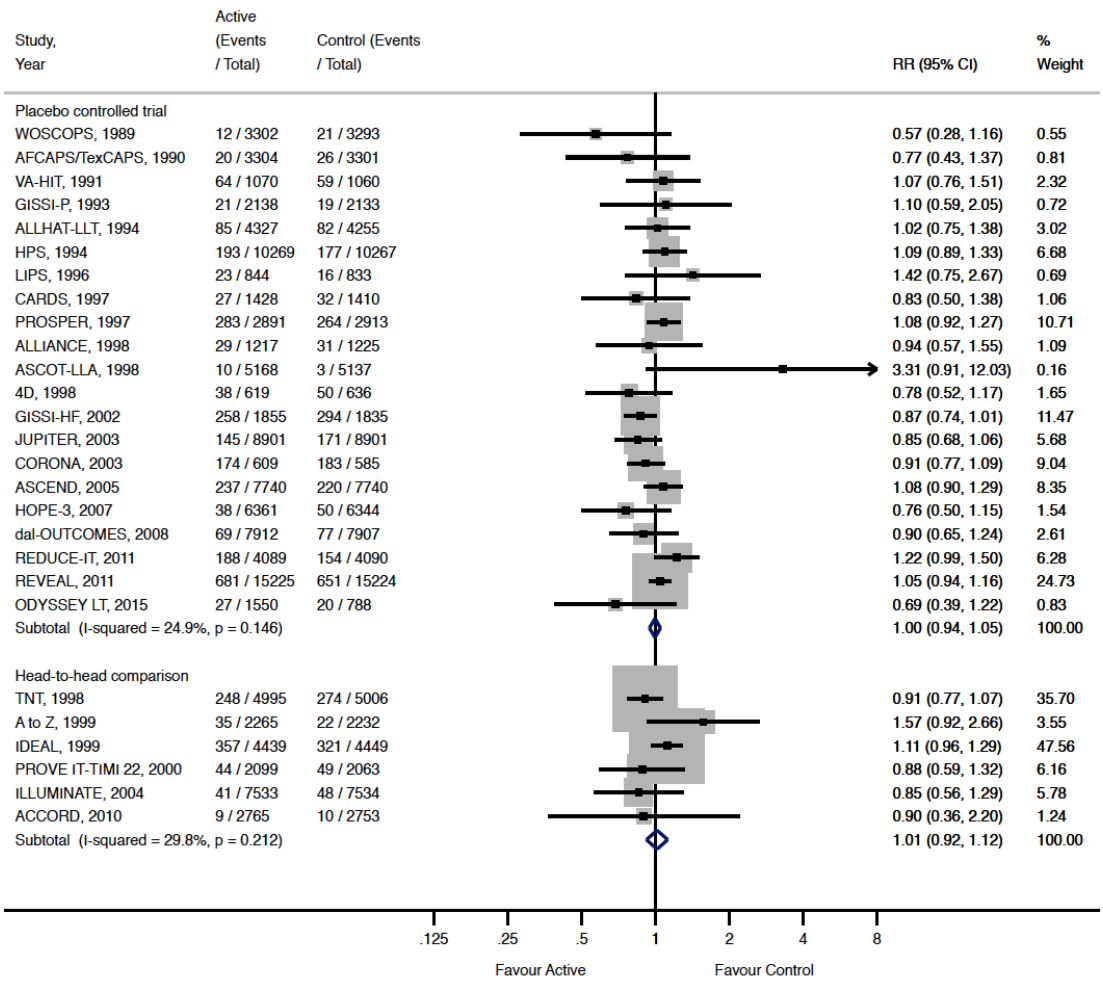


Figure S 2-16 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by types of comparison

Abbreviations: RR; Relative Risk, CI; Confidence Interval.

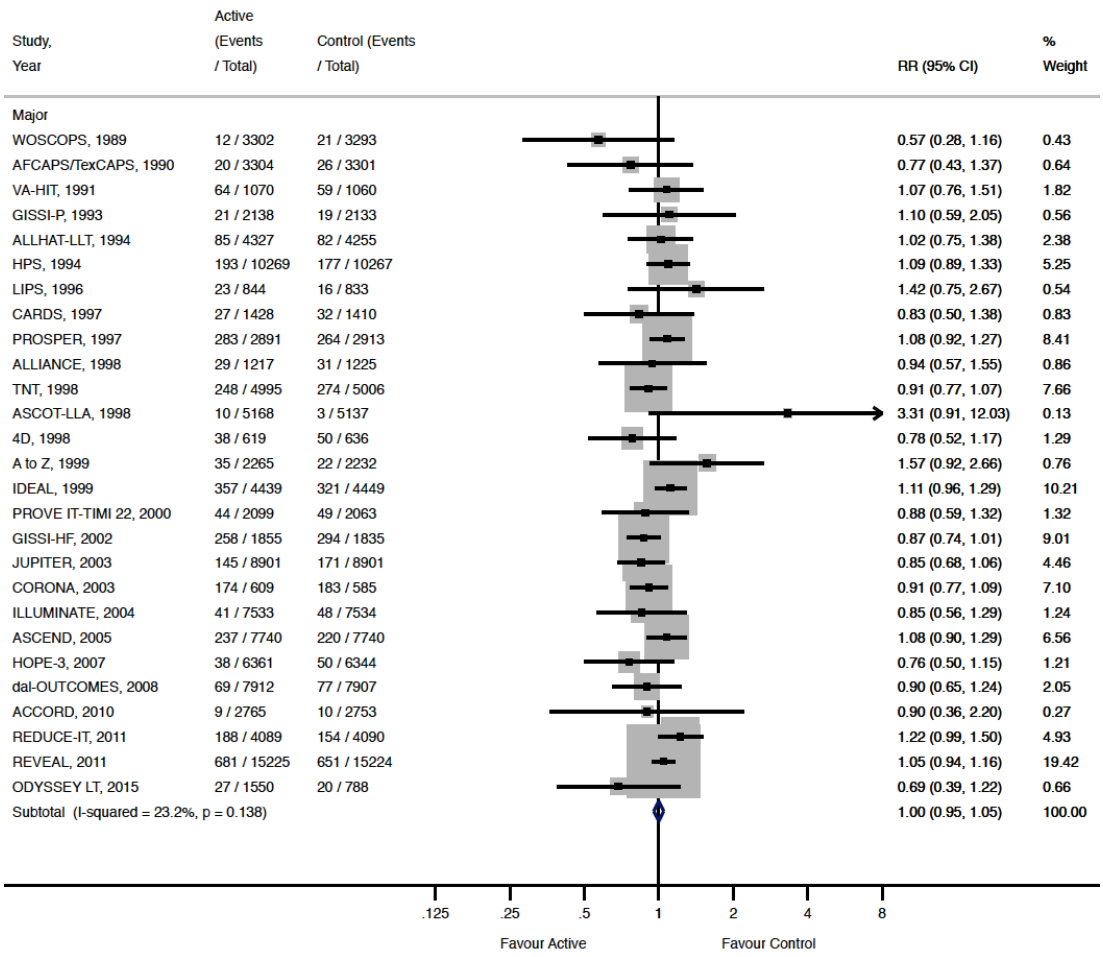


Figure S 2-17 Meta-analysis of RCTs (27 trials) on the association between lipid-lowering agents and the risk of AF stratified by sizes of trials

Note: No small trials reported AF outcome. **Abbreviations:** RR; Relative Risk, CI; Confidence Interval.

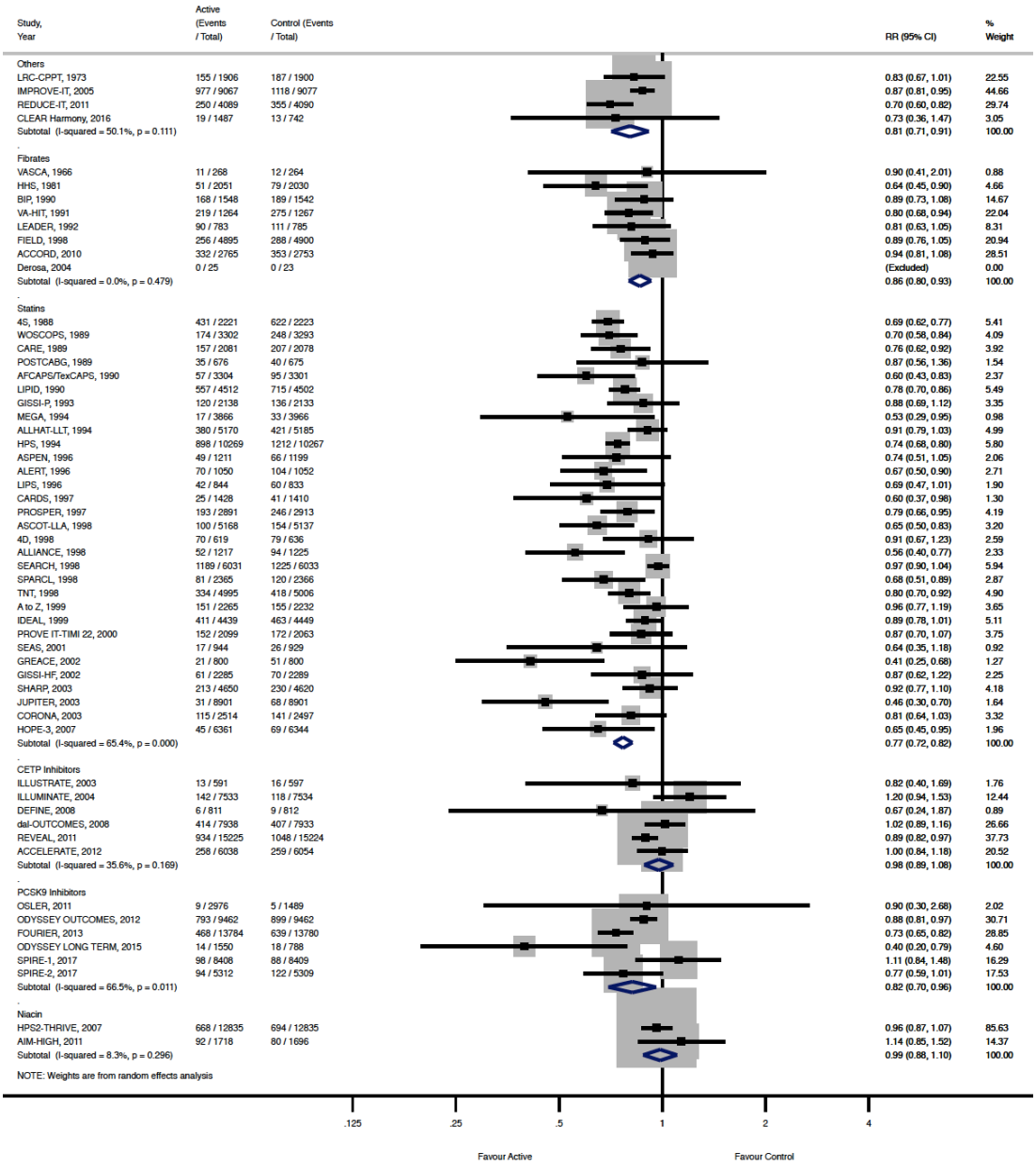


Figure S 2-18 Meta-analysis of RCTs (57 trials) on the association between lipid-lowering agents and the risk of MI stratified by classes of medications

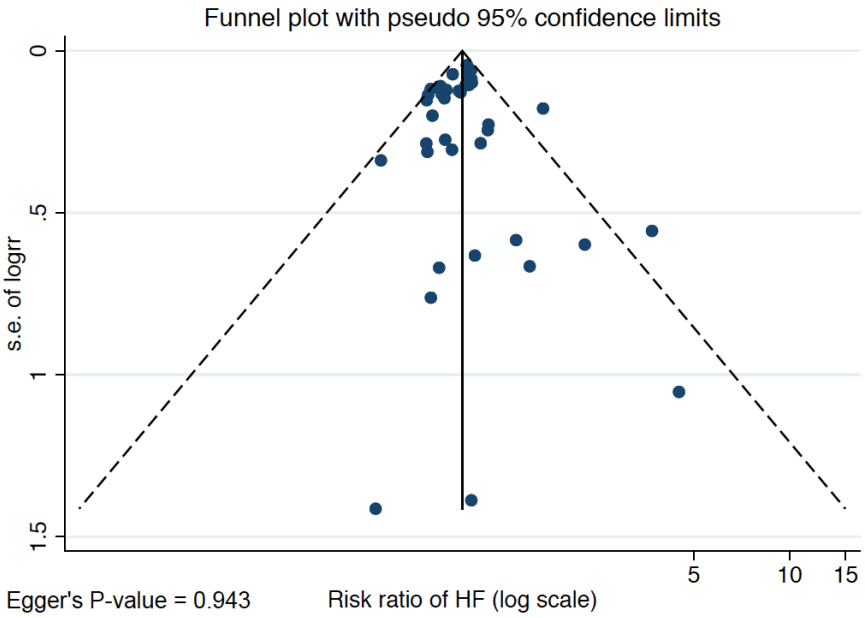


Figure S 2-19 Funnel plots of RCTs on lipid-lowering agents and the risk of HF (38 trials)

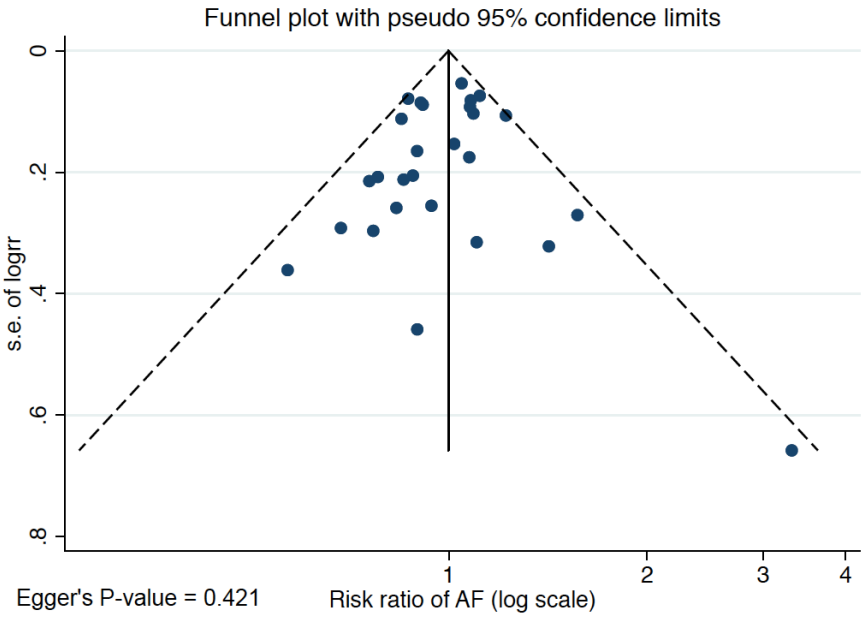


Figure S 2-20 Funnel plots of RCTs on lipid-lowering agents and the risk of AF (27 trials)

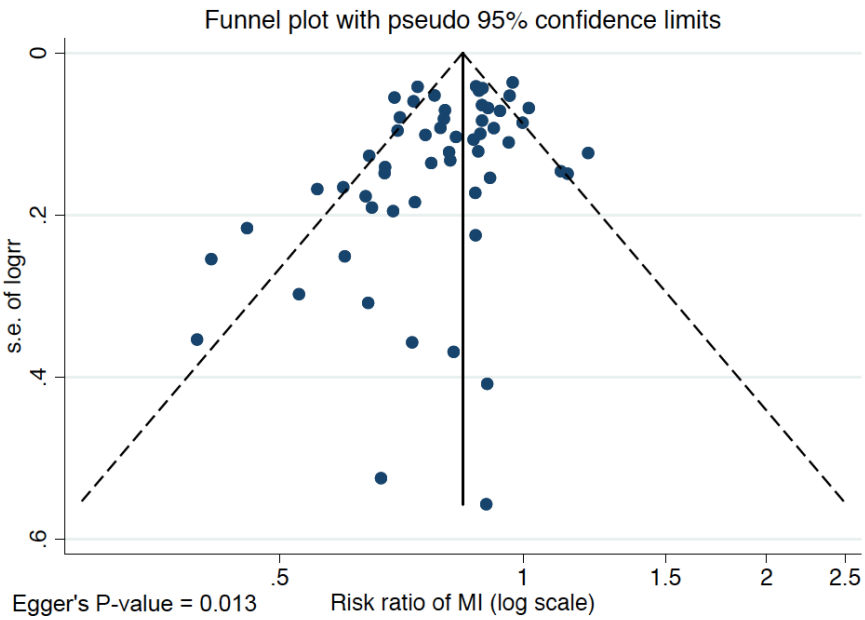


Figure S 2-21 Funnel plots of RCTs on lipid-lowering agents and the risk of MI (57 trials)

Table S 2-14 Summary of genetic studies on the association between lipid traits and the risk of MI and AF

Study, year	Population	Genetic instrument	Outcome	Summary of findings		
				LDL-C	HDL-C	TG
CHD or MI outcome						
Ridker, 2009[100]	Caucasians women age 45 years or older without history of CVD, CA, or other major chronic illness (n=18,245)	20 SNPs at CETP locus	Incident MI after 10 years of follow-up (full medical record review) (198 events)	NR	-	NR
Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration, 2010[159]	73,252 individuals without prior CVD at time of measurement (most were Caucasian) from 39 studies	rs662799 SNPs (–1131T>C) of the apolipoprotein A5 (APOA5) gene	CAD (20,842 cases and 35,206 controls)	NR	NR	+
Ference, 2012[160]	312,321 individuals from various studies (meta-analysis)	9 SNPs in 6 genes (SORT1, PCSK9, LDLR, HMGCR, ABCG8, and APOE)	CHD (i.e., cardiovascular death, nonfatal myocardial infarction, or coronary revascularization)	+	NR	NR
Haase, 2012[161]	54,500 individuals from general populations of 2 cohort studies in Denmark	rs4986970 SNPs in LCAT gene (R^2 0.1%)	MI (review hospital records and national registry) (6,195 events)	NR	0	NR
Voight, 2012[162]	Individuals (self-reported European or South Asian ancestry, not reported N) from 20 studies	14 SNPs (HDL-C at LIPG Asn396Ser gene), 13 SNPs (LDL-C, positive control)	MI (12,482 cases and 41,331 controls)	+	0	NR
Do, 2013[163]	95,454 individuals of European ancestry (GLGC consortium)	185 SNPs for LDL-C, HDL-C, and TG	CAD (86,000 individuals: 22,233 cases and 64,762 controls)	+	0	+
Holmes, 2015[164]	62,199 Individuals (Caucasians) from 17 studies	19 SNPs for LDL-C, 19 SNPs for HDL-C and 27 SNPs for TG (restricted to SNPs not related to other lipid traits)	CHD (12,099 combined incident [7,339] and prevalent cases)	+	0	0
Tragante, 2016[165]	2 Sample-MR method: LDL-C SNPs taken from GLGC: 95,454 individuals of European ancestry	197 SNPs for LDL-C (threshold pairwise $R^2 < 0.8$)	CAD from CARDIoGRAMplusC4D consortium (63,746 cases and 130,681 controls of European ancestry)	+	NR	NR
van Iperen, 2016[166]	2 Sample-MR method: Lipids SNPs were from 3 studies (limit to Caucasians)	54 SNPs for LDL-C, 74 SNPs for HDL-C, and 48 SNPs for TG	CAD from CARDIoGRAM consortium (22,233 cases and 64,762 controls)	+	-	+
White, 2016[62]	2 Sample-MR method: Lipids SNPs taken from GLGC (n=188,577) and CAD SNPs taken from CARDIoGRAMplusC4D	130 SNPs for LDL-C (R^2 7.9%), 140 SNPs for HDL-C (R^2 6.6%), and 140 SNPs for TG (R^2 5.9%)	CAD from CARDIoGRAMplusC4D consortium (63,746 cases and 130,681 controls of European ancestry)	+	0	+
AF outcome						
AFGen Consoritum, 2016[92]	64,901 Caucasian individuals without history of AF at baseline	37 SNPs for LDL-C (13 non-pleiotropic SNPs), 47 SNPs for HDL-C (14 non-pleiotropic SNPs), 27 SNPs for TG, and 52 SNPs for Total cholesterol	AF (5,434 incident cases) after the mean follow-up of 12 years	0	0	0

Note: -/0/+ represent inverse (lower risk per levels increase), null, and direct (increase risk per levels increase) association.

Abbreviations: AF; Atrial fibrillation, CAD; Coronary artery disease, CHD; Coronary heart disease, CVD; Cardiovascular disease, HDL-C; High-density lipoprotein cholesterol, LDL-C; Low-density lipoprotein cholesterol, MI; Myocardial infarction, NR; Not reported, TG; Triglyceride.

Table S 2-15 Summary of GRADE quality of evidence from the meta-analysis of RCTs

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with placebo	Risk with Medications				
Heart failure (HF) follow up: range 1 to 7.4 years	31 per 1,000	**29 per 1,000** (28 to 30)	**RR 0.94** (0.90 to 0.97)	297788 (38 RCTs)	⨁⨁◯◯ LOW [a,b,c]	
Atrial fibrillation follow up: range 1 to 7.4 years	29 per 1,000	**29 per 1,000** (28 to 31)	**RR 1.00** (0.95 to 1.05)	228829 (27 RCTs)	⨁◯◯◯ VERY LOW [c,d,e,f,g]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Explanations

a. Potential performance bias due to unclear blinding of participants or personnel (ALLHAT-LLT, ALLIANCE, IDEAL)

b. Potential attrition bias due to significant or incomparable drop-out or lost to follow-up (ALLHAT-LLT, ALLIANCE, CARE, CORONA, SEARCH)

c. Potential indirectness of evidence due to differences in the population (i.e., primary, secondary, mixed prevention setting), intervention (i.e., types of lipid-lowering agents), and comparison (i.e., head-to-head vs placebo)

d. Potential performance bias due to unclear blinding of participants or personnel (ALLHAT-LLT, IDEAL)

e. Potential attrition bias due to significant or incomparable drop-out or lost to follow-up (ALLHAT-LLT, CORONA)

f. Other bias due to self-reporting outcome and inadequate missingness handling (ASCEND)

g. Uncertainty of effect size of one with 95% CI crossing null-value of one

CHAPTER 3 ELECTRONIC HEALTH RECORD (EHR) COHORT: STRENGTHS, LIMITATIONS, AND APPROACH TO EHR PHENOTYPE VALIDATION

3.1 Abstract

The use of electronic health records (EHRs) in research has become increasingly popular. Despite the fact that EHRs are primarily designed for clinical care purposes, their applications have been expanded such that they are widely used in epidemiological research ranging from cross-sectional research in individual hospitals to longitudinal research cohorts at a national level.

In the previous chapter, I had shown that my PhD is going to be about HF, AF, MI, and blood lipids. This requires a large-scale cohort with a high resolution of disease and range of biomarkers. Therefore, the use of electronic health records (EHRs), which can provide all demanded features, is crucial.

In this chapter, I describe EHRs, going from a broad perspective to a specific example of the EHR platform in England (CALIBER), which I am using to produce my PhD work in this thesis. The overall objective of this chapter is to describe the cohort creation process and present the validity of the cohort used throughout the thesis based on different types of evidence. The content of this chapter, therefore, can be divided into three sections as follows. Firstly, I will briefly introduce EHR as a rich resource for clinical and epidemiological research. Secondly, the CALIBER platform is explained in terms of data sources and approach to data curation, which is a required process to transform the clinical and administrative data to the data that can be used in research, particularly the curation of the EHR phenotype. Thirdly, details of studied populations and phenotypes used in this thesis will be illustrated with the main focus on the validation of each component of the studied cohort (i.e., outcome, exposure, and covariates). In addition, limitations of the CALIBER platform will be raised, and suggestions for potential improvement will be given at the end of this chapter.

3.2 Introduction to Electronic Health Records (EHRs)

3.2.1 Background

Before the 1950s, vital statistics (i.e., government-recorded live births, deaths, fetal deaths, marriages, and divorces) were often used to conduct cross-sectional and time-series studies, mostly in relation to non-communicable diseases. However, the main problem due to temporal relationships limited causal inference, which led to the arising of funding in developing cohorts of individuals who were followed up longitudinally in the second half of the 20th century. Due to the issues of cost and time consumption of these prospective cohorts, in the 21st century, the use of EHRs as an alternative method of conducting research has been rising. A relatively low-cost way of accessing rich longitudinal data on large populations makes EHRs persuasive for a lot of researchers in the epidemiological field.[167]

EHRs were originally developed for billing purposes, but then in 2009, their uses were expanded by meaningful use requirements expressed in the Health Information Technology for Economic and Clinical Health (HITECH) Act, part of the 2009 American Recovery and Reinvestment Act. In 2012, more than two-thirds of primary care physicians in the US reported using EHRs, an increase of around one-fourths from 2009. In other countries, the current usage of EHRs ranges from lower levels in China and South Korea to nearly universal adoption in Australia, New Zealand, northern Europe, and the UK.[167] To date, the UK is the only country in the world that has both detailed electronic primary care records and cardiovascular disease (CVD) and procedure registries at a national scale, as well as more standard sources such as cause-specific hospitalisation and mortality records and census data.

Comparisons with traditional consent cohorts

EHRs can be used for research on health as much as we can do in traditional cohort studies. With the main strength in a very large scale with high dimensional data of EHRs, some research perspectives can even be explored more deeply with the use of EHRs. Furthermore, EHRs also provide high resolution of disease (i.e., we can study as many diseases as their codes are available) and a wide range of recorded biomarkers. For example, Kuan *et al*.

have developed a chronological map of 308 physical and mental health conditions from 4 million people in England, from which the case definition can be reusable in future research in related fields.[168]

In addition, given that the EHRs are derived from medical records of real-world practice, which are always being updated, they provide contemporary cohorts that enable investigators to examine the effect of the secular change in time that might impact diseases or treatments. For example, after year 2004, there was a skyrocket in the prescription of statins, leading to an improvement in cardiovascular disease morbidity and mortality worldwide.[169] The old cohort study that included participants and completed follow-up before this time could not capture the effect of this change and, therefore, might not be generalised to populations in the present time.

Compared with EHR research, a traditional cohort often facilitates high-quality data collection. However, its cost and time-consuming process limit the number of new cohort studies. Given that EHRs are not originally planned for research purposes, they usually have a relatively lower quality of data than classical cohorts. However, EHRs can provide a cheaper and faster way to conduct research, especially when we need to conduct a descriptive analysis (i.e., disease burden in real-world data) or hypothesis-generating research. However, more data management and more sophisticated statistical technique might be required to handle the incompleteness of EHRs.

3.3 Research platform of national linked EHRs: CALIBER

CALIBER (ClinicAl research using LInked Bespoke studies and Electronic health Records) is a research platform of the linkages between the UK EHR database called Clinical Practice Research Datalink (CPRD).[170] CPRD consists of i) longitudinal primary care data (i.e., general practice or GP); ii) Hospital Episodes Statistics (HES), a database of hospital admission and procedures); iii) Myocardial Ischaemia National Audit Project (MINAP), a national acute coronary disease registry); and iv) death registry from the Office of National Statistics (ONS).[171] The CALIBER data portal, which can be found at https://www.caliberresearch.org/portal, contains a comprehensive collection of all disease phenotypes and corresponding code lists developed

in the CALIBER and covers numerous noncommunicable and communicable disease areas.[172] The detailed features of the CALIBER platform have been shown in the Table 3-1.

Since CALIBER is an open platform with provided phenotyping tools, it is used as the main source for this PhD paper. The data used in my PhD contains approximately three million patients from 387 general practices across England that have consented to a linkage between 1997 and 2016.

3.3.1 CALIBER data sources

CALIBER links data from four main sources: Clinical Practice Research Datalink (CPRD), Hospital Episode Statistics (HES), Myocardial Ischaemia National Audit Project (MINAP), and Office for National Statistics (ONS). The Figure S 3-1 illustrated an example of the longitudinal nature of multiple linked data sources in CALIBER, and details of each source are as follows.

Clinical Practice Research Datalink (CPRD)

CPRD, previously known as the General Practice Research Database (GPRD), was founded in 1987 as a resource for collecting prospective primary care data of sample covering 7% of all UK populations. The name GPRD was then changed to CPRD in 2012 to imply the emphasis on data linkage with other data sources. General practitioners can enter data on patients—including demographics, blood test results, clinical biomarkers, disease diagnoses, prescribed medication, and patients' date of death.[171]

Diagnoses, results of clinical tests, and clinical procedures are coded using Read terms—a hierarchical coding system developed by Dr James Read and eventually adopted by the National Health Service (NHS). Compared with the ICD coding system, Read terms are more detailed and contain multiple terms for a single condition. CPRD developed a more refined coding system called 'medcodes', which is mapped to read terms in order to ease the transition between the coding systems.

Prescriptions are recorded according to the chapters of the British National Formulary (BNF), using BNF codes. Each chapter of the BNF refers to different disease areas. For example, chapter 2 contains all drugs used to treat cardiovascular diseases. The subsections within each chapter refer to

broad drug classes, such as chapter 2.9 antiplatelet drugs, and all drugs within the chapter will have the BNF code 2090000. In addition, all prescriptions have a prod code, which is a more detailed coding system than BNF codes, as it is specific to the drug substance, dosage, and manufacturer.

Hospital Episode Statistics (HES)

HES captures data for all admissions to NHS hospitals in England. The primary diagnosis for the admission, date of admission, date of discharge, and date of operational procedures while hospitalised are all recorded in HES. In contrast to CPRD, HES uses the International Classification of Disease (ICD) codes for all diagnoses; ICD–9 codes for all data recorded between 1997 and 2000 and ICD–10 codes for those from 2000 onwards. Procedures are recorded using the Office of Population, Censuses, and Surveys Classification of Surgical Operations and Procedures (OPCS) codes (the most recent version of OPCS is version 4).

ICD-10 codes can be grouped into 22 chapters, representing distinct disease types or particular anatomical sites. Compared with Read terms, which are more than 200,000 codes, ICD-10 are less detailed (i.e., 16,000 codes).

Myocardial Ischaemia National Audit Project (MINAP)

MINAP is a national registry for acute coronary events resulting in hospitalisation, covering 230 hospitals across England and Wales from the year 2000 onward. Data is collected on patient demographics, the MI subtypes (i.e., ST-elevation MI [STEMI] or non-ST-elevation MI [NSTEMI]), electrocardiogram results, cardiac biomarkers, complications, comorbidities, procedures, and treatment received prior to, during, and after hospitalisation.

Office for National Statistics (ONS)

ONS independently collects vital statistics regarding the population and economy of the UK. CALIBER has linked to the social deprivation and mortality data collected by the ONS. The social deprivation data includes the Index of Multiple Deprivation (IMD) score, which is determined by the deprivation statistics in the local geographical area of general practices and is used as a proxy for area-level socioeconomic status. The mortality data consists of the date of death as recorded on death certificates and the

underlying cause of death with 15 secondary causes of death, which are all recorded using the ICD coding system.

3.4 Approach to data curation in CALIBER platform

In the following section, the approaches for planning and preparing CALIBER data for analysis in order to achieve my PhD objectives and to answer my research questions (see Chapter 1) will be described in detail. In addition, a summary of the steps described in this section, including an estimated time required for each step, is shown.

3.4.1 Develop a study protocol

For a given research question and objective, it is crucial to develop a detailed study protocol that describes the background information of the research question, how to disentangle the question, and the potential implications of the findings.

Estimated duration of the process: 4 weeks

3.4.2 Exploratory/feasibility analysis

In this step, we have to provide the feasibility count to ensure sufficient power of statistics using available CALIBER data to answer a prespecified research question. These analyses are usually simple, for instance, initial counts of the study population, number of events of interest, and effect size to be detected to estimate the sample size which, in turn, can convert into power calculations. Such counts, except for estimated effect size, which usually derives from the literature review or from the best guessing based on worse-case scenarios, can be performed by the Data Lab before being granted access to the data. Generally, this step is a part of the stage of developing a study protocol since the ISAC applications require an estimated sample size and feasibility count in advance. Admittedly, once we are granted access to the data, we can reperform a power calculation based on existing data to re-ensure that our results are less likely to have an issue of type II error due to underpowered statistics.

Below is a sample size calculation for my study.

"For a single exposure affecting one fifth of the CPRD population (estimation of 350,000 from a total population of 1,750,000 individuals, based our previous work with the data) we are powered at the alpha=0.95, beta=0.2 level to detect heterogeneous relative effects across 10 endpoints which range evenly from 0.95 to 1.05 at the extremes, assuming that the baseline chance of an event for any endpoint during follow-up is 0.5% (equivalent to 500 events per 100,000 patients)."

Estimated duration: 1 week

3.4.3 Define study population inclusion and exclusion criteria

We need to define the study population to be included in our study. This can be based on some characteristics of patients (e.g., all patients with a medical history of a certain disease), availability of data (e.g., patients with complete records for some biomarkers of interest), or date range.

For my study, the sample population will include all patients aged at least 18 years old who are registered in their general practices in England, consenting to data linkage, with at least one year of up-to-standard pre-study follow-up and no history of any of cardiovascular diseases considered.

Estimated duration: 2 weeks

3.4.4 Define exposure, outcome, and the required variables

This usually includes required covariate data, such as patient characteristics, medical history of the disease, measurement of biomarkers, exposure, and study endpoints.

After listing all required exposure, outcome, and covariates, we have to, firstly, check whether there are available phenotypes that we require on the CALIBER portal, which can be accessed through https://www.caliberresearch.org/portal. If our required data matched with available phenotypes, we can simply refer to them as in the ISAC application and data request stage. However, for the data that is not available in the CALIBER portal, we have to generate codelists as a starting point to develop a new phenotype. Codelists can be generated using the R packages called 'CALIBERcodelists' and

'CALIBERlookups,' which allow researchers to perform searching by using search terms and keywords with Boolean operators (i.e., and, or, not).

Due to the fact that my PhD focuses on the association between lipid profiles (i.e., LDL-C, HDL-C, and TG) and the incidence of heart failure and atrial fibrillation, which are all available from CALIBER portal, therefore, I do not need to generate new codelists to define exposure, outcome, and variables of interest. However, Table S 3-1 provides the summary of meta-data of the outcome phenotype in my thesis (i.e., HF, AF, and MI).

Estimated duration: 1 week (maybe up to 2 weeks if new codelists is needed)

3.4.5 Apply for ISAC approval

For every study who used the CALIBER platform, it is compulsory for the protocol to grant approval from the Independent Scientific Advisory Committee (ISAC). ISAC approval is gained through submitting a protocol in which the study background and objectives, the data required, including justification and definitions (i.e., list of read terms and ICD-10 codes) used to define diseases or variables of interest, details of study population, exposure, outcome, covariates, statistical analysis plan, and limitations have to be outlined in the given form. Then, the members of the ISAC committee, who are from a multidisciplinary team of clinicians, statisticians, epidemiologists, health informaticians, data scientists, and lay members, will provide feedback and determine whether the submitted protocol is approved or requires revisions and resubmission or objection.

Lay summaries of approved studies by ISAC are available online. To get a study published in a journal, an approved ISAC protocol number is needed. In addition, any changes to an approved ISAC protocol needs to be reported to the ISAC committee with a letter of amendment. Moreover, major changes might need re-review and to grant re-approval before further implementation. For minor changes, the re-submission of the whole protocol is not required.In this case, submission of a cover letter with details of minor amendments, in order to inform the ISAC committee, is a good practice, but is still optional.

My study is one of many studies based on an already-developed and -approved ISAC (protocol number 12_153RARMnAR). Due to the fact that I

did not originally develop this protocol, and in the interest of not going over the content limit, the protocol is not included in this thesis.

Estimated duration: 4 weeks

3.4.6 Request the data

After the submitted study has been granted approval by ISAC, details of the study population and variables required can be compiled in a simple spreadsheet, which will then be processed by a member of the data lab who will work in cooperation with CPRD to extract the data. In brief, the process will start with a researcher completing a linkage request form and submitting it to the data lab team. Then, a member of the data lab team will generate patient identifiers and send them to CPRD. Finally, the data set from CPRD will be uploaded in the data safe haven (DSH) environment from which researchers can get access to the data, run analyses, and export results.

Estimated duration: 3-5 days

3.4.7 Receive the linked data

All data sets are in the format of Comma Separated Values (.csv) files. Each file corresponds to longitudinal records for individual variables from each data source. For example, heart failure outcome is from both the GP (heart_failure_cprd.csv) and hospital (heart_failiure_hes.csv) files. The format of the file name is ‘variable_datasource’; for instance, the file name ‘heart_failure_cprd.csv’ contains all heart failure diagnoses from GP records, while the one named ‘heart_failure_hes.csv’ contains all hospitalised admissions due to heart failure.

We also receive a general cohort file from which we can find each patient’s anonymous identifier, general practice (GP) identifier, date of birth, gender, date of entry and exit to their GP, and date of death from GP records (which may or may not be the same as that recorded in ONS).

Estimated duration: 4 weeks (depends on sample size of the study)

3.4.8 Construct cohort: applying inclusion and exclusion criteria

The data is usually extracted for a broader cohort from which we have to apply our inclusion and exclusion criteria in order to get cohorts that match our prespecified criteria.

Estimated duration: 1 week

3.4.9 Develop new phenotypes (if necessary)

In case the required data does not have a phenotype defined in the CALIBER portal, it is necessary to develop a new phenotype based on a rule-based approach in which patients without diagnostic coding might have symptoms, a laboratory parameter, or on prescribed medications suggesting potential diseases or conditions of interest so that case identification can be improved. In brief, the process of developing the new phenotype starts from reviewing the nature of disease or condition of interest and defining code lists (e.g., ICD-10 or Read terms) of the disease or condition, which will be grouped as a definite case. For patients without diagnostic codes, we will logically consider potential symptoms, laboratory parameters, or prescribed medications that suggest the likelihood of having that disease or condition, which are usually referred to as an inferred case. A newly developed phenotype is usually amended and agreed among clinicians specialised in that field. Due to the fact that my PhD work is based on existing phenotypes for heart failure and atrial fibrillation, there is no necessity for new phenotypes. Examples of phenotyping of HF and AF are shown in Figure S 3-2 and Figure S 3-3.

To validate the developed phenotype, there are six approaches, including (1) case-note review (then calculating a positive predictive value), (2) cross-referencing against different EHR sources, (3) replication of known aetiology, (4) genetic and (5) prognostic associations, and (6) portability across health systems and countries. Next section is going to fully explain each approach and what have been done so far on HF and AF.[173,174]

Estimated duration: 2 weeks

3.4.10 Generate study exposure and covariates

In general, the longitudinal records might need to transform into a single variable per patient to describe their characteristics and disease status at baseline.

Baseline blood lipids measurements (i.e., LDL-C, HDL-C, and TG) were taken from primary care data. Since an individual patient can have a measurement of each lipid component on different dates, I, therefore, generate three cohorts separately for LDL-C, HDL-C, and TG cohorts. Potential outlier value, plasma lipid measurement, duplications, and repeated measurements require specific data cleaning and management process. I also calculate a yearly average value in order to compare the findings between the use of single measurement and that of yearly average measurement (sensitivity analysis).

For patients' biomarker data, such as C-reactive protein (CRP), we generally select the nearest record to their baseline data (e.g., the date of first lipid measurement) within an appropriate time window, for instance, within one or two years prior to baseline. For patients with no record in the specified time window, their data for that biomarker is recorded as missing. Other approaches which generate baseline biomarker data might be averaging multiple records, instead of selecting a single value. Further missing value might be replaced by using a technique, such as multiple imputations, which is also used in my study. Below is a Stata script I used to create a covariate for CRP. It might be noticeable that I defined baseline CRP as the records nearest to patients' baseline lipid measurement within one year or two years, whichever is closer to the baseline date, either prior to or after baseline lipid measurement.

Estimated duration: 1 week

3.4.11 Generate endpoints

Before performing the planned analyses, we need to generate endpoints for patients. I used time-to-event analysis in this thesis; therefore, the endpoint information requires patients' follow-up time (i.e., the length of time between patient entry and the occurrence of an event of interest or patient loss to follow-up) and the outcome variable to indicate whether an individual had an

event or was censored (i.e., died, transferred out of their registered GP, or lost to follow-up).

Estimate duration: 1 week

3.5 Preliminary results and validity of data used in this thesis

After the curation process described in the previous section, herein, I describe the preliminary results by showing the incidence of 12 CVDs. Then the validity of the created cohort, variables used, and endpoints used in this thesis will be explained to ensure the reliability of the findings of my work. Details of the study population can be found in each chapter separately. A summary of data sources for risk factor phenotypes can be found in the Table 3-2, and details of code lists used to define outcomes and blood lipids in my thesis have been shown in the Table 3-3 and Table 3-4, respectively.

3.5.1 Preliminary results: Simple count and incidence of CVDs in my cohort

According to Table S 3-1, I identified 3,340,437 individuals from CALIBER who were free of CVD at baseline (i.e., one year after the first general practice registration, the date of being 18 years old, or 1st January 1997, whichever occurred last). These people were followed up until the end of the follow-up period (30th June 2016) or being censored (i.e., died or transferred out of practice), whichever occurred first.

Among these, 491,948 CVD events were identified in which 283,953 were the first event cases during the median follow-up time of seven years (interquartile range of 3–13 years). We observed that AF, HF, and MI are among the top three CVDs, accounting for approximately half of all cases, regardless of counting method (i.e., first VS any event cases). Moreover, AF (23–24%) and HF (13–15%) made up around one-third of all identified cases (Figure 3-1). Therefore, the data suggest that AF, HF, and MI are among the most common CVDs that should be firstly prioritised in order to reduce the health and economic burden due to CVDs. Table S 3-3 illustrates the incidence rate per 1,000 person-years of each CVD.

3.5.2 Different approach designs contributing evidence of validity

Generally, there are six approaches to validating the EHR phenotypes (i.e., ways of defining diseases of interest from linkage EHRs, which may later be used as an outcome or covariate in research). These include 1) Cross-EHR sources, 2) Case-note review, 3) Aetiological association, 4) Prognostic association, 5) Genetic association, and 6) External validation.[172] Details of each approach can be explained as follows:

1) Cross-EHR sources: In this approach, cases identified across sources will be quantified by the percentage in each source and the overlap between sources. Then, per-source completeness and positive predictive value (PPV) based on a reference source (mostly the disease registry) are calculated.
2) Case-note review: In this approach, cases identified from the phenotyping algorithm (using ICD-10 and read code) will be randomly selected. Then, the whole medical records of selected individuals will be reviewed by at least two independent physicians who will not have prior knowledge of the patients' ICD-10s (or read code). PPV, negative predictive value (NPV), sensitivity, and specificity of cases identified from the algorithm are then calculated using the physicians' reviews as a gold standard.

   Among all validation approaches, case-note review is the most important. However, this approach sometimes seems implausible to implement since a separate study is required for re-contacting participants or clinicians to confirm diagnoses or review records, which is a costly process. Moreover, the anonymity of the CALIBER data to protect patients' privacy makes it difficult for researchers to trace back to patients.
3) Aetiological association: This approach required prior knowledge or previous reports from the non-EHR study, since the effect size of known risk factors (e.g., the hazard ratio from Cox-model) estimated from the EHR study will be compared with that from the non-EHR study. Consistency of both direction and magnitude is then compared between EHR and non-EHR studies (or compared EHR results with prior knowledge if the association has been well-established).

4) Prognostic association: This approach is similar to 3). The only difference is, for this approach, prognostic parameters, such as cumulative incidence of disease stratified by EHR sources, will be compared with previous non-EHR works, instead of comparing the effect size of risk factor.
5) Genetic association: In this approach, the cases identified by the EHR phenotyping algorithm will be extracted for their genetic variants (i.e., single nucleotide polymorphism [SNPs]) from the UK Biobank. Then, the association between SNPs and outcomes are analysed, and the results will be compared for the concordant direction of effect with the results from a publicly available consortium.
6) Across countries: In this approach, the EHR phenotyping algorithm from one country will be applied to different populations (or external data sources). The consistency of the results between countries is then evaluated.

3.5.3 Validity of the EHR phenotype for MI, HF, and AF

In my thesis, the endpoints used throughout are myocardial infarction (MI), heart failure (HF), and atrial fibrillation (AF). The following are the validation results from previous studies on these three outcomes.

Myocardial infarction (MI) phenotype

The MI outcome is the most validated phenotype because it has been validated through five approaches (except in a case-note review due to the limitations of CALIBER, as mentioned earlier). Another reason is that there is a disease registry (MINAP), which is considered as a reference, making it relatively easier to validate compared with other phenotypes. In cross-EHR sources using MINAP as a reference, the precision is very high in both primary care (CPRD) and secondary care (HES) sources, corresponding to PPVs of 92.2% (91.6%–92.8%) and 91.5% (90.8%–92.1%), respectively.[175] Further calculation reveals the sensitivity of 70.6% and 72.6% for MI cases identified from CPRD and HES sources, respectively.

In terms of aetiological association, MI cases identified from CPRD, HES, and MINAP had a similar prevalence of CV risk factors.[175] In addition, prognostic association has shown that at one year, the mortality rate was similar in all

three sources at around 20%.[175] In terms of genetic association, using the EHR AMI phenotype can identify 69 SNPs from 3,408 cases from which their SNP-trait association was replicated in 67 SNPs (97%) of the previous report.[176] Regarding the cross-country validation, it has been shown that 12 prognostic factors associated with AMI—such as age, gender, hypertension, stroke, and an adjusted AMI risk—were comparable to those in Sweden, the USA, and France.[177]

Heart failure (HF) phenotype

For HF phenotype—due to lack of disease registry—it has been mainly validated through the aetiological association and prognostic association approach (Table 3-5). In the cross-EHR source approach, among 89,554 HF patients who were identified by EHR phenotype, 26% and 34% were recorded only in primary care (GP) source and secondary care (hospital) source, respectively. Of these, 27% were found to overlap between these two sources, and 13% of patients were identified only from the death registry data source.[178] In terms of aetiological association approach, it has been shown that incident HF identified using the EHR phenotype was associated with a list of risk factors that are consistent with previous work using traditional cohorts.[52]

Regarding the prognostic association, it has been shown that HF patients identified only from secondary care records had the highest probability of death from all-causes within 90 days (Figure S 3-4). Also, HF patients were associated with an increased risk of death from any causes (hazard ratio ranged from 7.01 [95%CI 6.83, 7.20] to 15.38 [95%CI 15.02, 15.83]), compared with general populations.[178]

Atrial fibrillation (AF) phenotype

Regarding the AF outcome, the aetiological association approach is, again, used for the purpose of validation. It has been shown that the associations between the AF outcome identified from the CALIBER EHR phenotype and prespecified risk factors, including MI, hypertension, and HF, were consistent with findings from those of the Framingham Heart Study (FHS) and the Malmo Diet and Cancer Study (MDCS), which are traditional non-EHR cohorts[179] (Table S 3-4 and Figure S 3-5).

In terms of prognostic association of AF, Allan *et al.* have shown that AF patients identified from the CALIBER EHR phenotype had consistently increased the risk of ischaemic stroke as their CHA_2D_2-VASc score increased (Figure S 3-6).[180] This ensures the validity of the case definition, as the CHA_2D_2-VASc score is the well-validated tool to predict the risk of thromboembolic events, and it has been widely used in clinical practice.[5,30]

In conclusion, among the three outcomes used in my PhD, MI is the most valid outcome, since it has shown a good validation through five approaches, partly due to the availability of disease registry (MINAP) being used as a reference (gold standard). For the HF and AF outcomes, validation was performed by the aetiological and prognostic approaches, which also shown well-validated results for both outcomes. Therefore, the outcome definitions used throughout my thesis are reasonably validated. Table 3-5 summarises the validation processes for MI, HF, and AF. Figure S 3-7 to Figure S 3-10 provides an example of the interface of the CALIBER platform, HF phenotyping tools, and HF validation results, respectively. All CALIBER HER phenotypes are all publicly available and can be found at https://caliberresearch.org/portal/phenotypes.

After applying the EHR phenotype for disease definition to my data, the incidence rate per 1,000 person-years for each disease in each group has been calculated and plotted (Figure 3-2). It can be seen that the incidence of HF, AF, and MI increased as age increased, especially from the age of 60 onwards. This pattern is relatively similar to those in previous reports from the UK,[24] Europe,[20] and the US.[181] Additionally, comparing the three diseases, AF has the highest incidence rate, followed by HF and MI (Table S 3-2 and Table S 3-3).

3.5.4 Validity of EHR phenotype for blood lipids

To assess the validity of a phenotype for blood lipids, after extracting data and creating a cohort, we ran a Cox-proportional model to test for the association between blood lipids and the risk of myocardial infarction. The method for cleaning and managing the data is the same as in Chapter 4-7, but in this chapter, we used complete-case analysis and different reference groups were

chosen: the lowest group for LDL-C and TG analysis and the highest group for HDL-C analysis. Results were then compared with previous findings from the Emerging Risk Factors Collaboration (ERFC), which analysed data based on 302,430 individuals from 68 longitudinal cohort studies across Europe and North America.[95]

Our results have indicated that the phenotype for blood lipids are reasonably valid as we can reproduce the similar findings with ERFC. In terms of LDL-C, although the strength of the association from our cohort was weaker than that from ERFC, the direction of the association is robust and consistent with the one from ERFC (Table 3-6). For HDL-C and TG, our results were nicely concordant to those from ERFC (Figure 3-3).

3.5.5 Validity of created cohort and variables used in this thesis

In the previous section, I have shown good validity of outcome definitions. In this part, I had adapted the validation approaches to my cohort that I specifically created for my PhD work. My aim is to show the validity of the cohort and covariates used for further analyses in Chapters 4 to 7, and the aetiological association approach was used due to the availability of previous findings that can be used as a reference.

According to Table 3-7, we performed multivariable adjustment using the Cox model to examine the aetiological association between risk factors and incident MI from 1.1 to 1.3 million individuals (with 17,000 to 22,000 new cases of MI identified during the median follow-up period of five years between 1997 and 2016). There are three points worth noticing. First, the direction and magnitude of the associations are robust across three cohorts (LDL-C, HDL-C, and TG). Second, we found that current smokers, type 2 diabetes, increased systolic blood pressure (SBP), increased LDL-C, and increased CRP levels, but decreased HDL-C levels are significantly associated with the increased risk of incident MI, and these findings are strongly supported by previous evidence, such as Framingham Heart Study[182] and Physicians Health Study-II.[183]

Concerning the age variable, we found that age and age squared are negatively and positively, respectively, associated with the risk of incident MI,

which is consistent with results from the development of the pooled cohort equation (PCE) for 10-year CVD risk prediction in Caucasians.[184] However, the discrepancy in the magnitude of the associations might be a result of various reasons, such as different scales and transformation used in different studies and the impact of different variables included in multivariable adjusted models. Therefore, our cohort and variables created using CALIBER seem reasonably valid and can be used for further analysis.

3.6 Main limitations of CALIBER and further suggestions

Although EHR data can provide a very large number of samples (e.g., more than a million) and allow the investigation of multiple risk factors or endpoints simultaneously, there are some limitations mostly due to the nature of data that initially was not designed for research purposes. Among many limitations, there are three main issues that I was struggling with when using CALIBER data that are worth mentioning.

1. Selection bias due to indication: Although blood lipids can be added as an annual check-up plan in healthy individuals, it is undeniable that lots of available blood lipids were from patients who had some indications for lipid profile measurement, such as higher weight, higher blood pressure, higher prevalence of diabetes, higher proportion of receiving statins, etc. Moreover, it is impossible to trace back the anonymous data whose results are from healthy check-ups or indicated patients.

   Interestingly, I found from my cohorts that individuals with LDL-C measurements were likely to have lower renal functions (in terms of eGFR) but higher prevalence of cancer and COPD, compared with those in the same age but without LDL-C measurement. Also, those with LDL-C measurement tend to be prescribed with statins, antihypertensive, and antiplatelet agents (Table S 3-4). Therefore, the generalisability of my findings might be limited and should not be extrapolated to healthy or community cohorts.

   However, it can be argued that it is relatively rare to see absolutely healthy individuals, even in people who do blood tests for their annual

health check-up. Our study can ensure that, at least, patients included in the cohort are free of CVD disease at baseline lipid measurement, and this might be more representative of the real-world population in that sense.

2. Temporal change in disease definition and clinical practice: Due to the fact that clinical practices are based on evidence-based medicine which can be change over time as new findings are revealed, and the follow-up period of my cohort is between 1997 and 2016. This change in disease definition and clinical practice over time might affect the validity of my findings. For example, in the past, LDL-C levels were calculated based on Friedewald formulae, whereas, now, LDL-C levels tend to be directly measured. This might result in a misclassification bias, as it has been suggested that calculated LDL-C levels can significantly differ from directly measured values as TG levels are higher than 1.7 mmol/L (150 mg/dL).[185]

   Another example is the pattern of statins use. There should not be much of statins prescription until the year 2002-2004, the time when many clinical trials on statins had revealed their findings and clinical guideline (NCEP ATP-III) suggested the use of statins as the first-line therapy.[186]

3. Missingness: Due to the nature of EHR data that is not originally designed for research purposes, there is likely to be a high amount of missingness for each variable. For instance, ethnicity and C-reactive protein have shown the missing values of around 40%, and more than 50%, respectively (Table S 3-5) in which missing not at random (MNAR) mechanism is likely to explain the missingness.

   However, multiple imputations (MI) might currently be considered to be the best method to deal with missing data. Moreover, it has been suggested that the process of MI that includes auxiliary variables might increase plausibility to meet MAR assumption (i.e., some variables that are not included in the main analysis might be strongly associated with missing variables and, therefore, including these variables in the

process of MI can improve prediction of the missing value), and MI might still provide unbiased results even though the MAR assumption has been violated.[187] Then, we can perform further sensitivity analysis to compare the results from the complete-case approach with those from multiple imputations. Admittedly, it has been suggested that using ethnicity data from 2006 onwards is reliable and representative of the UK population.[188]

4. Conflicts or discrepancies of the data between sources: In my PhD work, for example, approximately 10% of people who had the date of death recorded in primary care source differed from that recorded in ONS. Handling such a situation depends on researchers. Exclusion of conflicting data might be the easiest solution but can cause losing samples and worsening the power of statistics. An alternative way is to use the earliest date of death in the main analysis; perform sensitivity analysis by excluding conflicted data and compare the results with the main one. However, the difference in the date of death recorded in two data sources might be a result of the delay in the record. Therefore, the use of the earliest date of death in order to retain sample size and power of statistics seems to be reasonable.

5. Limitations of extracting phenotype from EHRs and potential consequences.

   There are several limitations of extracted phenotype as follows:

   - Extracted phenotypes of outcome are mainly based on signs and symptoms but not based on an objective confirmation, such as echocardiogram results. Therefore, we found small amount of cases of HF had echo results (i.e., ~ 3%, see page 445-446) and this makes it difficult to subgroup HF cases.
   - Diagnosis taken from EHR is based solely from one physician not by adjudication committee, and this might vary according to level of expertise of physicians. Therefore, this prone to misclassification bias.

These two limitations above would resulting in having more cases, and some might be falsely positive cases (i.e., patients might have signs and symptoms that mimic HF and then had HF diagnosis but actually they might had only GERD, COPD exacerbation, or other health conditions). In other words, this would lead to decrease in specificity due to increasing false positive cases leading to type I error, which could bias the findings towards observation.

- Lipid measurement taken from EHR is impossible to trace back whether it derived from Friedewald equation or direct measurement. This is more evident in LDL-C. Moreover, after checking the codes, we are unable to identify whether lipids were measured during the fasting state or not. This might be more evident in TG.

This can cause measurement error and lead to misclassification bias (i.e., patients with low lipid levels might be assigned to high lipid levels or vice versa). This would unpredictably bias results in either directions (bias toward null or toward observation).

However, some methods to control for this bias, such as excluding outlier levels, using one-year averaged lipid levels (and compared with the results from using single lipid levels), and stratifying results by practice levels, can be used to minimise the impact of measurement error and misclassification bias. In addition, previous study and a recent clinical guideline has shown that using Friedwald formulae and non-fasting levels of lipid do not clinically differ from direct measurement[104] and fasting levels.[36]

3.7 Conclusions

EHR data is an electronic form of health care records from routine clinical practices which provides an opportunity for researchers to conduct their clinical research on very large and representative samples (e.g., more than 1 million) with relatively cheaper and shorter time process compared with a traditional cohort study. These three main strengths (i.e., size, cost, and time)

made an EHR study increasingly popular over the last decade. In the UK, there is an EHR platform that links four sources of data (i.e., CPRD, HES, MINAP, and ONS) altogether called CALIBER, from which health information is captured from approximately 10 million people across England, representing 7% of all UK populations.

Although CALIBER is an excellent resource for health care research, and all parts of the cohort created from the CALIBER EHR phenotype for use in this thesis are reasonably valid, there are some limitations that should be considered. These include indication bias in the selection of variable measurement, the incompleteness of the data, the temporal change in disease definition and clinical practice, and conflict of data from different sources.

Moreover, data cleansing and management skill are challenging when dealing with large-scale data, and advanced statistical techniques are usually required. In the next four chapters, I am going to demonstrate the use of CALIBER data to disentangle my research questions previously mentioned in the first chapter.

Table 3-1 Characteristics of CALIBER platform

General practices	
Number of	
Participating GPs	
CPRD GOLD	548
CPRD Aurum	540
Participating linking GPs	
CPRD GOLD	411
CPRD Aurum	232
Eligible patients for linkage (up to June 2018)	
CPRD GOLD	10,553,586
CPRD Aurum	6,566,869
Were GPs paid to take part?	Yes
Quality framework	Up to standard
Number of general practices	387
Number of participants age more than 18 years old	3,580,229 (98.42%)
Vendor (software system)	
CPRD GOLD	Vision
CPRD Aurum	EMIS
Drug data	Prescription
Distribution of practice region participating in linked data (% of data contributed)	London: 16.52% North West: 13.59% South East Coast: 12.89% South Central: 12.81% South West: 12.41% East of England: 11.62% West Midlands: 10.92% Yorkshire & The Humber: 4.09% East Midlands: 3.18% North East: 1.98%
Information governance	
Legal basis	Section 251 of Health and Social Care Act 2012
Environment	Data safe haven (DSH)
Unique ID	NHS number
Linkage method	Deterministic
What record linkages have been carried out?	NHS-Digital + CPRD
Data	
Primary care data	
Ontology	Read code
Covering period	From January 1997 up to June 2018
Secondary care data	
Registry system	HES
Ontology	ICD-10
Operational procedure code	OPCS 4.6
Covering period	
HES APC	April 1997 to December 2017
HES OP	April 2003 to December 2017
HES A&E	April 2007 to December 2017
HES DID	April 2012 to October 2017
Median (range) ICD-10 code per patient per visit	
Death registration data	

Ontology	ONS (using ICD codes)
Covering period	January 1998 to February 2018
Access and tools for research, including phenotyping	
Portal for access	https://www.caliberresearch.org/portal Patient level datasets can be extracted for researchers, following protocol approval from the ISAC.
Phenotyping method	Rule-based phenotyping approach with more than 70 phenotypes (code lists and logic) on the CALIBER portal
Validation for phenotypes	Up to 6 layers of validation reported on the portal*

Note: *Phenotypes are codes (often from multiple ontologies) and the logic relating them into a clinically (human) readable definition. Validations are being collected in up to six layers of evidence: case-note review, cross referencing against different EHR sources, replication of known aetiological, genetic and prognostic associations, and portability across health systems and countries.

Abbreviations: CPRD; Clinical Practice Research Datalink, CPRD GOLD; GP On-Line Database (CPRD's primary care data collection database), HES A&E: HES Accident and Emergency, HES APC; HES Admitted Patient Care, HES DID; HES Diagnostic Imaging Dataset, HES OP; HES Outpatient, ICD-10; International Classification of Diseases version 10, IMD; Index of Multiple Deprivation; OPCS; ISAC; Independent Scientific Advisory Committee, UK Office of Population, Census and Surveys classification.

Table 3-2 Summary of data sources used to define CALIBER EHR phenotypes in my PhD thesis

EHR Phenotype	CPRD diagnosis	CPRD biomarker	CPRD prescription	CPRD procedure	HES admission	HES procedure	ONS
Demographics							
Age	○	○	○	○	○	○	○
Gender	○	○	○	○	○	○	○
Ethnicity	○	○	○	○	○	○	○
Index of multiple deprivation	○	○	○	○	○	○	○
Practice region	○	○	○	○	○	○	○
Health behaviours							
Smoking status	●	○	●	○	●	○	○
Physical examination							
Body mass index	○	●	○	○	○	○	○
Systolic blood pressure	○	●	○	○	○	○	○
Diastolic blood pressure	○	●	○	○	○	○	○
Laboratory biomarkers							
C-reactive protein	○	●	○	○	○	○	○
LDL cholesterol	○	●	○	○	○	○	○
HDL cholesterol	○	●	○	○	○	○	○
Triglyceride	○	●	○	○	○	○	○
Cardiovascular disease history							
Heart failure	●	○	○	●	●	○	●
Atrial fibrillation	●	●	○	○	●	●	○
Myocardial infarction	●	○	○	○	●	●	●
Coronary revascularisation	●	○	○	○	○	●	○
Unstable angina	●	○	○	○	●	●	○
Stable angina	●	●	○	○	●	○	○
Ischaemic stroke	●	○	○	○	●	●	●
Transient ischaemic attack	●	○	○	○	●	○	○
Subarrachnoid haemorrhage	●	○	○	○	●	○	●
Intracerebral haemorrhage	●	○	○	○	●	○	●
Abdominal aortic aneurysm	●	○	○	○	●	●	●
Peripheral arterial disease	●	○	○	○	●	●	●
Ventricular fibrillation	●	○	○	○	●	●	●
Non-cardiovascular disease							
Diabetes	●	○	○	○	●	○	○
COPD	●	○	○	○	●	○	○
Cancer	●	○	○	○	●	○	○
Chronic kidney disease	●	○	○	○	●	○	○
Prescribed medications							
Antihypertensive medications	○	○	●	○	○	○	○
Statins	○	○	●	○	○	○	○

Note: ● indicated 'yes' and ○ indicated 'no'.

Abbreviations: CALIBER; ClinicAl research using LInked Bespoke studies and Electronic health Records, COPD; Chronic Obstructive Pulmonary Disease, CPRD; Clinical Practice Research Datalink, HES; Hospital Episode Statistics, MINAP; Myocardial Ischaemia National Audit Project, ONS; Office for National Statistics.

Table 3-3 Codelists used to define each cardiovascular endpoint and data sources

Endpoint	CPRD – Read codes	MINAP – specific disease registry	HES – OPCS 4 hospital procedures	HES – ICD 10 hospital diagnoses	ONS – ICD 10 Primary causes of death
To define outcomes of interest					
Heart failure	G58.·00: Heart Failure + 40 other Read codes for heart failure. 585f·00: Echocardiogram shows left ventricular systolic dysfunction. 585g·00: Echocardiogram shows left ventricular diastolic dysfunction.	Not used.	Not used.	I11·0: Hypertensive heart disease with (congestive) heart failure. I13·0: Hypertensive heart and renal disease with (congestive) heart failure. I13·2: Hypertensive heart and renal disease with both (congestive) heart failure and renal disease. I50: Heart failure.	I11·0 Hypertensive heart disease with (congestive) heart failure I13·0: Hypertensive heart and renal disease with (congestive) heart failure I13·2: Hypertensive heart and renal disease with both (congestive) heart failure and renal disease I50 Heart failure
Atrial fibrillation	G573000: Atrial fibrillation + 7 other Read codes for paroxysmal atrial fibrillation, persistent atrial fibrillation and atrial flutter. 7936A00: Implant intravenous pacemaker for atrial fibrillation + 10 other Read codes for atrial fibrillation devices. 7914000: Allograft replacement of valve of heart NEC + 15 other Read codes for heart valve replacement. Warfarin or Digoxin prescription.	Not used.	K521: Open ablation of atrioventricular node. K571, K575, K621-4: Percutaneous transluminal ablation of atrial wall. K291-4: Replacement of valve of heart NEC.	I48·0: Atrial fibrillation and flutter	Not used.
Myocardial Infarction (MI)	G30X000: Acute ST segment elevation myocardial infarction. G307100: Acute non-ST segment elevation myocardial infarction. G30.·15: MI Acute myocardial infarction + 53 other Read codes 7929100: Percutaneous transluminal coronary thrombolysis with streptokinase + 3 other Read codes for coronary thrombolysis. Elevated cardiac markers, troponin or CKMB results associated with 16 Read codes.	MI with or without ST elevation based on initial ECG findings, raised troponins and clinical diagnosis.	K50·2: Percutaneous transluminal coronary thrombolysis using streptokinase. K50·3: Percutaneous transluminal injection of therapeutic substance into coronary artery NEC.	I21: Acute MI. I22: Subsequent MI.	I21: Acute MI. I22: Subsequent MI.
To define baseline CVDs for exclusion (also include HF, AF, and acute MI)					

Endpoint	CPRD – Read codes	MINAP – specific disease registry	HES – OPCS 4 hospital procedures	HES – ICD 10 hospital diagnoses	ONS – ICD 10 Primary causes of death
Coronary revascularisation	57 Read codes for coronary artery bypass graft. 28 Read codes for percutaneous coronary intervention.	Not used.	K40-K46: Coronary artery bypass graft. K49, K50 and K75: Percutaneous coronary intervention	Not used.	Not used.
Unstable angina	G311500: Acute coronary syndrome. G311100: Unstable angina + 10 other Read codes for unstable angina.	Discharge diagnosis of acute coronary syndrome without raised troponin. .	Lack of coronary artery bypass graft or percutaneous coronary intervention record in the same hospital spell as I20·9 implies admission for unstable angina.	I20·0: Unstable angina. I24·0: Coronary thrombosis not resulting in MI. I24·8: Other forms of ischemic heart disease. I24·9: Acute ischemic heart disease, unspecified. 'I20·9: Angina pectoris, unspecified' without coronary artery bypass graft or percutaneous coronary intervention in the same hospital spell.	Not used.
Stable angina	G33z400: Ischemic chest pain. G33.·00: Stable Angina. G33z·00: Angina pectoris NOS + 19 other Read codes for diagnosis of stable angina pectoris. Test results coded associated with 33 Read codes for coronary angiography, or 139 Read codes for myocardial ischemia tests (resting ECG, exercise ECG, stress echo, radioisotope scan). Two or more successive prescriptions for anti-anginal drugs.	Not used.	Not used.	I20·1: Angina pectoris with documented spasm. I20·8: Other forms of angina pectoris. I20·9: Angina pectoris, unspecified.	Not used.
Ischemic stroke	G64.·11: CVA – cerebral artery occlusion + 9 other Read codes. 7A20311: Carotid endarterectomy and patch + 4 other Read codes for carotid endarterectomy within 90 days of stroke not otherwise specified denote ischemic stroke.	Not used.	L29·5: Endarterectomy of carotid artery NEC + 3 other codes for carotid endarterectomy or stenting within 90 days of stroke not otherwise specified denote ischemic stroke.	I63: Cerebral infarction.	I63: Cerebral infarction.
Unclassified stroke	G66.·00: Stroke and cerebrovascular accident unspecified + 14 other Read codes.	Not used.	U54·3 Delivery of rehabilitation for stroke.	I64: Stroke, not specified as haemorrhage or infarction. G46·3-G46·7: Stroke syndromes.	I64: Stroke, not specified as haemorrhage or infarction. I67·2: Cerebral atherosclerosis. I67·9: Cerebrovascular disease, unspecified.

Endpoint	CPRD – Read codes	MINAP – specific disease registry	HES – OPCS 4 hospital procedures	HES – ICD 10 hospital diagnoses	ONS – ICD 10 Primary causes of death
Transient ischemic attack (TIA)	G65.·12: Transient ischemic attack + 5 other Read codes.	Not used.	Not used.	G45·8-G45·9: Transient cerebral ischemic attack.	Not used.
Subarachnoid haemorrhage	G60X·00: Subarachnoid haemorrhage from intracranial artery, unspecified + 2 other Read codes for subarachnoid haemorrhage.	Not used.	Not used.	I60: Subarachnoid haemorrhage.	I60: Subarachnoid haemorrhage.
Intracerebral haemorrhage	G61.·00: Intracerebral haemorrhage + 16 other Read codes for intracerebral haemorrhage.	Not used.	Not used.	I61: Intracerebral haemorrhage.	I61: Intracerebral haemorrhage.
Abdominal aortic aneurysm (AAA)	G714·00: Abdominal aortic aneurysm without mention of rupture + 12 other Read codes.	Not used.	L18-20: Emergency or other replacement of aneurysmal segment of aorta. L254: Operations on aneurysm of aorta NEC. L27: Transluminal insertion of stent graft for aneurysmal segment of aorta. L28: Transluminal operations on aneurysmal segment of aorta.	I71·3: Abdominal aortic aneurysm, ruptured. I71·4: Abdominal aortic aneurysm, without mention of rupture. I71·5: Thoracoabdominal aortic aneurysm, ruptured. I71·6: Thoracoabdominal aortic aneurysm, without mention of rupture. I71·8: Aortic aneurysm of unspecified site, ruptured. I71·9: Aortic aneurysm of unspecified site, without mention of rupture.	I71·3: Abdominal aortic aneurysm, ruptured. I71·4: Abdominal aortic aneurysm, without mention of rupture. I71·5: Thoracoabdominal aortic aneurysm, ruptured. I71·6: Thoracoabdominal aortic aneurysm, without mention of rupture. I71·8: Aortic aneurysm of unspecified site, ruptured. I71·9: Aortic aneurysm of unspecified site, without mention of rupture.
Peripheral arterial disease (PAD)	63 codes for Lower limb peripheral arterial disease diagnosis (including diabetic PAD, gangrene and intermittent claudication). 136 Read codes for peripheral arterial disease procedures. 2 Read codes for abnormal lower limb angiogram.	Not used.	L50-L54: Bypass, reconstruction and other open operations on iliac artery. L58-L60, L62: Bypass, reconstruction, transluminal operations or other open operations of femoral artery. L65: Revision of reconstruction of artery.	I73·1: Thromboangiitis obliterans. I73·8: Other specified peripheral vascular diseases. I73·9: Peripheral vascular disease, unspecified. I74·3: Embolism and thrombosis of arteries of lower extremities. I74·4: Embolism and thrombosis of arteries of extremities, unspecified. I74·5: Embolism and thrombosis of iliac artery.	I73·1: Thromboangiitis obliterans. I73·8: Other specified peripheral vascular diseases. I73·9: Peripheral vascular disease, unspecified. I74·3: Embolism and thrombosis of arteries of lower extremities. I74·4: Embolism and thrombosis of arteries of extremities, unspecified. I74·5: Embolism and thrombosis of iliac artery.
Ventricular fibrillation	G574000: Ventricular fibrillation + 30 other Read codes for Ventricular tachycardia, asystole, cardiac arrest, cardiac resuscitation.	Not used.	X50: External resuscitation. K59: Cardioverter defibrillator introduced through the vein.	I49·0: Ventricular fibrillation and flutter. I47·2: Ventricular tachycardia. I47·0: Re-entry ventricular arrhythmia.	I49·0: Ventricular fibrillation and flutter. I47·2: Ventricular tachycardia. I47·0: Re-entry ventricular arrhythmia.

Endpoint	CPRD – Read codes	MINAP – specific disease registry	HES – OPCS 4 hospital procedures	HES – ICD 10 hospital diagnoses	ONS – ICD 10 Primary causes of death
				I46·0: Cardiac arrest with successful resuscitation. I46·9: Cardiac arrest, unspecified.	I46·0: Cardiac arrest with successful resuscitation. I46·9: Cardiac arrest, unspecified.

Note: Details of how these codes are combined are given in the CALIBER portal (https://www.caliberresearch.org/portal).

Table 3-4 Codelists used to define blood lipids from primary care data

Read code	Clinical term	Frequency	Percentage
LDL-C (6,073,795 observations)			
44P6.00	Serum LDL cholesterol level	4,581,426	75.43
44PI.00	Calculated LDL cholesterol level	1,254,920	20.66
44dB.00	Plasma LDL cholesterol level	173,725	2.86
44PD.00	Serum fasting LDL cholesterol level	29,617	0.49
44PE.00	Serum random LDL cholesterol level	19,562	0.32
44d5.00	Plasma fasting LDL cholesterol level	10,010	0.16
44d4.00	Plasma random LDL cholesterol level	4,535	0.07
HDL-C (7,664,506 observations)			
44P5.00	Serum HDL cholesterol level	7,375,290	96.23
44dA.00	Plasma HDL cholesterol level	231,486	3.02
44PB.00	Serum fasting HDL cholesterol level	28,156	0.37
44PC.00	Serum random HDL cholesterol level	18,838	0.25
44d3.00	Plasma fasting HDL cholesterol level	9,590	0.13
44d2.00	Plasma random HDL cholesterol level	1,146	0.01
TG (7,101,840 observations)			
44Q..00	Serum triglycerides	6,760,612	95.20
44e..00	Plasma triglyceride level	235,721	3.32
44Q4.00	Serum fasting triglyceride level	62,385	0.88
44Q5.00	Serum random triglyceride level	15,701	0.22
44Q1.00	Serum triglycerides normal	10,206	0.14
44e1.00	Plasma fasting triglyceride level	6,722	0.09
44QZ.00	Serum triglycerides NOS	5,206	0.07
44Q3.00	Serum triglycerides raised	4,439	0.06
44Q2.00	Serum triglycerides borderline	807	0.01

Abbreviations: CPRD; Clinical practice research datalink, HDL-C; High-density lipoprotein cholesterol, LDL-C; Low-density lipoprotein cholesterol, TG; Triglyceride

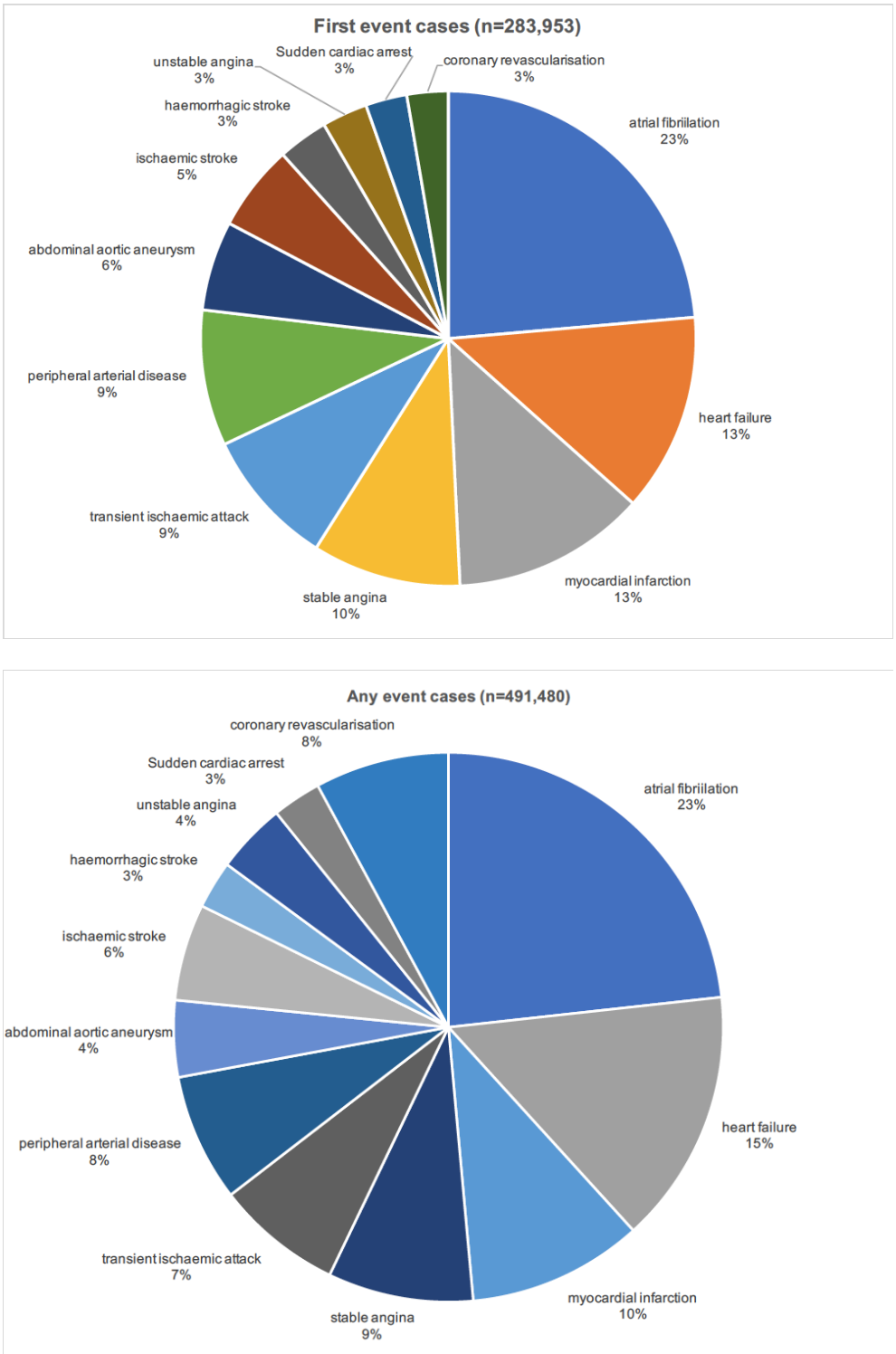


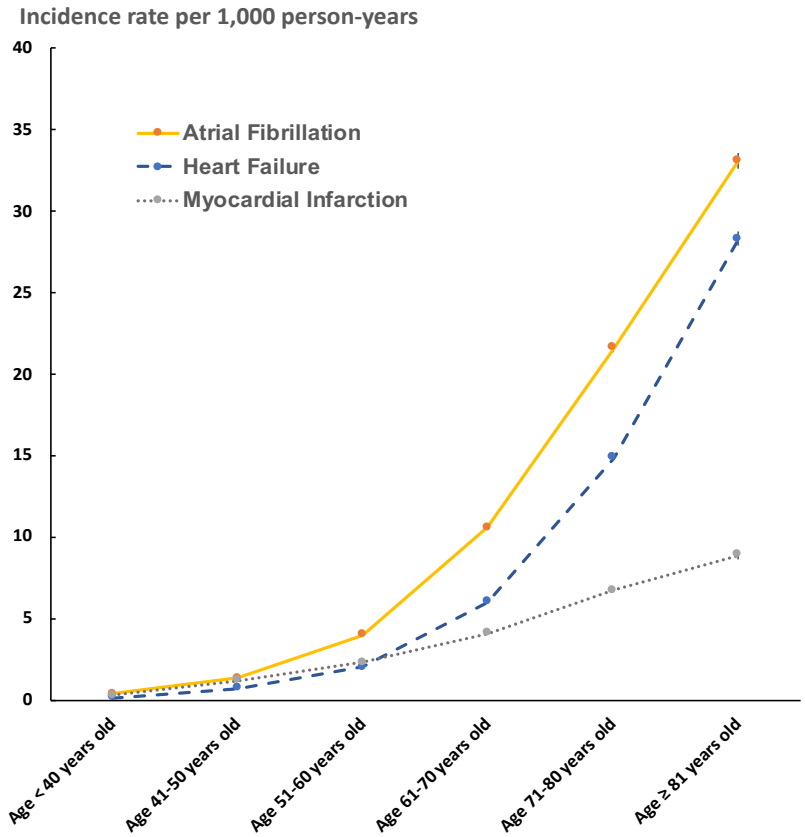
Figure 3-1 Pie charts of 12 CVDs from the total of 3,340,437 patients: First event cases (n=283,953, Top), any event cases (n= 491,480, Bottom)

Table 3-5 Validation process of EHR phenotype for MI, HF, and AF

Phenotype	Validation process[Reference]					
	Cross-EHR sources	**Case-note review**	**Aetiological association**	**Prognostic association**	**Genetic association**	**Across countries**
MI	PPV from CPRD was 92.2% (91.6-92.8%) PPV from HES was 91.5% (90.8%-92.1%) using MINAP as a reference[175]	Not performed	Overall, the cohorts identified from the primary care, hospital, and disease registry sources had a similar prevalence of CV risk factors and comorbidities. However, patients recorded by the death registry were older than patients recorded in the other sources and had a higher burden of risk factors reflecting their age.[175]	Patients with myocardial infarction identified in the disease registry had lower crude 30-day mortality (10.8%, 95%CI 10.2% to 11.4%) than those identified in HES (13.9%, 13.3% to 14.4%) or in CPRD (14.9%, 14.4% to 15.5%). At one year, however, mortality was similar in all three groups, at around 20%.[175]	Using an EHR AMI phenotype, 3,408 cases and 108,734 controls were identified, and 69 SNPs were discovered on chromosomes 6 and 9 (e.g. 9p21 loci) showing genome-wide significance ($p<5x10-8$, $\lambda=1.02$). Consistent direction and magnitude of associations were replicated in 67 (97%) of previously reported genetic variants [176]	Across each of the four countries (Sweden, n= 54,841), (USA, n=53 909), (England, n=4,653), and (France, n= 961), there was consistent associations with 12 baseline prognostic factors, such as age, gender, DM, HF, stroke, etc.[177]
HF	Among 89, 554 patients identified with newly recorded HF, of whom 23,547 (26%) were recorded in CPRD but never HES, 30,629 (34%) in HES but not known in CPRD, 23,681 (27%) in both, and 11 697 (13%) in death certificates only. [178]	Not performed	Overall, patient characteristics were similar to those observed in traditional HF registries.[178] Moreover, incident HF identified using EHR phenotyping algorism are associated with a list of risk factors that are consistent with prior knowledge.	Corrected for age and sex, HF was strongly associated with mortality, and its 90-days mortality is high with higher probability of death in patients identified from HF hospital admission than that in those identified from primary care records (Figure S 3-4). [178]	Under way in BigData@Heart[189]	Under way in BigData@Heart[189]
AF	Using the phenotype algorithm for AF, almost half the patients with a diagnosis code (39.6%; 28,795 individuals) had diagnoses recorded in both primary and secondary care. [179]	Not performed	The associations between pre-specified risk factors and incident AF were consistent in magnitude across EHR sources and with estimates from traditional consented cohorts (Figure S 3-5)[179]	Using AF cases identified from CALIBER EHR phenotype, it has been showed that the risk of ischaemic stroke consistently increased as the CHA_2DS_2-VAsc score increased (Figure S 3-6).[180]	Under way in BigData@Heart[189]	Under way in BigData@Heart[189]

Abbreviations: MI; myocardial infarction, AF; atrial fibrillation, CPRD; Clinical Practice Research Datalink, EHR; Electronic health record, HES; Hosptial episodic statistic, HF; heart failure, HR; Hazard ratio, MINAP; Myocardial infarction national audit programme, PPV; Positive predictive value,

(Source: https://www.caliberresearch.org/portal/phenotypes)



Age	< 40	41-50	51-60	61-70	71-80	≥ 81
AF	0.39 (0.37, 0.40)	1.38 (1.35, 1.41)	4.01 (3.96, 4.07)	10.61 (10.49, 10.73)	21.64 (21.41, 21.87)	33.12 (32.62, 33.63)
HF	0.18 (0.17, 0.19)	0.75 (0.73, 0.78)	2.05 (2.01, 2.09)	6.03 (5.94, 6.12)	14.91 (14.73, 15.10)	28.31 (27.86, 28.77)
MI	0.32 (0.31, 0.33)	1.24 (1.22, 1.27)	2.35 (2.31, 2.40)	4.10 (4.03, 4.17)	6.78 (6.66, 6.91)	8.90 (8.65, 9.16)

Figure 3-2 Incidence rate of HF (73,795 cases), AF (114,394 cases), and MI (50,691 cases) per 1,000 person-years according to age groups (n=3,340,437)

Table 3-6 Validity of EHR phenotype for LDL-C

LDL cholesterol	CALIBER	ERFC[95]
	HR (95%CI) per 35 mg/dL (1SD) higher	HR (95%CI) per 33 mg/dL (1SD) higher
Adjusted for age and sex	1.19 (1.17, 1.21)	1.39 (1.09, 1.78)
Plus systolic blood pressure	1.18 (1.16, 1.20)	1.37 (1.08, 1.74)
Plus smoking status	1.19 (1.17, 1.21)	1.34 (1.03, 1.73)
Plus body mass index	1.19 (1.17, 1.21)	1.40 (1.10, 1.80)
Plus history of diabetes	1.26 (1.24, 1.28)	1.41 (1.11, 1.81)
Plus log TG	1.16 (1.14, 1.17)	1.37 (1.09, 1.73)
Plus HDL cholesterol	1.19 (1.17, 1.21)	1.38 (1.09, 1.73)

Note: CALIBER included 1,142,656 indiviudals with 17,571 MI cases, ERFC included 8 studies (44,234 individuals with 2,076 CHD cases)

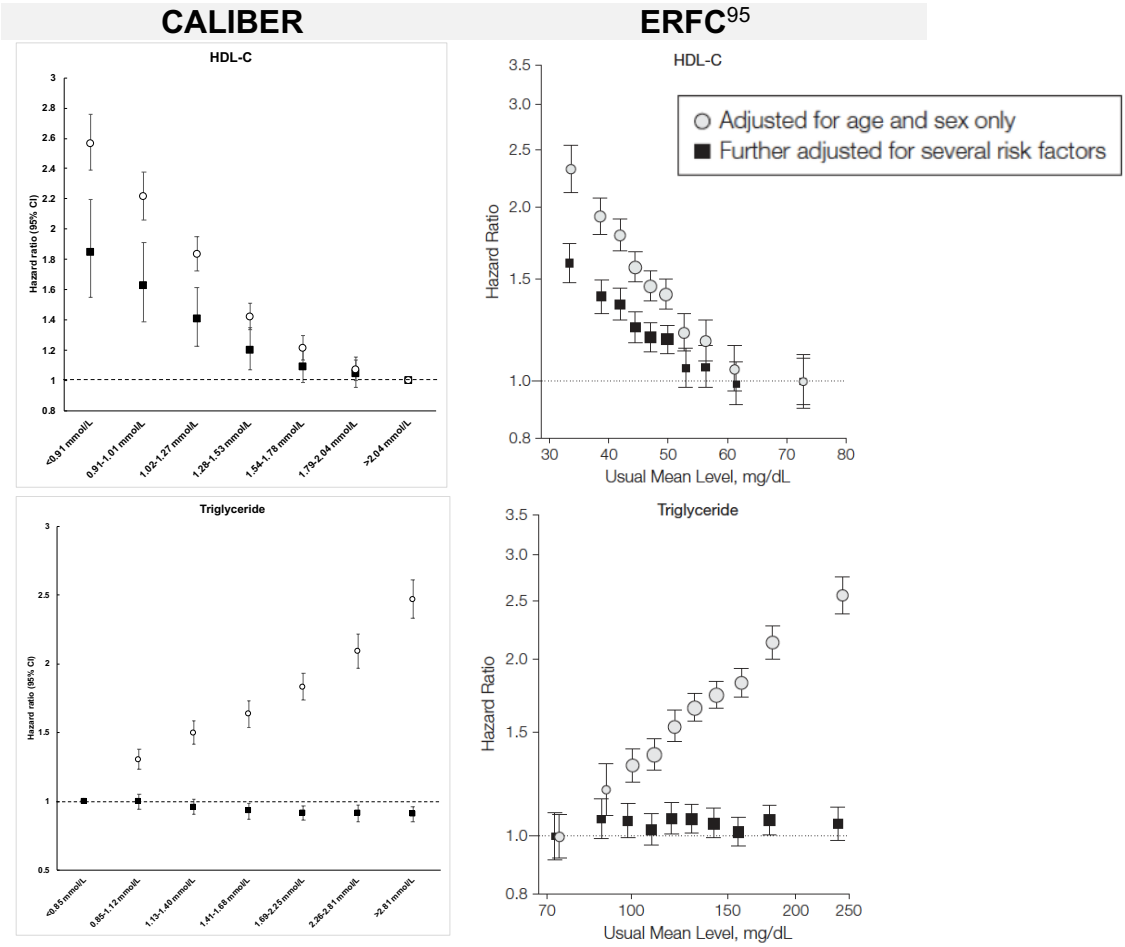


Figure 3-3 Validity of EHR phenotype for HDL-C and TG

Note: To convert from mmol/L to mg/dL, multiplies the values with 38.67 (for LDL-C and HDL-C) and 88.57 (for TG). **Abbreviations**: ERFC; Emerging Risk Factors Collaboration, HDL-C; High-density lipoprotein cholesterol, TG; Triglyceride

Source (ERFC): Di Angelantonio E, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009;302(18):1993–2000.[95]

Table 3-7 Hazard ratio (HR) point estimates and 95% confidence intervals for selected risk factors and incident MI using the cohort created from CALIBER

Risk factors	Hazard Ratio (95% CI) per 1 SD increase in risk factors		
	LDL-C cohort (n=478,703 8,967 MI cases)	HDL-C cohort (n= 446,613 8,480 MI cases)	TG cohort (n= 442,530 8,309 MI cases)
Age	0.25 (0.20, 0.30)	0.25 (0.21, 0.31)	0.26 (0.21, 0.32)
Age^2	6.07 (5.08, 7.26)	5.93 (4.94, 7.11)	5.78 (4.81, 6.95)
BMI	0.94 (0.91, 0.96)	0.94 (0.91, 0.96)	0.94 (0.91, 0.96)
Smoking status			
Non-smokers	Reference	Reference	Reference
Ex-smokers	1.16 (1.10, 1.21)	1.18 (1.13, 1.24)	1.18 (1.13, 1.25)
Current smoking	1.73 (1.63, 1.83)	1.70 (1.60, 1.80)	1.71 (1.61, 1.82)
SBP	1.10 (1.07, 1.13)	1.09 (1.06, 1.12)	1.10 (1.06, 1.13)
DBP	0.95 (0.92, 0.98)	0.95 (0.92, 0.98)	0.95 (0.92, 0.98)
LDL-C	1.09 (1.07, 1.11)	1.06 (1.04, 1.09)	1.07 (1.05, 1.09)
HDL-C	0.85 (0.82, 0.87)	0.85 (0.82, 0.87)	0.84 (0.82, 0.87)
TG	0.98 (0.95, 1.00)	0.97 (0.95, 1.00)	0.97 (0.95, 1.00)
CRP	1.13 (1.13, 1.15)	1.18 (0.87, 1.61)	1.13 (1.11, 1.15)
Diabetes mellitus (DM)			
T1DM	1.14 (0.83, 1.55)	1.18 (0.87, 1.61)	1.03 (0.74, 1.45)
T2DM	1.23 (1.16, 1.31)	1.18 (1.10, 1.26)	1.21 (1.13, 1.29)
Use of HTN Medications	0.97 (0.93, 1.02)	0.96 (0.92, 1.01)	0.99 (0.94, 1.03)
Use of statins	11.54 (10.62, 12.54)	12.24 (11.22, 13.34)	11.92 (10.93, 12.99)

Note: All models were stratified by gender and based on complete-case analysis. 1 SD = 13 years (age), 6 kg/m^2 (BMI), 17 mmHg (SBP), 9 mmHg (DBP), 0.9 mmol/L (LDL-C), 0.4 mmol/L (HDL-C), 0.55 mmol/L (log TG), and 1.18 mg/L (log CRP).

Abbreviations: BMI; Body mass index, CI; Confidence interval, CRP; C-reactive protein, DBP; Diastolic blood pressure, HDL; high density lipoprotein, HTN; Hypertension, LDL; low density lipoprotein, SBP; Systolic blood pressure, SD; Standard deviation, TG; Triglyceride, T1DM; Type 1 Diabetes mellitus, T2DM; Type 2 Diabetes mellitus

3.8 Chapter Supplementary

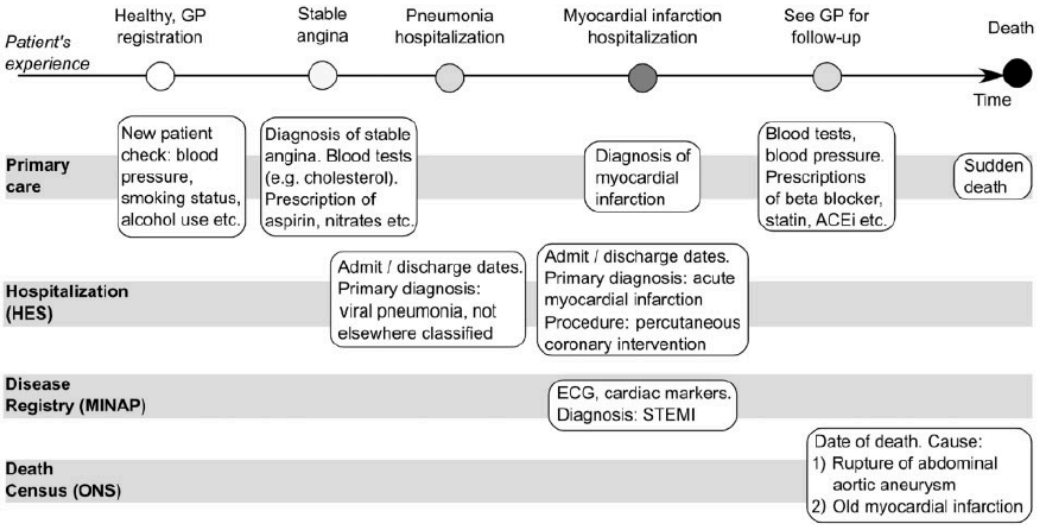


Figure S 3-1 Example of prospective nature of linked data from multiple sources in CALIBER

Source: Denaxas SC, et al. Data resource profile: Cardiovascular disease research using linked bespoke studies and electronic health records (CALIBER). Int J Epidemiol. 2012;41(6):1625–38.

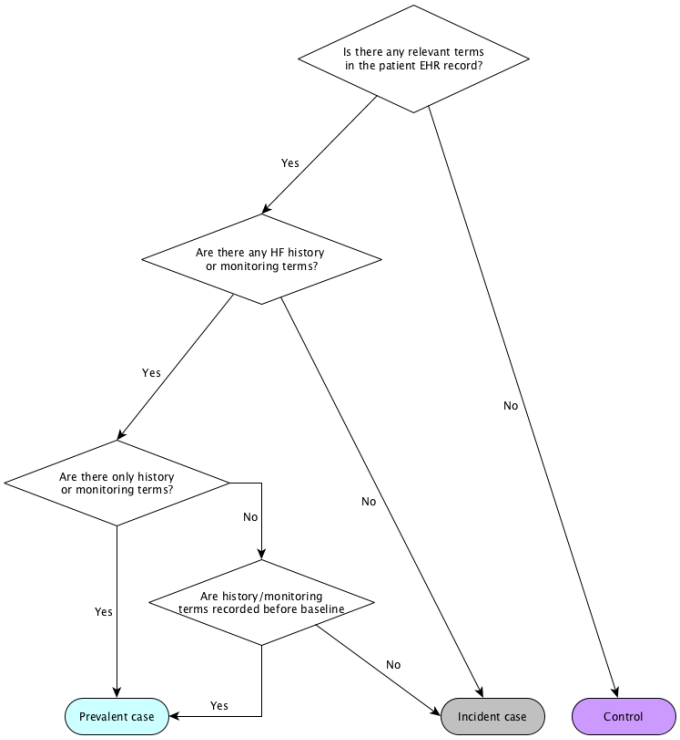


Figure S 3-2 Phenotyping algorithm for HF

Source: https://www.caliberresearch.org/portal/phenotypes/heartfailure

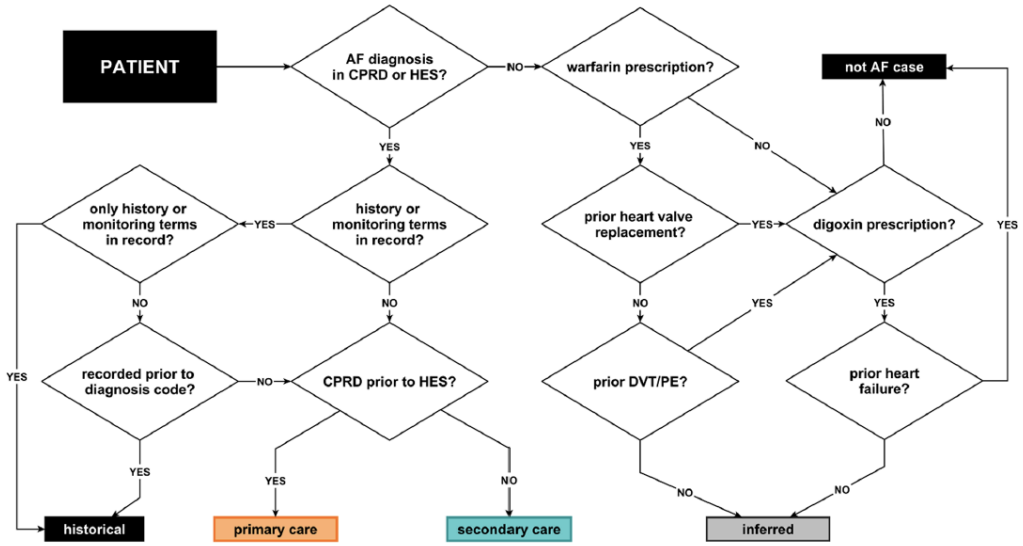


Figure S 3-3 Phenotyping algorithm for AF

Source: Morley KI, Wallace J, Denaxas SC, Hunter RJ, Patel RS, Perel P, et al. Defining disease phenotypes using national linked electronic health records: A case study of atrial fibrillation. PLoS One. 2014;9(11):e110900.

Table S 3-1 Metadata of outcome phenotype

Phenotype	Heart failure	Atrial fibrillation	Acute myocardial infarction
Type	Disease or syndrome	Disease or syndrome	Disease or syndrome
Data sources	Primary care (CPRD), hospital admission data (HES), mortality (ONS)	Primary care (CPRD), hospital admission data (HES)	Primary care (CPRD), hospital admission data (HES), mortality data (ONS)
Clinical terminologies	Read, ICD-10, ICD-9	Read, ICD-10, ICD-9, OPCS-4	Read, ICD-10, ICD-9, OPCS-4
Valid event date range	01/01/1999 - 01/07/2016	01/01/1999 - 01/07/2016	01/01/1999 - 01/07/2016
Sex	Female/Male	Female/Male	Female/Male
Agreed	05.05.2016 (Revision 3)	23.11.2012 (Revision 2)	23.11.2012 (Revision 2)
Authors	Koudstaal S, et al.	Morley KI, et al.	George J, et al.
Digital object identified (DOI)	10.6084/m9.figshare.7152197	Not available	Not available

Source: https://www.caliberresearch.org/portal

Table S 3-2 Numbers of new cases with 12 CVDs identified from a cohort of 3,340,437 participants who were free of CVDs at baseline and were followed-up from 1st Jan 1997 to 30th Jun 2016 with a median follow-up time of 7 years (interquartile range: 3-13 years)

Cardiovascular diseases	First event cases[$]		Any event cases[$]	
	Number	%	Number	%
Atrial fibrillation	67,081	24%	114,394	23%
Heart failure	36,841	13%	73,795	15%
Myocardial infarction	35,922	13%	50,691	10%
Stable angina	27,578	10%	42,117	9%
Transient ischaemic attack	25,596	9%	36,587	7%
Peripheral arterial disease	25,225	9%	37,000	8%
Abdominal aortic aneurysm	16,624	6%	22,158	5%
Ischaemic stroke	16,001	6%	28,013	6%
Haemorrhagic stroke	9,317	3%	13,852	3%
Unstable angina	8,458	3%	20,390	4%
Sudden cardiac arrest	7,676	3%	14,156	3%
Coronary revascularisation	7,634	3%	38,795	8%
Total	283,953	100%	491,948	100%

Note: [$] First event is defined as the initial presentation of the disease (i.e., no intercurrent CVD of any types). For example, 67,081 patients with first AF were individuals with their first CVD diagnosis of any type. Any event is defined as any diagnosis made during the follow-up period, regardless of the order of presentation, which can be repeatable (i.e., one patient might have more than one disease throughout the follow-up period).

Table S 3-3 Incidence rate per 1,000 person-years of 12 CVDs

Cardiovascular diseases	First event cases[$]		Any event cases[$]	
	Person-year	Incidence rate (95%CI)	Person-year	Incidence rate (95%CI)
Atrial fibrillation	25,159,421	2.67 (2.65, 2.69)	26,166,808	4.37 (4.35, 4.40)
Heart failure	25,159,421	1.46 (1.45, 1.48)	26,365,595	2.80 (2.78, 2.82)
Myocardial infarction	25,159,421	1.43 (1.41, 1.44)	26,365,402	1.92 (1.91, 1.94)
Stable angina	25,159,421	1.10 (1.08, 1.11)	26,298,046	1.60 (1.59, 1.62)
Transient ischaemic attack	25,159,421	1.02 (1.00, 1.03)	26,411,911	1.39 (1.37, 1.40)
Peripheral arterial disease	25,159,421	1.00 (0.99, 1.02)	26,416,144	1.40 (1.39, 1.42)
Abdominal aortic aneurysm	25,159,421	0.66 (0.65, 0.67)	26,486,822	0.83 (0.84, 0.85)
Ischaemic stroke	25,159,421	0.64 (0.63, 0.65)	26,499,962	1.06 (1.04, 1.07)
Haemorrhagic stroke	25,159,421	0.37 (0.36, 0.38)	26,544,191	0.52 (0.51, 0.53)
Unstable angina	25,159,421	0.34 (0.33, 0.34)	26,478,328	0.77 (0.76, 0.78)
Sudden cardiac arrest	25,159,421	0.31 (0.30, 0.31)	26,558,486	0.53 (0.52, 0.54)
Coronary revascularisation	25,159,421	0.30 (0.30, 0.31)	26,381,123	1.47 (1.46, 1.49)

Note: [$]First event is defined as the initial presentation of the disease (i.e., no intercurrent CVD of any types). Any event is defined as any diagnosis made during the follow-up period, regardless of the order of presentation, which can be repeatable (i.e., one patient might have more than one disease throughout the follow-up period).

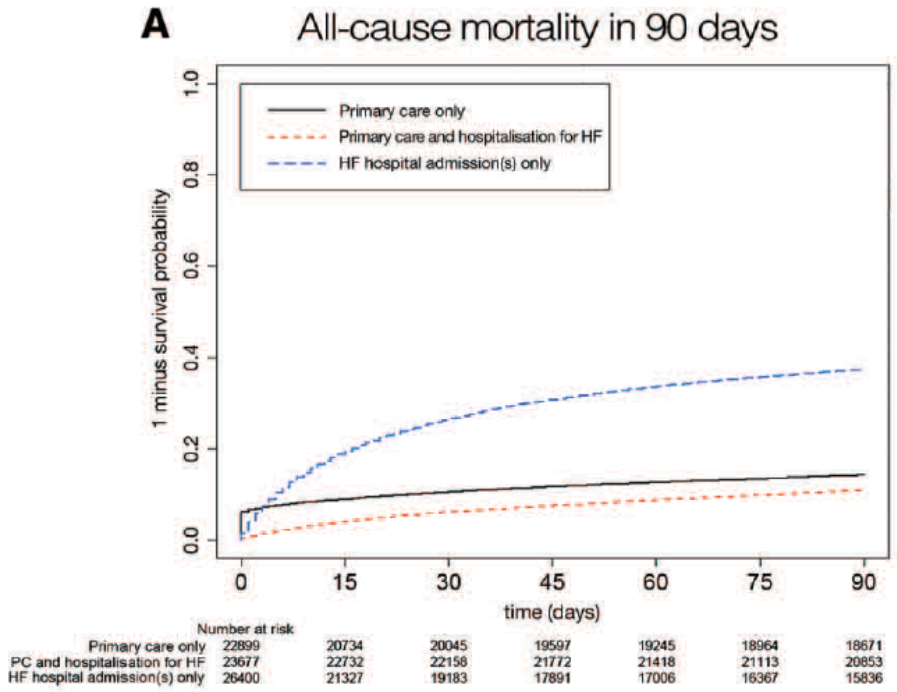


Figure S 3-4 Probability of all-cause deaths in 90 days of HF patients

Source: Koudstaal S, et al. Prognostic burden of heart failure recorded in primary care, acute hospital admissions, or both: a population-based linked electronic health record cohort study in 2.1 million people. Eur J Heart Fail. 2017;19(9):1119–27.

Table S 3-4 Hazard ratio point estimates and 95% confidence interval for selected risk factors and incident AF among 3 cohort studies

Study	Analysis	Risk factors		
		Heart failure	Hypertension	Myocardial infarction
CALIBER	Primary care	2.07 (1.95-2.19)	1.74 (1.70-1.78)	1.53 (1.46-1.60)
	Secondary care	2.31 (2.21-2.43)	1.80 (1.76-1.84)	1.75 (1.68-1.82)
	Inferred	N/A	1.72 (1.68-1.77)	1.69 (1.61-1.77)
	Combined	2.35 (2.25-2.46)	1.80 (1.77-1.84)	1.70 (1.64-1.76)
FHS		3.20 (1.99-5.16)	1.80 (1.48-2.18)	1.44 (1.02-2.03)
MDCS	Women	8.70 (3.60-20.94)	1.74 (1.42-2.13)	1.84 (1.26-2.69)
	Men	4.53 (2.34-8.75)	1.78 (1.48-2.14)	2.03 (1.65-2.49)

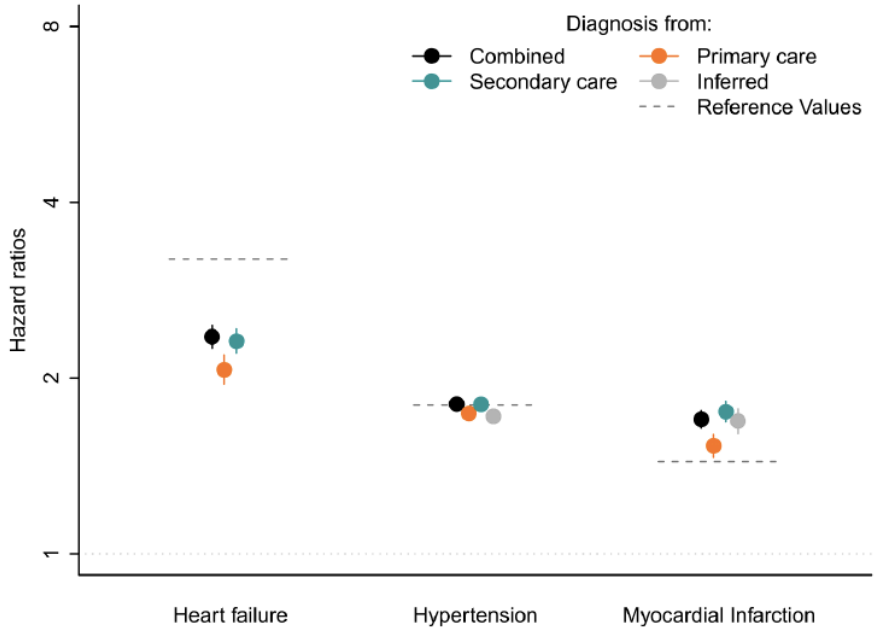


Figure S 3-5 Hazard ratio estimates and 95% confidence intervals for selected risk factors and incident AF across EHR sources

Note: Results are shown separately for associations between each risk factor and incident AF, defined according to each source of cases and for a composite using all sources. All analyses were adjusted for age, sex, and practice ID. Note that the use of heart failure diagnosis in the algorithm for inferred AF precludes estimation of the hazard ratio. The dashed lines are point estimates of hazard ratios from the Framingham Heart Study for the same risk factors, adjusted for age and sex

Source: Morley KI, Wallace J, Denaxas SC, Hunter RJ, Patel RS, Perel P, et al. Defining disease phenotypes using national linked electronic health records: A case study of atrial fibrillation. PLoS One. 2014;9(11):e110900.

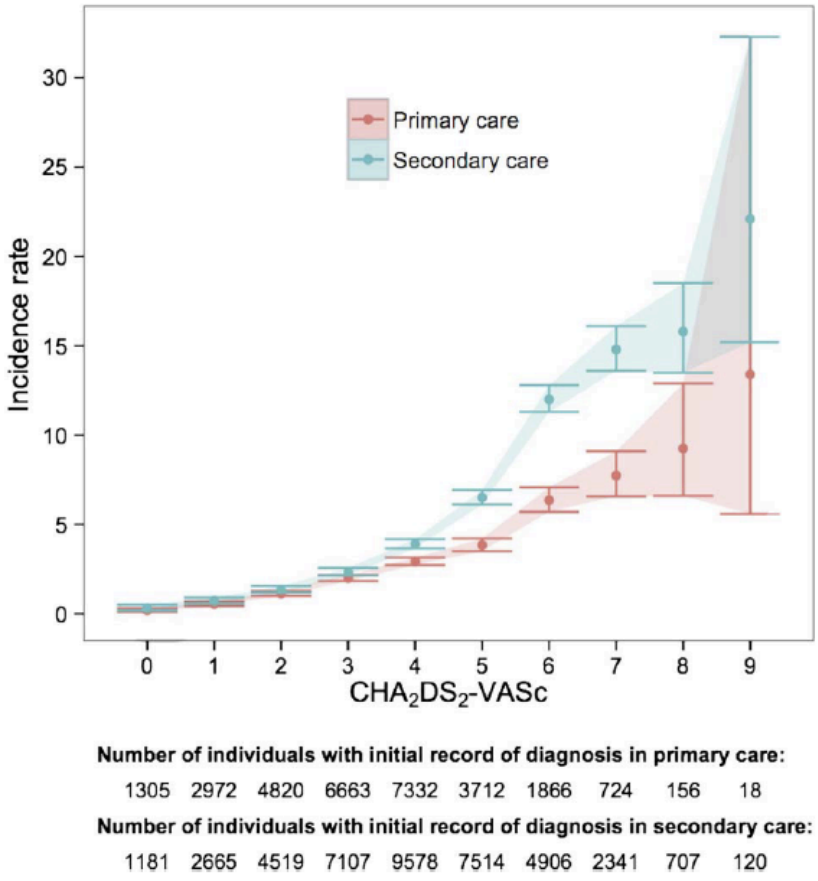


Figure S 3-6 The risk of ischaemic stroke according to CHA_2DS_2-VASc score based on AF cases identified from AF phenotyping algorithm

Source: Allan V, et al. Net clinical benefit of warfarin in individuals with atrial fibrillation across stroke risk and across primary and secondary care. Heart. 2017;103(3):210–8.

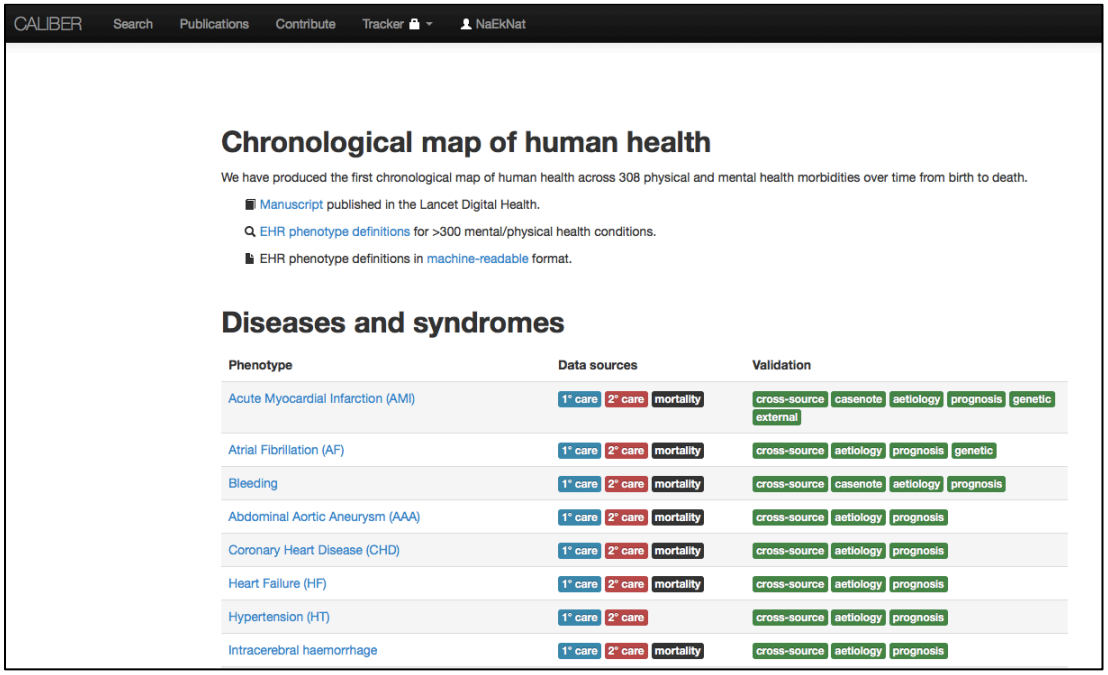


Figure S 3-7 Example of the interface of CALIBER platform

Source: https://caliberresearch.org/portal/phenotypes

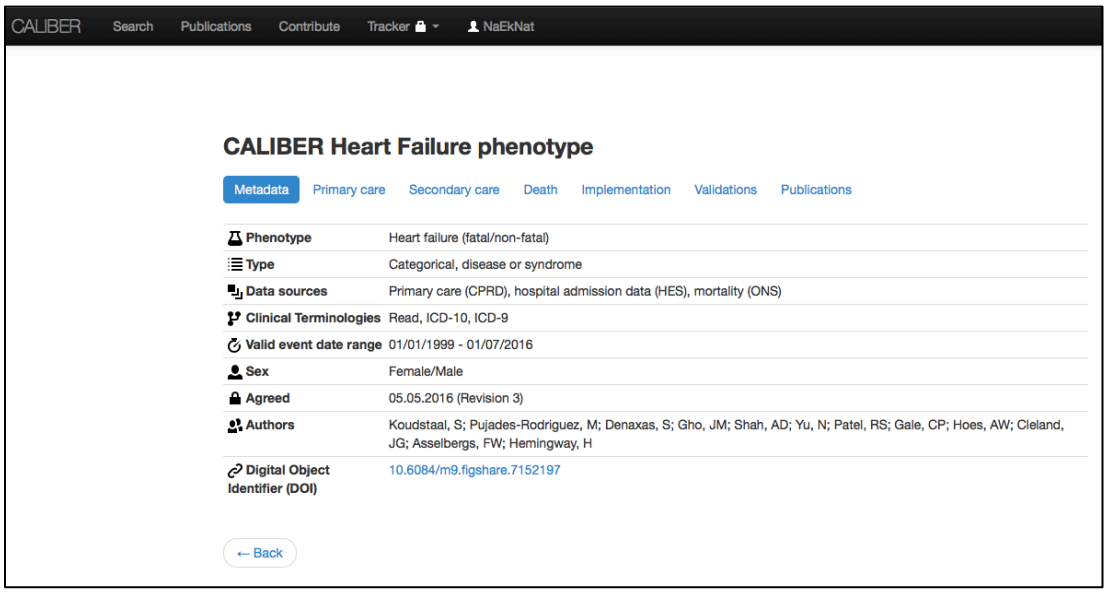


Figure S 3-8 Example of CALIBER phenotyping tools for HF outcome

Source: https://caliberresearch.org/portal/phenotypes

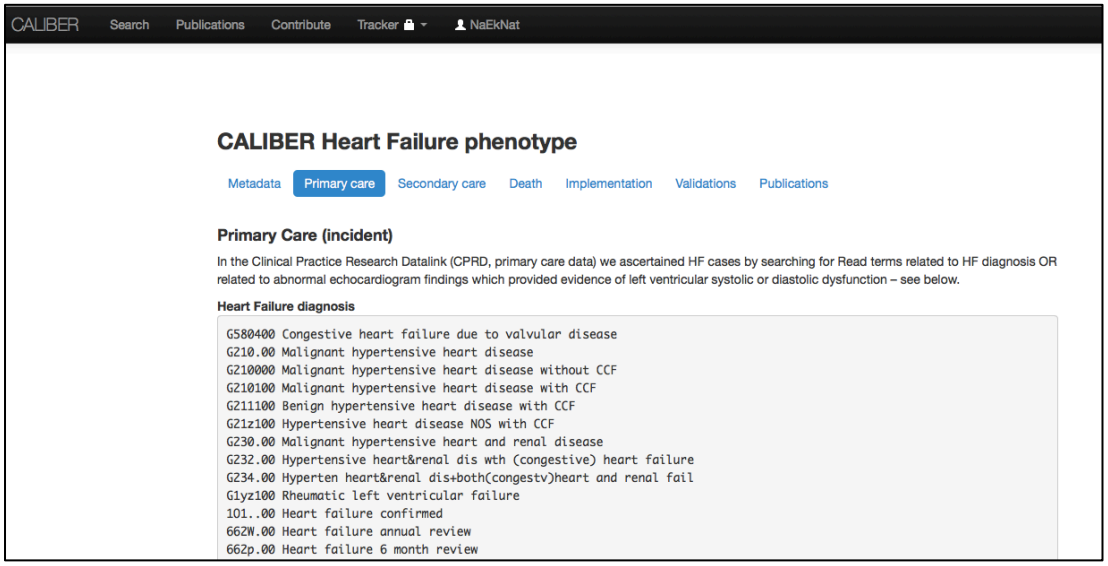


Figure S 3-9 Example of CALIBER EHR phenotyping codes for HF outcome

Source: https://caliberresearch.org/portal/phenotypes

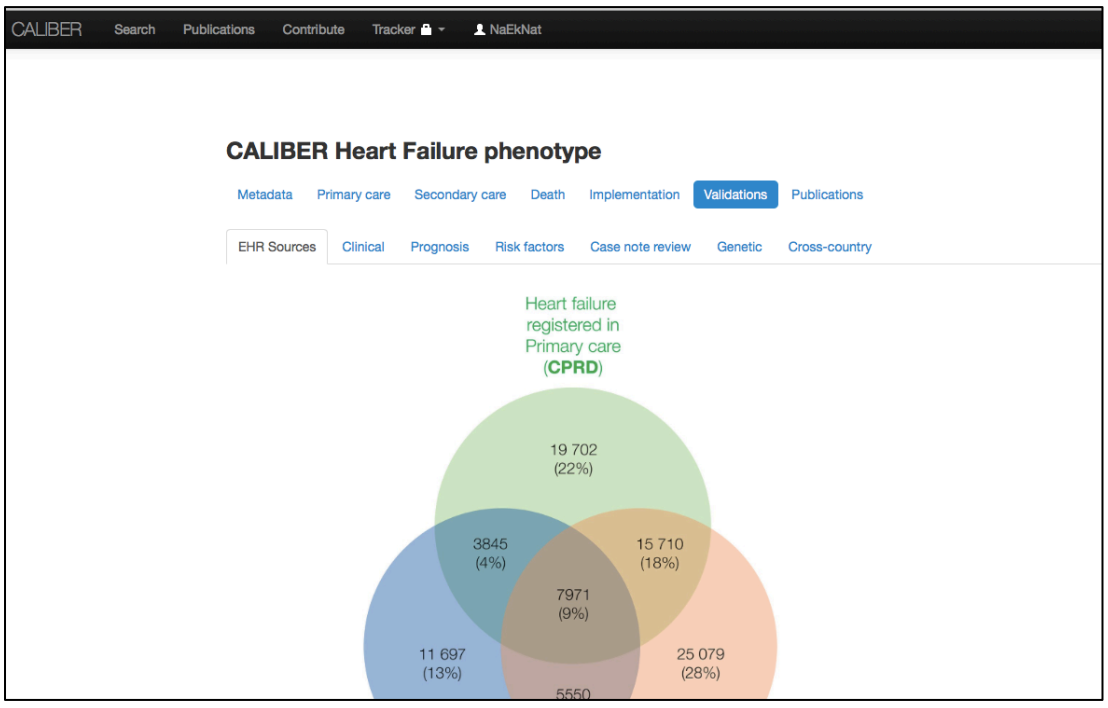


Figure S 3-10 Validation results of HF outcome

Source: https://caliberresearch.org/portal/phenotypes

Table S 3-5 Example of clinical characteristics of populations aged 45-50 years with and without LDL-C measurement at baseline (n=1,496,635)

Clinical characteristics	With LDL-C (n=149,232)	Without LDL-C (n=1,347,403)	Total (n=1,496,635)	P-value
Age (years)	47.5 (1.4)	47.0 (0.0)	47.0 (0.5)	<0.001
Female (%)	75,409 (51%)	649,079 (48%)	724,488 (48%)	<0.001
Smoking status (%)				
Non-smokers	83,877 (58%)	183,058 (50%)	266,935 (53%)	<0.001
Ex-smokers	31,681 (12%)	72,545 (20%)	104,226 (21%)	
Current smokers	28,740 (20%)	108,838 (30%)	137,578 (27%)	
Body mass index (kg/m^2)	28.5 (5.9)	26.9 (5.4)	27.4 (5.7)	<0.001
Systolic blood pressure (mmHg)	131.3 (15.6)	126.2 (14.5)	127.7 (15.0)	<0.001
Diastolic blood pressure (mmHg)	81.8 (9.9)	78.5 (9.5)	79.5 (9.7)	<0.001
eGFR (ml/min/1.73m^2)	93.4 (13.6)	102.5 (14.9)	100.3 (15.1)	<0.001
CRP, median (IQR) (mg/L)	3.3 (1.7 to 6.0)	3.2 (1.5 to 7.0)	3.3 (1.6 to 6.6)	<0.001
HDL cholesterol (mmol/L)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	0.511
Triglyceride, median (IQR) (mmol/L)	1.3 (0.9 to 1.9)	1.3 (0.9 to 2.0)	1.3 (0.9 to 1.9)	<0.001
Total cholesterol (mmol/L)	5.4 (1.0)	5.2 (1.0)	5.4 (1.0)	<0.001
Diabetes type 1 (%)	335 (0.2%)	564 (<0.1%)	899 (0.1%)	<0.001
Diabetes type 2 (%)	4,382 (3%)	3,765 (0.3%)	8,147 (0.5%)	<0.001
Diagnosed cancer (%)	1,962 (1%)	6,948 (0.5%)	8,910 (0.6%)	<0.001
Chronic obstructive pulmonary disease (%)				
Possible COPD	34,217 (23%)	214,484 (16%)	248,701 (17%)	<0.001
Definite COPD	743 (0.5%)	2,483 (0.2%)	3,226 (0.2%)	
Statins use at baseline (%)	11,998 (8%)	2,080 (0.2%)	14,078 (0.9%)	<0.001
Other lipid-lowering agents (%)	889 (0.6%)	173 (0.01%)	1,062 (0.07%)	<0.001
Antihypertensive agents (%)	28,373 (19%)	9,091 (0.7%)	37,464 (2.5%)	<0.001
Antiplatelet agents (%)	3,027 (2%)	1,218 (0.1%)	4,245 (0.3%)	<0.001

Note: Value represents mean (standard deviation) unless specified elsewhere.

P-values from chi-squared test, Independent T-test, or Mann-Whitney U test as appropriate.

Table S 3-6 Missingness profile of each cohort

Variables	Percentage of missing values		
	LDL-C cohort (n=1,142,656)	HDL-C cohort (n=1,338,276)	TG cohort (n=1,262,280)
Age	0%	0%	0%
Gender	0%	0%	0%
Ethnicity	41.70%	42.02%	41.84%
General practice region	0%	0%	0%
Index of multiple deprivation	0%	0%	0%
Body mass index	4.42%	4.60%	4.62%
Smoking status	0.59%	0.65%	0.80%
Systolic blood pressure	0.99%	1.04%	1.04%
Diastolic blood pressure	0.99%	1.04%	1.04%
Diabetes at baseline	0%	0%	0%
Use of antihypertensive medications	0%	0%	0%
Use of statins	0%	0%	0%
LDL-C	0%	19.19%	15.62%
HDL-C	0.30%	0%	8.22%
TG	0.86%	12.52%	0%
C-reactive protein	55.86%	55.99%	56.49%

CHAPTER 4 LOW-DENSITY LIPOPROTEIN CHOLESTEROL AS A CAUSE OF HEART FAILURE AND ATRIAL FIBRILLATION: COHORT, TRIAL, AND GENETIC EVIDENCE

4.1 Key messages

What is already known?

- The incidence of myocardial infarction (MI) has declined over the last two decades; by contrast, the prevalence of heart failure (HF) and atrial fibrillation (AF) remains the same or even increased, especially in elderly.
- Evidence has shown the causal relevance of LDL-C to MI, which leads to the occurrence of HF. However, the association between LDL-C and the incidence of HF in patients without pre-existing MI is less known.
- Whether LDL-C is causally relevant to HF and AF and thus might be applicable to primary prevention of these conditions, is not known. Specifically, there have been no evaluations of LDL-C and HF and AF across observational cohorts, randomised controlled trials, and genetic evidence (Mendelian randomisation: MR).
- Previous cohort studies have suggested that higher LDL-C levels might be paradoxically associated with the lower risk of incident AF, but findings were less consistent for HF.

What does this study add?

- This study presents the largest observational cohorts (total cohorts = 1,142,656) to date on the incidence of both AF and HF, which had never been studied simultaneously before.
- We found that LDL-C levels are inversely associated with the risk of HF and AF across a wide range of LDL-C levels.
- The observed paradoxical associations found between LDL-C and HF and AF are significantly heterogeneous when taking into account the intercurrent CVDs and the use of antihypertensive medications at

baseline, suggesting the role of both factors as a mediator. Besides, baseline statins use might be a major confounder for the association between LDL-C and both diseases.

- Results from trials (meta-regression) do not support a causal role of LDL-C on both HF and AF.
- Although findings from the large GWAS (HERMES) have shown a causal role of LDL-C on HF, controlling for coronary artery disease (CAD) attenuates the association towards the null hypothesis, supporting our conclusion that LDL-C is not causally relevant to HF.

4.2 Abstract

Objective: To evaluate the causal relevance of low-density lipoprotein cholesterol (LDL-C) for the risks of heart failure (HF) and atrial fibrillation (AF).
Design: We compared the evidence from three study designs: 1) a population-based cohort free of diagnosed cardiovascular diseases using linked electronic health records (CALIBER platform), 2) meta-regression of major randomised controlled trials of lipid-lowering agents, and 3) Mendelian randomisation (MR) using UK Biobank (UKB) and the HERMES consortium (47,309 HF cases).
Main exposure: LDL-C
Main outcome measures: HF and AF
Results: During the median follow-up of 5 years (CALIBER), we identified new 25,352 HF and 46,235 AF cases among 1,142,656 participants. For HF, there was an inverse association with LDL-C with a hazard ratio (HR) per 1 SD lower LDL-C of 1.09 (1.08 to 1.11). In 33 trials (8,591 HF cases) and MR, LDL-C was not associated with HF with a relative risk (RR) and an odds ratio (OR) per 1 SD decrease in LDL-C of 1.07 (0.97 to 1.19) and 0.79 (0.61 to 1.02), respectively. Besides, we found consistent MR results from the HERMES consortium (OR adjusted for coronary artery disease was 1.00 [0.96 to 1.03]). For AF, we also observed a paradoxically inverse association with LDL-C levels with an HR of 1.11 (1.10 to 1.12). However, the meta-regression of 25 trials (6,122 AF cases) and MR-UKB did not show significant association

between LDL-C and the risk of AF. Further subgroup and sensitivity analyses did not change our main findings.

Conclusion: There are strong paradoxical associations between lower LDL-C levels and higher risks of HF and AF. However, these are likely confounded because observational findings are not supported by either meta-regression of lipid-lowering trials or genetic evidence. Therefore, our findings do not support the role of LDL-C in the cause or primary prevention of the two most common cardiac diseases.

4.3 Introduction

4.3.1 Importance

Low-density lipoprotein cholesterol (LDL-C) has an established role in the causation of myocardial infarction (MI) based on concordance across three forms of evidence: observational cohort studies,[93,94] randomised clinical trials of lipid-lowering interventions,[60,61] and, more recently, genetic studies (Mendelian randomisation).[62] In high-risk populations without established CVD, lowering LDL-C is also beneficial on major cardiovascular events and all-cause mortality.[190] The causal understanding underpins successful primary prevention of AMI, and there have been major declines in the incidence of MI during the past twenty years.[191]

MI is a major cause of HF and AF, and these two cardiac diseases are associated with major morbidity, mortality, and hospitalisation costs.[192,193] Despite the decrease in MI, the incidence of HF and AF has shown no evidence of decline, and both diseases have now become the most common cardiac diseases globally.[11,57] Current primary prevention guideline of CVD makes no recommendations on the role of LDL-C in the primary prevention of HF and AF.[4,5,58,59] Therefore, it is important to understand whether LDL-C is causally related to HF and AF and whether the effect is independent of MI. Such understanding may inform primary preventive strategies and inform endpoint selection in clinical trials based on known associations.

4.3.2 Uncertainty and opportunity

However, it is not known whether LDL-C plays a causal role in initially CVD-free populations in the incidence of HF and AF, with a separate role in causing MI. There have been no previous large-scale cohort studies examining both conditions, which often co-exist and share common risk factors and pathophysiological mechanisms.[7,8,42,194]

Although there are observational cohorts examining populations without CVD at baseline showing the association between LDL-C and the risks of incident AF[32,69,70,73,76] and that of HF[108,112,113], the results were mixed and had some limitations. Previous observational cohorts have lacked the powerful sample size to examine the lower levels of LDL-C relevant in contemporary populations. For instance, the largest sample size of 103,860 (2,146 incident HF cases) and 88,785 (328 incident AF cases) covered the LDL-C range from 2.6 to 3.8 mmol/L[113] and from 0.8 to 4.4 mmol/L[73], respectively. Moreover, some previous studies did not take into account the effect of intercurrent MI,[69,76] and no previous studies had compared the estimation of LDL-C to incident HF, AF, and MI in the same cohorts. However, the recent availability and accessibility of the linked EHRs from general practices and hospitals across England enables us to investigate a broader range of LDL-C in a vast and representative sample size (i.e., N > 1 million).[170,171]

Regarding trial evidence, for HF, a meta-analysis of LDL-C lowering with statins suggested a modest reduction in the risk of non-fatal HF hospitalisations (not HF deaths).[66] For AF, trial evidence from long-term studies, conducted mainly in the secondary prevention setting, showed no beneficial effects of statins.[65] However, none of these trials directly investigated the impact of LDL-C on incident HF and AF, and other groups of lipid-lowering agents affecting LDL-C levels were not included in the analyses. Therefore, we cannot distinguish whether the observed benefits were from statins, lowering LDL-C levels, or both. Additionally, previous genetic evidence (AFGen Consortium) has indicated that LDL-C is not causally associated with AF.[92] However, it is not confirmed whether such null findings are due to low statistical power. Also, there has been no such genetic evidence on HF outcomes.

We hypothesised that LDL-C might play a causal role in HF and AF, independent of intercurrent MI because of the following reasons: i) some observational cohorts reported an association (see Table S 2-8, Table S 2-9 in chapter supplementary); and ii) a meta-analysis of trials had shown the effect of statins on the reduction of non-fatal HF.[66] Moreover, since LDL-C is targetable by various lipid-lowering medications, such as statins, ezetimibe, and PCSK-9 inhibitors, and its role on both cardiac diseases is not well established, it is more compelling to investigate any causal associations.

4.3.3 Objectives

Here we provide new observational, trial, and genetic (Mendelian randomisation) evidence of the association between LDL-C and HF and AF using MI as a positive control with three specific objectives. First, we aim to conduct large observational cohorts examining incident HF and AF in the same cohort based on electronic health records (EHRs) across a wide range of LDL-C levels (i.e., LDL-C 0.1 mmol/L (3.88 mg/dL) to 10.2 mmol/L (394.43 mg/dL)). Also, we aim to further evaluate the roles of intercurrent MI and CVDs on the association. Second, we aim to examine whether the change in the risks of HF and AF is associated with the difference in LDL-C levels between active and control group over the follow-up period in randomised controlled trials of lipid-lowering agents. Third, we aim to investigate whether the genetic evidence using the Mendelian randomisation (MR) approach supports findings from both observational cohorts and trials.

4.4 Methods

4.4.1 EHR-longitudinal cohort study

In the cohort part of this study, we used a longitudinal cohort design from which participants were followed-up over the period between 1998 and 2016. Exposure and outcomes were ascertained through the linked electronic health records (EHRs) among general practices (GP), hospital records, and national death registry. A report on the cohort section has followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[195,196] and the REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) recommendations[197] (see supplementary materials).

Data sources and studied population

The studied populations in cohort study were from the CALIBER (ClinicAl research using LInked Bespoke studies and Electronic health Records) platform.[170] In brief, the CALIBER platform provides access to longitudinal linked EHRs between primary care data (general practices [GP] from clinical practice research datalink [CPRD]), secondary care data (hospital admission), and cause-specific mortality. CPRD is a database of electronic health records of 10.5 million populations from 548 GPs across the UK from which 411 GPs consented for the linkage.[171] Therefore, the population drawn from CRPD was unselected and representative in terms of age, sex, and overall death of the general English populations.[171,188] Details of the CALIBER platform can be found in supplementary materials.

In this study, we initially included 3.6 million patients from 387 GPs across England from the CPRD database. We identified all patients aged 18 years or older who registered between 1st January 1998 and 30th June 2016 and had been followed-up with their GPs for at least one year. We excluded individuals who had history of cardiovascular diseases, including heart failure, atrial fibrillation, ventricular fibrillation, myocardial infarction, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, and peripheral arterial disease, or had previously undergone coronary revascularisation at the baseline LDL-C measurement (see the supplementary appendices for details).

Approval of this study was granted by the Independent Scientific Advisory Committee of the Medicines and Healthcare products Regulatory Agency (protocol number 12_153RARMnAR) and was registered at ClinicalTrial.gov (NCT01687686).

Exposure: LDL-C as an EHR phenotype

We used ambulatory care low-density lipoprotein cholesterol (LDL-C) measurements sampled in clinics and hospital out-patients and electronically recorded in the primary care. The raw data included plasma and serum samples (6,073,818 records, multiple records per person), and plasma levels

(3.10 % of all records) were multiplied by the factor of 1.03 to convert to serum levels before analysis.[198] We excluded outlier values (i.e., LDL-C < 0.1 or ≥ 10 mmol/L) from our analysis (3.04 % of all records). Since 27% of all patients had more than one LDL-C measurement within a year of study entry, we, therefore, used a yearly-averaged value, referred to 'baseline LDL-C'. The earliest date of LDL-C measurement was used as the start of patient follow-up. For individuals with more than one measure on a given day (0.06 % of all LDL-C records), we aggregated the values by taking the mean.

Covariates

Baseline covariates taken from the closest record to the baseline date (within a one-year interval) were selected based on their association with LDL-C, HF, and AF from previous studies.[74,178,199,200] These included age, socioeconomic status (i.e., quintiles of index of multiple deprivation), smoking (non-smoker, ex-smoker, and current smoker), body mass index, systolic blood pressure, diastolic blood pressure, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), C-reactive protein (CRP: not specified assay method), diabetes, use of antihypertensive medications, and use of statins. Any missing value of covariates was imputed using multiple imputations by chained equations (MICE) (supplementary appendices). A complete list of codes and algorithms used to identify all exposures and covariates used in this study can be found at https://www.caliberresearch. org/portal.

Follow up for incident HF, AF, MI, and intercurrent diseases

Endpoints including HF, AF, and MI were evaluated based on diagnosis codes (ICD-10 and Read codes), which included hospitalised- (HES) and non-hospitalised (CPRD) cases. For incident events, we considered only patients who had the first presentation with the outcome of interest. For intercurrent events, we defined them as any events that occurred during the period after baseline LDL-C measurement, but before the first occurrence of the event of interest. For example, intercurrent HF in MI endpoint can be defined as any HF events that occurred after baseline LDL-C measurement but before the first MI diagnosis. The validity of MI[175], AF[179], and HF endpoints[178] has been demonstrated in Chapter 3.

Statistical analysis

We use Cox proportional hazard model to calculate the hazard ratio (HR) from the time of blood sampling LDL-C measurement to the time of the incident event, censored (i.e., death or transfer out of practice), or end of the follow-up, whichever occurred first.

All models were adjusted for baseline covariates and stratified by sex and primary care practice. We analysed baseline LDL-C as both continuous and categorical variables to avoid presuming a particular shape of the association.

For categorisation, we used the cut-off for LDL-C according to a clinical guideline[201] as follows: less than 1.81 mmol/L (< 70 mg/dL), 1.88-2.32 mmol/L (70-89 mg/dL), 2.33-2.83 mmol/L (90-109 mg/dL), 2.84-3.35 mmol/L (110-129 mg/dL), 3.36-3.87 mmol/L (130-149 mg/dL), 3.88-4.39 mmol/L (150-169 mg/dL), and 4.39 mmol/L (170 mg/dL) or higher. We chose the middle category (i.e., LDL-C 2.84-3.35 mmol/L) as a reference group so that we could avoid the impact of an outlier, if there was any, on an overall shape of the association. The associations of each endpoint with baseline LDL-C were reported as per continuous (per 1 standard deviation [~ 38.67 mg/dL] lower in LDL-C) or categorical baseline LDL-C. Sensitivity analyses were carried out and have been explained in the supplementary appendices.

All analyses in the cohort part had been done using STATA version 13 (MP version, StataCorp). A two-tailed P-value of < 0.05 was accepted as a statistically significant value. The Bonferroni method was used, if applicable, for multiple comparison adjustment.

4.4.2 A trial-level meta-analysis of randomised controlled trials

Study selection and outcome data

We included randomised controlled trials of lipid-modifying agents published up to September 2018 with at least 1,000 participants who were followed up for at least one year to perform a meta-analysis. The trials included in this analysis are already listed in the Cholesterol Treatment Trialists (CTT) Collaboration[65,66] and previous works[60,67] (Table S 2-10). Depending on the availability of reported data, HF and MI were defined as both fatal and non-

fatal cases. AF was defined as either reported atrial fibrillation or cardiac arrhythmia.

Statistical analysis

We calculated relative risk (RR) of the outcome of interest per one mmol/L (38.67 mg/dL) reduction in LDL-C levels between active and control groups at the end of the follow-up period using the random-effect meta-regression method. For visualisation, we created scatter plots for the association between the RR of disease on the y-axis versus absolute reduction (in mmol/L) of LDL-C between active and control groups on the x-axis. Each dot on the plot represents an individual trial with the size depending on its variance. Then the association between the change in LDL-C and the risk ratio of outcome was the beta-coefficient of slope from the linear equation derived from the estimated straight line.

For trials with no events in both active or control arms, a nominal amount (0.5 cases) was added to the results for both trial groups. Main results were derived from univariable meta-regression models. Potential publication bias was assessed by visualising a funnel plot of log risk ratio (x-axis) and standard error (y-axis) of a model without a moderator (i.e., absolute change in LDL-C variable) and further evaluating p-value from the Egger's test. We also analysed multivariable meta-regression models and performed sensitivity analyses (supplementary appendices). All analyses in this part were done by using the 'metafor' and the 'CALIBERdatamanage' packages in R version 3.3.2.

4.4.3 Mendelian randomisation (MR)

Data Sources

We used summary-level data for lipids from the Global Lipids Genetics Consortium (GLGC),[202] MI data were from both the Coronary Artery Disease Genome-wide Replication and Meta-analysis plus Coronary Artery Disease Genetics (CARDIoGRAMplusC4D)[203] and UK biobank.[204] In addition to the UK Biobank resource, where AF and HF data were mainly taken from, HF data were obtained from the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) consortium.[205] Details of the consortia are provided in the

supplementary appendices. All data sets were limited to individuals of European ancestry, and beta-coefficients and standard errors were obtained for the per-allele association of each SNP with all exposures and outcomes from these data sources. Because this report used published genome-wide association studies data available in the public domain, specific ethnical review and consent from study participants was not sought but had been obtained in the original studies. For the use of the UK Biobank data, this study was approved by the committee with the application number 12113.

Selection of SNPs and MR Analyses

We used 185 lipid-associated SNPs identified by Willer *et al* to generate a series of genetic instruments for LDL-C.[202] This process was conducted by restricting to a set of SNPs in low linkage disequilibrium (pairwise $R^2 < 0.2$), with an exception for HERMES from where GWAS-significant SNPs (P-value $< 5x10^{-8}$) for LDL-C were pruned ($R^2 < 0.05$; LD window of 10,000kb; using the UKB10K LD reference). SNPs across consortia were matched and aligned to ensure the same strand used throughout the analysis, and effect allele frequencies were checked for concordance. We used the two-sample MR approach with various methods, including i) inverse variance weighted (IVW) MR; ii) MR-Egger; iii) weighted median MR; and iv) multivariate MR (MVMR) analyses. MVMR, the method in which simultaneously takes into account for genetic variability of other lipids components (i.e., HDL-C and TG), will be presented as the main analysis and the rest as a sensitivity analysis. Also, MR-Egger will be used to test for horizontal pleiotropy. Details about the MR assumption testing and power calculation are provided in the supplementary appendices. All analyses in the MR part were performed using the 'TwoSampleMR' package in R (version 3.3.2).

4.5 Results

4.5.1 Observational associations

At baseline, the study population of 1,142,656 had a mean age of 55.8 years, and 15% were receiving statins or other lipid-modifying agents. Mean LDL-C was 3.30 mmol/L (127.61 mg/dL) and about one-third (n=360,170) had baseline LDL-C < 2.84 mmol/L (110 mg/dL). In our CALIBER cohorts, we

observed that lower LDL-C levels were associated with younger age, lower blood pressure, lower total cholesterol and triglyceride levels, but higher prevalence of type 2 diabetes, chronic kidney disease, use of lipid-lowering medications, blood-pressure-lowering medications, and antiplatelets (Table 4-1 and Table S 4-2). More than 40% of participants received statins either at baseline or during the follow-up period. In total, first cardiovascular events (fatal or non-fatal) were recorded during a median follow-up of 5.1 years (interquartile range [IQR] 2.4 to 8.4 years) for the HF endpoint, 5.0 years (IQR: 2.3 to 8.3 years) for the AF endpoint, and 5.1 years (IQR: 2.4 to 8.5 years) for the MI endpoint from which 25,352 HF cases, 46,235 AF cases and 17,571 MI cases were identified (Figure S 4-1). We also noticed that the cumulative incidence of HF and AF increased as LDL-C levels lowered while that of MI showed the opposite pattern (Figure S 4-2).

We found an inverse association between LDL-C and incident HF and AF: Every 1 SD lower in LDL-C levels (~ 1 mmol/L or 38.67 mg/dL) was associated with an increase in the incidence of both HF (HR 1.09, 95%CI 1.08 to 1.11) and AF (HR 1.11, 95%CI 1.10 to 1.12) (Figure 4-1). These paradoxical findings were still consistent and even more obvious after LDL-C levels were categorised. A significantly positive association of LDL-C levels with an incidence of MI was confirmed in our cohort with HR of 0.89 (0.88 to 0.91) per one SD lower in LDL-C (Figure 4-1).

Subgroup analyses have shown the robustness of our results regardless of values of LDL-C used (i.e., yearly averaged value or single value), gender, age groups, and intercurrence of CVDs. Also, excluding the first four years events did not significantly change the direction of the associations. However, having intercurrent CVD or having hypertension at baseline significantly modified the results (P-value for heterogeneity < 0.05). Further exclusion of statin use strengthened the associations between LDL-C and HF and AF, suggesting a potential confounding role of the use of statins in both diseases (Figure 4-2). Schoenfeld residual plots and proportional hazard plots indicated that hazard functions in our analyses were constant over time. Our results, therefore, are valid and reliable (Figure S 4-5 and S 4-6).

Interestingly, further analysis on subtype of HF and AF (Figure 4-3) have shown that the association of LDL-C became inflated with only HF due to chronic respiratory disease. Additional adjusting for competing risk did not significantly deviate the results from the main findings (Figure 4-3).

4.5.2 Results from meta-analysis of major randomised controlled trials

Among 53 included RCTs, 33 and 25 studies had reported heart failure and atrial fibrillation (including cardiac arrhythmia and atrial flutter) as their endpoints, respectively (Table S 2-10). Our meta-regression results were based on 8,591 heart failure events (i.e., hospitalisation or death) and 6,122 cardiac arrhythmia events out of 265,473 and 198,282 total populations, respectively. In pooled analyses, we found no significant associations between change in LDL-C levels and the risks of HF (RR 1.05, 95% CI 0.95 to 1.15) and AF (RR 0.89, 95% CI 0.77 to 1.02) (Figure 4-4).

The source of small to moderate degree of statistical heterogeneity (I^2 25-50%) observed in our meta-regression results had been examined further by stratified analyses according to types of analyses (i.e., univariate vs multivariate), types of lipid-modifying agents, and types of prevention. Although most of the stratified results did not significantly deviate from our main findings, types of lipid-lowering agents might be a potential source of the noticed heterogeneity (Figure S 4-7). The visually symmetrical shape of funnel plots for both HF and AF endpoints with corresponding Egger's p-value > 0.05 indicated that publication bias should not be a major concern for both outcomes (Figure S 4-8).

4.5.3 Results from the genetic study

From Figure 4-5, the pooled data set includes 63,746 individuals with a diagnosis of coronary heart disease from the CARDIoGRAMplusC4D consortium corresponding to 177 SNPs, which explained 8% of the variability in LDL-C levels. From UK biobank, we extracted 181 SNPs from 512 cases of first diagnostic HF, 3,349 cases of first diagnostic AF, and 1,761 cases of first diagnostic MI, which also explained 8% of the variability in LDL-C levels. The odds ratio for the risk of HF and AF per genetically determined 1-SD decrease in LDL-C from multivariate models (MVMR) were 0.79 (0.61 to 1.02) and 0.91

(0.80 to 1.04), respectively. This suggested no causal association between LDL-C and both diseases and all sensitivity MR methods also showed robust results.

Interestingly, if any event cases were analysed instead of first event individuals, the results suggested a causal role of LDL-C on both HF and AF corresponding OR (95%CI) of 0.74 (0.60, 0.90) and 0.85 (0.76, 0.96), respectively. These were consistent with those from HERMES on the risk of HF in which genetically-determined 1 SD reduction in LDL-C from an unadjusted generalised summary-based MR (GSMR) model was associated with a lower risk of HF (OR 0.85 [95%CI: 0.83 to 0.88]). Conditioning on AF did not change the results. The causal role of LDL-C on HF, however, attenuated to null upon controlling for coronary artery disease (CAD) (OR 1.00 [95%CI: 0.96, 1.03]) (Figure 4-5).

Moreover, the genetic instrument for LDL-C showed neither the evidence of horizontal pleiotropy with all corresponding p-values for Egger's intercept coefficient > 0.05 (Table S 4-3), nor a potential violation of the InSIDE assumption (i.e., not highly correlated between instrumental variable effect and SNPs-exposure effect, see Table S 4-5). For MI, MVMR models had shown consistent direct associations between genetically determined-LDL-C and first presentation of MI, with OR of 0.72 (0.60 to 0.86) and 0.64 (0.60 to 0.69) per 1-SD (approximately 1 mmol/L or 38.67 mg/dL) genetically instrumented lower LDL-C from UK Biobank and CARDIOGRAMplus-C4D, respectively. In our study, although more than 80% of the power of statistics could be ensured from the association between LDL-C and MI, those between LDL-C and HF and AF are still uncertain (Table S 4-4).

4.6 Discussion

We have reported the first evaluation of the causal relevance of LDL-C in the two most common cardiac diseases globally, HF and AF. We have presented the first study on HF and AF in the same cohorts, which had never been done before. We compared high-resolution observational cohorts (more than ten times more participants than previous reports),[113] a new meta-regression of lipid-lowering trials and new MR with the largest GWAS on HF outcomes

(HERMES consortium). We found strong inverse associations extending into low LDL-C levels with incident HF and AF. The drug trials and the genetic evidence (MR) did not support a causal role for LDL-C and HF and AF, suggesting that the observational associations reflect residual confounding or reverse causation. Taken together, these three forms of evidence do not support a role for lowering LDL-C in the primary prevention of HF and AF.

4.6.1 Heart failure - what is new about our observation?

We found strongly inverse association extending from low (<1.81 mmol/L or <70 mg/dL) to high (>4.39 mmol/L or >170 mg/dL) LDL-C levels and incident HF (25,352 cases). To date, only a few (much smaller) observational cohorts have reported the association of LDL-C with the risk of HF, and their findings were mixed. Most studies showed a null association,[72,108,109] but one showed a positive association,[112] whereas another one showed an inverse association among patients without ischaemic heart disease.[113] Unlike previous studies, we stratified the associations with intercurrent MI and other CVDs rather than adjusting for them as in previous works.[31,32,71,73,77,78,106,108,110,112,113] We found that using antihypertensive medications at baseline significantly attenuated the magnitude, not the direction, of the association between LDL-C and HF (Figure 4-2). Excluding the use of statins at baseline even strengthened the paradoxical association. Moreover, we observed that intercurrent MI did not have mediating effect on the association between LDL-C and the risk of HF and AF (supplementary appendices). Instead, MI might play a role as an effect modifier on the risk of HF as we found the significant paradoxical association between LDL-C and incident HF only among patients without intercurrent MI.

4.6.2 LDL-C and HF: How have we extended knowledge from the trials and genetic studies?

This was the first report making a direct inference between change in LDL-C levels and the risks of HF and AF from trials of lipid-lowering agents. Our meta-regression of randomised controlled trials did not reveal any associations of LDL-C with HF, regardless of drug classes, except for fibrates that might be linked with the lower risk of HF. The previous meta-analysis had shown that statins could reduce non-fatal hospitalisation due to HF in patients without

preceding MI.[66] This suggests that statins might prevent HF through a mechanism not related to an LDL-C-lowering (pleiotropic) effect.[206]

No genetic study on the association between LDL-C and HF has been previously reported. We are the first to show that LDL-C is not causally relevant to HF, while the results from HERMES also indicated that CAD might confound the association observed between LDL-C and HF.

4.6.3 If not causally relevant, what could be the alternative explanation?

Alternative explanations include frailty (reverse causality), asymptomatic cardiac hypertrophy, or brain natriuretic peptide (BNP) (collectively as a residual confounder). Frailty may co-present with HF, which, in turn, can cause low LDL-C levels.[207,208] Nevertheless, the robustness of findings after stratification by age group and comorbidity or restrictive analyses by excluding patients who developed HF within the first four years after baseline LDL-C measurement, suggests that reverse causality was minimal. Additionally, we found high prevalence rates of diabetes and chronic kidney disease, and use of antihypertensive medications (a proxy of hypertension) among the cohorts in the low LDL-C strata; these are risk factors for cardiac hypertrophy: a condition that often precedes the development of symptomatic HF.[209] Therefore, patients in the low LDL-C groups seemed to have some characteristics that increase their risk of further development of HF, and these might residually confound our observed associations. However, this argument is not supported by the results from the subgroup analysis of the use of antihypertensive medication showing that the association was even stronger among participants who were not prescribed antihypertensive medication at baseline.

Regarding BNP, we did not control our findings for BNP levels. High BNP levels are associated with low BMI[210] and high CKD prevalence[211], which were collective characteristics of individuals in the lower LDL-C strata. However, it has been shown that BNP (NT-proBNP in particular) may be casually protective of incident HF by protecting against collagen accumulation and the cardiac remodelling leading to progressive HF.[212] In other words, if we consider only some characteristics linked to BNP levels, patients in the low LDL-C strata should have higher BNP levels than those in the upper LDL-C

strata. Admittedly, the protective effect of BNP against HF should reduce the risk of HF rather than increase it among patients in the low LDL-C strata as we observed. Therefore, a confounding effect of BNP in our findings was less likely to occur.

4.6.4 Atrial fibrillation – what does the observation add?

We found strong, monotonic inverse associations extending from low (i.e., < 1.81 mmol/L or < 70 mg/dL) to high (i.e., > 4.39 mmol/L or > 170 mg/dL) LDL-C levels and incident AF (46,235 cases). Our findings were consistent with some small cohorts[32,69,70,73,76,107] but not with others that showed null[31,74,75,110] or positive associations[68] (Table S 2-9). Alternative explanations for an observed inverse association are hyperthyroidism and natriuretic peptide, which might play a role as a residual confounder. Hyperthyroidism can reduce LDL-C levels and simultaneously increase the risk of AF.[32,70,76] Even though our observational analyses were not adjusted for thyroid disease at baseline, previous work had performed the adjustment but failed to support thyroid hormone as a confounder.[70] Furthermore, we observed in some studies that after adjusting for natriuretic peptide (e.g., ANP and BNP), the results were likely to become null despite a significant association observed in an unadjusted model. Therefore, the natriuretic peptide may mediate or confound the association between LDL-C and risk of AF.[31,74]

4.6.5 What does trial and genetic evidence add on LDL-C and AF?

To our knowledge, there is no previous meta-analysis on LDL-C *per se* because all have focused on the effect of statins on AF or ventricular tachyarrhythmia (VT) with inconsistent findings.[65,213–216] For instance, statins lowered the risk of AF only in short-term (i.e., less than six months of following-up) trials[65] and trials of postoperative cardiac surgery.[214]

Findings from our genetic study are consistent with previous work in which gene score for LDL-C was not associated with the incidence of AF in seven large cohorts, which included 5,434 incident AF cases among 64,901 individuals (HR per allele score increase in LDL-C: 0.98, 95% CI 0.94 to 1.02).[92] Our meta-regression findings, again, suggest that the benefit of statins

on AF is potentially due to other pathways that are not relevant to the LDL-C lowering effect.[206,217]

4.6.6 Positive control: myocardial infarction and further insight

The direction of the association between LDL-C and incident MI was robust across study designs. This is consistent with the well-established knowledge that high LDL-C levels can cause MI through the atherosclerotic pathway,[36] and a reduction of LDL-C is associated with the reduced risk of major coronary events.[61,62] Moreover, a recent genetic study of 438,952 participants enrolled in UK Biobank had confirmed our findings that genetically determined LDL-C levels were inversely associated with risks of major coronary events.[218] Nevertheless, we observed that the magnitude of the association was strongest among genetic findings, followed by trials and observational studies, which is counterintuitive and cannot be explained by the difference in LDL-C exposure time in different designs (Figure S 4-3).

According to a Venn diagram (Figure S 4-1), it is worth noticing that HF and AF were more common than MI. In our cohorts, we identified 17,571 new cases of MI, whereas 25,352 and 46,235 new cases of HF and AF were identified, respectively, over the median follow-up of 5 years. Furthermore, in terms of concurrent CVD, we found that only a few HF cases (2,174 out of 25,352 HF cases [8.6%]) who also had intercurrent MI during follow-up.

4.6.7 Strengths

This was the first study that comprehensively investigated the association and causation of LDL-C on the two most common, but less-well studied, CVDs using three different study designs (i.e., cohort, trials, and genetic). The strengths of our study are as follows: 1) our observational results were derived from huge and representative cohorts (N = 1,142,656). Having such a substantial sample also allowed us to study HF, AF, and MI together, which improved the ability to evaluate intercurrent disease.

Moreover, this was the first time that we could examine an association of the disease with a very low LDL-C level; i.e., LDL-C < 1.81 mmol/L (< 70mg/dL), in the observational cohort for HF, AF, and MI. 2) this was the first meta-regression focusing on the change in LDL-C levels (not drugs) on the risk of

HF and AF and 3) we applied numbers of MR sensitivity analyses in different data sources (consortium and UK biobank) to ensure the validity and robustness of findings. 4) MR analysis on a large independent consortium (HERMES) had shown the robustness and secured generalisability, especially in European populations.

4.6.8 Limitations

However, each of the study designs had important limitations. The observational cohort based on EHRs: ascertainment of AF or HF cases in our study was based solely on ICD-10 or Read Codes. Since the nature of AF is asymptomatic or patients may have prior asymptomatic left-ventricular hypertrophy, using diagnostic codes is likely to underestimate the true incidence of both diseases. However, it has been shown that the use of diagnostic codes from CPRD can provide highly accurate case identification; an overall positive predictive value (based on case-note review approach) of 96% and 98% for HF[219] and AF[220], respectively, with 92% of completeness, compared with national registry data.[221] Moreover, a combination of EHR sources (e.g., primary care, secondary care, and national registry) can further improve the case identification and yield representative AF[179] and HF[178] cases.

Second, the randomised controlled trials in our meta-regression may underestimate the incidence of HF and AF since these diseases are often not pre-specified as a primary outcome of interest; 14 out of 27 statin trials pre-specified HF in their protocols but no trials pre-specified AF as their primary endpoint. Although most of the trials had excluded patients with severe heart failure (NYHA class III or IV) or uncontrolled symptomatic AF before the randomisation process, we cannot exclude prevalent cases of HF and AF. Also, we lacked trials in healthy population. Our meta-regression results cannot be generalised to the incident cases, and, therefore, to the implication of the primary prevention role.

Lastly, although our MR results showed no apparent evidence of horizontal pleiotropy, we still had an issue of low statistical power, especially from UKB (Table S 4-4). However, consistent findings from the largest GWAS (HERMES) on HF to date, and previous genetic evidence on AF (AFGen consortium), have shown no causation between LDL-C and AF[92] supporting our conclusion.

Potential bias from the observational study design are as follows:

Selection bias: patients who were recruited in my analysis are the individuals with lipid measurement. Therefore, only patients with an indication to measure blood lipids will be included. This would limit the generalisibility of my findings rather than artifact the results. Also, the selection bias can be arisen when there is a discrepancy in the quality of care at practice level. For example, the same patient might be eligible to have lipid measured in one GP but ineligible if he or she goes to another GP. To minimise the bias due to the variation of practice level, I have stratified all analysis by gender and practice level.

Misclassification and information bias:

- Extracted phenotypes of outcome are mainly based on signs and symptoms but not based on an objective confirmation, such as echocardiogram results. For instance, there were only 3% of HF cases who had codes for echocardiography that confirmed HF. Therefore, it is likely to include other health conditions that are mimic signs and symptoms of HF, such as chronic respiratory disease exacerbation as HF cases (i.e., false positive cases).
- Diagnosis taken from EHRs is based solely from one physician (not by adjudication committee), and this might vary according to level of expertise of physicians. Therefore, this prone to misclassification bias.

These two scenarios above would result in misclassification bias and increasing false positive cases. In other words, this would lead to decrease in specificity due to increasing false positive cases, which leads to type I error, and could inflating the observation. Furthermore, subgroup analysis of HF based solely on echocardiography codes (i.e., systolic and diastolic HF) had shown no associations with LDL-C, which are inconsistent with the main findings (Figure S). Therefore, the impact of misclassification bias cannot be excluded.

- Lipid measurement taken from EHRs is impossible to be traced back whether it derived from Friedewald equation or direct measurement, which is more evident in LDL-C. This scenario can cause measurement error and lead to information bias and would unpredictably bias results in either directions (inflating or attenuating findings). However, some

methods to control for this bias, such as excluding outlier lipid levels, using one-year averaged lipid levels (and compared with the results from using single lipid levels), and stratifying results by practice levels, can be used to attenuate the impact of measurement error and misclassification bias. Moreover, although using Friedewald formulae may resulting in significantly lower LDL-C, comparing with direct measurement especially when TG level > 1.7 mmol/L, the absolute difference was between 0.13 and 0.35 mmol/L,[185] which might not be clinically important.

Attrition bias (i.e., bias due to loss to follow-up or dropping out): In my study, there were 5% of studied populations who were censored due to death from other causes, and this might compete the outcome of interest. However, in age and sex-adjusted model, further adjusting for competing risk (Figure 4-3) did not significantly change the findings.

4.6.9 Implications of findings

Clinical implications

Clinical guidelines have no current recommendations for primary prevention of HF and AF, and our results do not support LDL-C lowering as a strategy for the primary prevention of the two most common cardiac diseases. Nonetheless, lowering LDL-C is still likely to be beneficial through the prevention of MI and, therefore, could indirectly prevent HF and AF.

Also, we raised a general point on the research for causation by illustrating how misleading an observation might be. Our study showed the discordance between observational (inverse association) and genetic and trial evidence (null). If observational CVD epidemiology started with HF and AF (not MI), then maybe we would never have developed lipid-lowering drugs because we were misled to believe that low LDL-C levels might be associated with increasing risks of HF and AF.

Research implications

Our study is an example of the simultaneous use of different study designs (e.g., EHRs cohort, a meta-regression of trials, and MR) to triangulate and tackle a particular research question.[222] This should be encouraged since we

can strengthen the evidence and make it more conclusive from the best use of available data sources. Furthermore, we observed a gap in CVD study in that the temporal relationship among the three most common CVDs (i.e., MI, HF, and AF) requires further investigation. Also, we are still in need of research with large-scale definitions of HF subtypes (e.g., HF with reduced or preserved ejection fraction), and AF (e.g., paroxysmal, persistent, or permanent AF).

4.7 Conclusion

We found strong, inverse (paradoxical) observational associations between LDL-C levels and incident HF and AF, which were not supported either by meta-regression of clinical trials of various lipid-lowering agents or by Mendelian randomisation. LDL-C *per se* is, therefore, unlikely to be causally relevant to the onset of HF and AF, and the observed associations likely reflect residual confounding or reverse causation. In summary, the evidence here does not support the benefit of lowering LDL-C for the primary prevention of HF and AF.

Table 4-1 Observational cohort: participant characteristics of population-based EHR cohort (n=1,142,656)

Baseline	LDL cholesterol at baseline (mmol/L)				P-values
	< 1.81	2.84-3.35	> 4.39	Total	
N	53,675	246,965	149,808	1,142,656	
Female	51.6%	52.5%	55.1%	53.0%	P < 0.001
Age (year)	56.0 (15.0)	55.3 (13.2)	57.5 (12.1)	55.8 (13.2)	P < 0.001
White	85.4%	87.6%	93.1%	88.6%	P < 0.001
Missing*	36.2%	41.7%	43.4%	41.7%	
Health behaviors, physical and laboratory measurements at baseline					
Non-smokers	55.4%	59.2%	57.1%	58.5%	P < 0.001
Missing*	0.5%	0.6%	0.7%	0.6%	
Body mass index (kg/m^2)	27.8 (6.3)	27.8 (5.7)	27.9 (5.0)	27.8 (5.6)	P < 0.001
Missing	3.9%	4.5%	4.6%	4.4%	
Systolic blood pressure (mmHg)	133.4 (16.3)	135.5 (16.6)	138.5 (16.7)	135.8 (16.7)	P < 0.001
Missing	1.0%	1.1%	0.7%	1.0%	
Diastolic blood pressure (mmHg)	78.6 (9.1)	81.2 (9.2)	82.6 (9.1)	81.2 (9.3)	P < 0.001
Missing	1.0%	1.1%	0.7%	1.0%	
eGFR (mL/min/1.73m^2)	83.4 (31.1)	82.5 (19.7)	83.8 (18.6)	82.8 (20.7)	P < 0.001
Missing	2.2%	3.2%	3.6%	3.2%	
CRP (mg/L), median (IQR)	4.0 (2.0-8.0)	4.0 (2.0-7.5)	4.0 (2.0-7.7)	4.0 (2.0-7.6)	P < 0.001
Missing	55.3%	55.8%	56.8%	55.9%	
LDL cholesterol (mmol/L)	1.5 (0.3)	3.1 (0.1)	4.9 (0.5)	3.3 (0.9)	P < 0.001
HDL cholesterol (mmol/L)	1.5 (0.6)	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)	P < 0.001
Missing	0.2%	0.3%	0.5%	0.3%	
Total cholesterol (mmol/L)	3.8 (0.7)	5.2 (0.5)	7.0 (0.7)	5.5 (1.0)	P < 0.001
Missing	6.7%	6.0%	5.1%	5.8%	
Triglyceride (mmol/L), median (IQR)	1.2 (0.8–2.0)	1.2 (0.9-1.8)	1.6 (1.2-2.1)	1.3 (0.9-1.9)	P < 0.001
Missing	0.6%	0.8%	1.3%	0.9%	
Health conditions at baseline					
Diabetes type 2	16.7%	4.6%	2.0%	5.1%	P < 0.001
Chronic kidney disease	7.6%	3.4%	2.9%	3.6%	P < 0.001
Cancer	4.4%	3.4%	3.3%	3.4%	P < 0.001
COPD	2.8%	1.7%	1.5%	1.8%	P < 0.001
Medication					
Statins (at baseline)	40.9%	11.4%	13.1%	14.5%	P < 0.001
Statins (during follow-up)	44.8%	26.7%	46.4%	32.4%	P < 0.001
Other lipid-lowering drugs	2.2%	0.7%	0.7%	0.8%	P < 0.001
Antihypertensive drugs	43.1%	29.7%	28.6%	30.6%	P < 0.001
Antiplatelet drugs	17.3%	5.6%	4.3%	6.4%	P < 0.001

Note: Values are presented as numbers (percentage) or mean (standard deviation) or median (interquartile range) as appropriate. *Percentages of missing category were separately calculated from complete cases. Corresponding values for LDL-C are: 1.81 mmol/L = 70 mg/dL; 2.33 mmol/L = 90 mg/dL, 2.84 mmol/L = 110 mg/dL, 3.36 mmol/L = 130 mg/dL, 3.88 mmol/L = 150 mg/dL, 4.39 mmol/L = 170 mg/dL. To convert mmol/L of HDL, total cholesterol and triglyceride to mg/dL, multiply by the factor of 38.67, 38.67, and 88.57, respectively.

Abbreviations: BMI; Body mass index, COPD; chronic obstructive pulmonary disease, CRP; C-reactive protein, DBP; Diastolic blood pressure, eGFR; estimated glomerular filtration rate, HDL; high density lipoprotein, LDL; low density lipoprotein, SBP; Systolic blood pressure

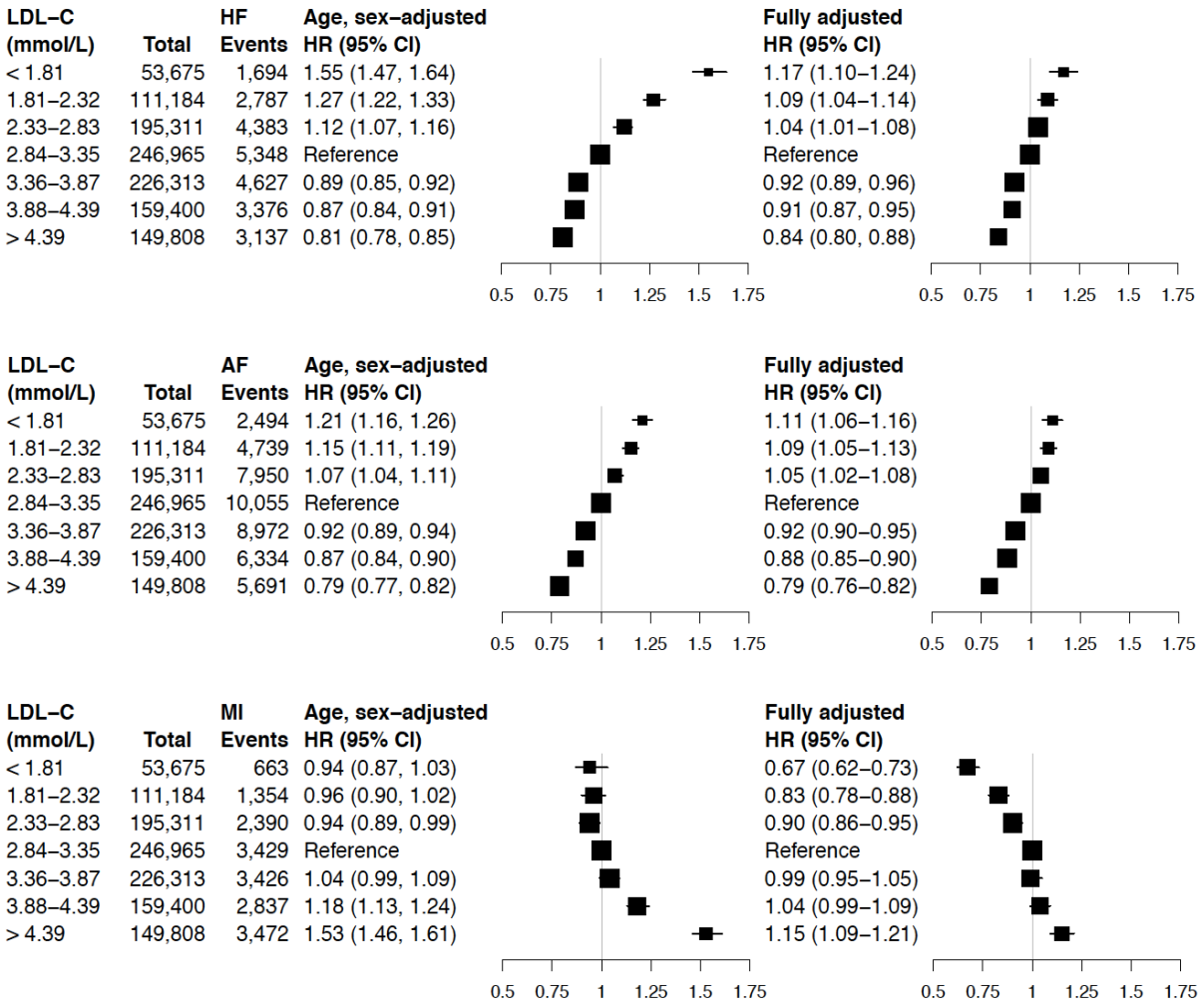


Figure 4-1 Observational cohort: The associations of different levels of LDL-C and incident HF (total 6,472,147.27 person-years), AF (total 6,393,476 person-years), and MI (total 6,475,969.07 person-years) among 1,142,656 individuals without diagnosed CVD at baseline over the median follow-up of 5 years (interquartile range: 2-8 years)

Note: Fully adjusted models were stratified for gender and primary care practice and adjusted for age, socioeconomic status, smoking, body mass index, systolic blood pressure, diastolic blood pressure, high-density lipoprotein cholesterol, triglyceride, C-reactive protein, diabetes, use of blood pressure lowering drugs and use of statins. The size of the boxes varies based on inverse variance of the data in each category. Corresponding values for LDL-C are: 1.81 mmol/L = 70 mg/dL; 2.33 mmol/L = 90 mg/dL, 2.84 mmol/L = 110 mg/dL, 3.36 mmol/L = 130 mg/dL, 3.88 mmol/L = 150 mg/dL, 4.39 mmol/L = 170 mg/dL.

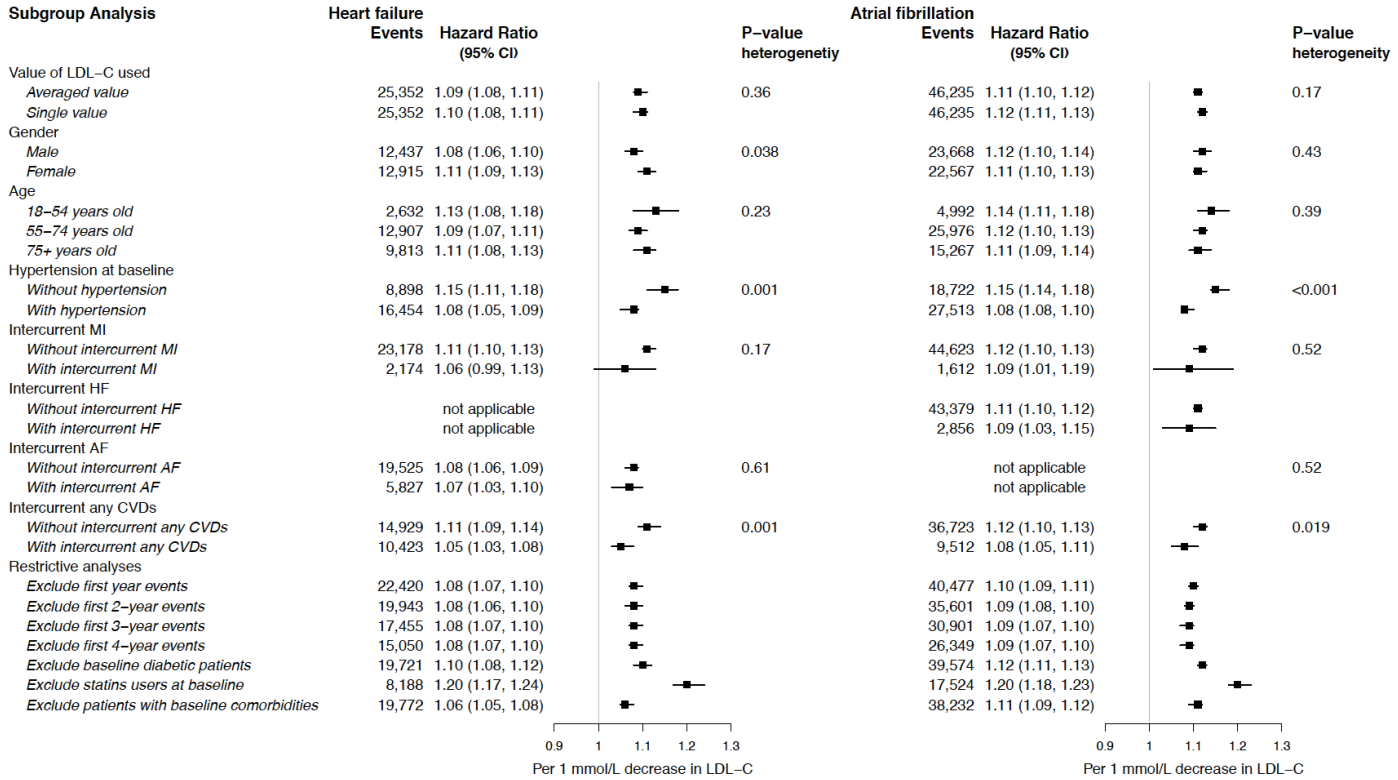
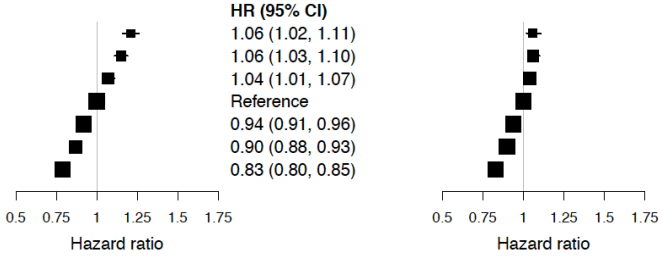


Figure 4-2 Subgroup analyses for associations between LDL-C and incident HF and AF.

Note: All models were stratified for gender and primary care practice and adjusted for age, body mass index, smoking, systolic blood pressure, diabetes, socioeconomic status, high-density lipoprotein cholesterol, triglyceride, use of blood pressure lowering drugs and use of statins. Baseline comorbidities include having been diagnosed with cancer, kidney disease, and COPD at baseline LDL-C measurement.

LDL–C (mmol/L)	Total	HF Events	Age, sex–adjusted HR (95% CI)	Competing risk adjusted HR (95% CI)
< 1.81	53,675	1,694	1.55 (1.47, 1.64)	1.37 (1.29, 1.44)
1.81–2.32	111,184	2,787	1.27 (1.22, 1.33)	1.17 (1.12, 1.23)
2.33–2.83	195,311	4,383	1.12 (1.07, 1.16)	1.08 (1.04, 1.12)
2.84–3.35	246,965	5,348	Reference	Reference
3.36–3.87	226,313	4,627	0.89 (0.85, 0.92)	0.91 (0.88, 0.95)
3.88–4.39	159,400	3,376	0.87 (0.84, 0.91)	0.91 (0.87, 0.95)
> 4.39	149,808	3,137	0.81 (0.78, 0.85)	0.86 (0.82, 0.90)

LDL–C (mmol/L)	Total	AF Events	Age, sex–adjusted HR (95% CI)	Competing risk adjusted HR (95% CI)
< 1.81	53,675	2,494	1.21 (1.16, 1.26)	1.06 (1.02, 1.11)
1.81–2.32	111,184	4,739	1.15 (1.11, 1.19)	1.06 (1.03, 1.10)
2.33–2.83	195,311	7,950	1.07 (1.04, 1.11)	1.04 (1.01, 1.07)
2.84–3.35	246,965	10,055	Reference	Reference
3.36–3.87	226,313	8,972	0.92 (0.89, 0.94)	0.94 (0.91, 0.96)
3.88–4.39	159,400	6,334	0.87 (0.84, 0.90)	0.90 (0.88, 0.93)
> 4.39	149,808	5,691	0.79 (0.77, 0.82)	0.83 (0.80, 0.85)



HF Subtype	Events	HR (95% CI) per one SD decrease in LDL–C
Overall HF	25,352	1.10 (1.08, 1.11)
Systolic HF	588	1.06 (0.96, 1.14)
Diastolic HF	139	0.99 (0.83, 1.19)
HF due to chronic respiratory disease	293	1.49 (1.31, 1.71)
Hypertensive HF	189	1.09 (0.93, 1.27)
Other HF	24,143	1.09 (1.08, 1.11)
AF Subtype		
Overall AF	46,233	1.11 (1.10, 1.12)
Paroxysmal AF	4,318	1.13 (1.09, 1.16)
Persistent/Permanent AF	56	1.14 (0.85, 1.55)
Atrial flutter	1,157	1.16 (1.09, 1.24)
Other AF	40,704	1.11 (1.10, 1.12)

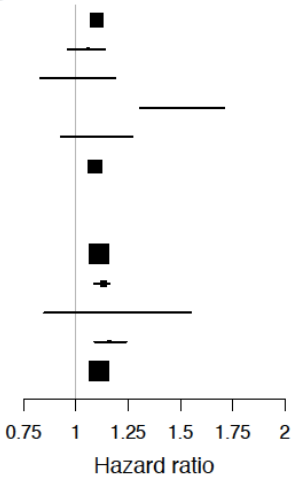
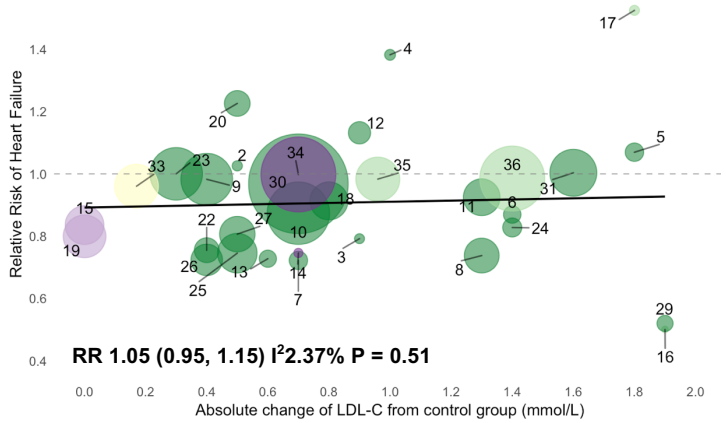
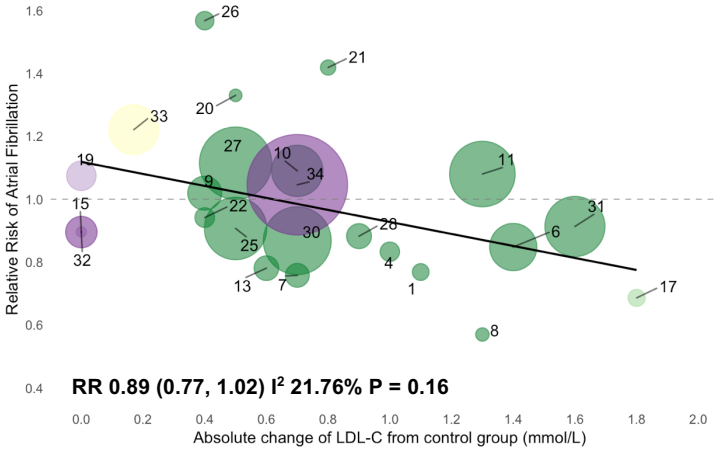


Figure 4-3 Competing risk adjustment and subtype analysis

Heart failure (N = 33 trials: 8,591 events and 265,473 total participants)



Atrial fibrillation (N = 25 trials: 6,122 events and 198,282 total participants)



Myocardial infarction (N = 53 trials: 27,630 events and 429,962 total participants)

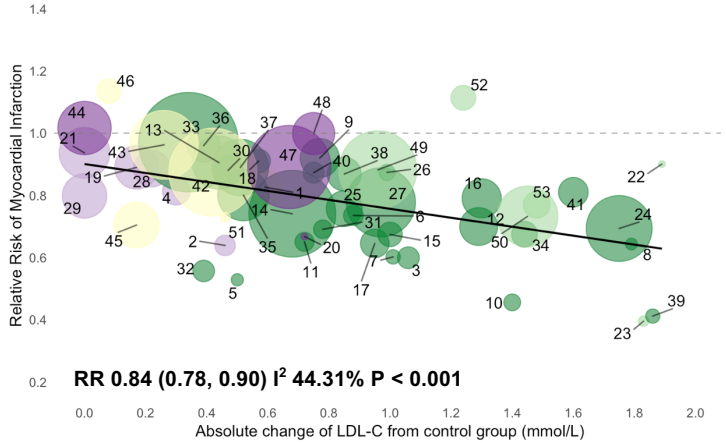
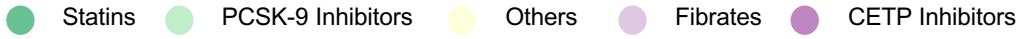


Figure 4-4 RCTs of lipid-lowering agents: Univariable meta-regression of the risk of HF, AF, and MI per 1 mmol/L reduction in LDL-C.

Note: Size of plots is proportional (weighted) to inverse-variance. Designated numbers represent study identification (see chapter supplementary Table S 2-10 and Table S 2-11).


Statins
PCSK-9 Inhibitors
Others
Fibrates
CETP Inhibitors

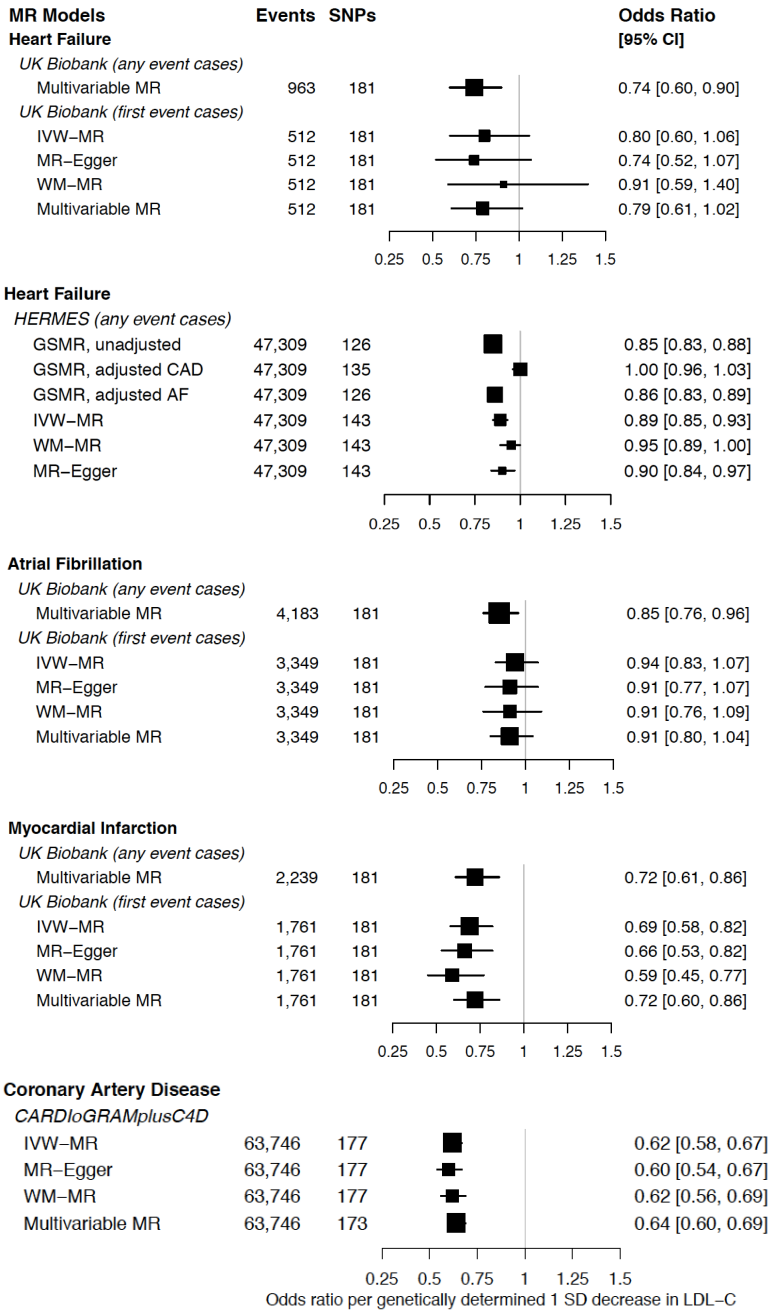


Figure 4-5 Genetic (MR) study of associations between 1 SD decrease in LDL-C and the risk of HF, AF, MI, and CHD

Note: 1 SD = 1 mmol/L (or 38.67 mg/dL) of LDL-C levels. The size of the box varied according to the data's variance (inverse variance weighted).

Abbreviations: CARDIoGRAMplusC4D; Coronary Artery Disease Genome-wide Replication and Meta-analysis plus Coronary Artery Disease Genetics, CI; Confidence interval, HERMES; Heart Failure Molecular Epidemiology for Therapeutic Targets Consortium, IVW; Inverse variance weighted, MR; Mendelian randomisation, OR; Odds ratio, SNPs; Single nucleotide polymorphisms, WM; Weighted median.

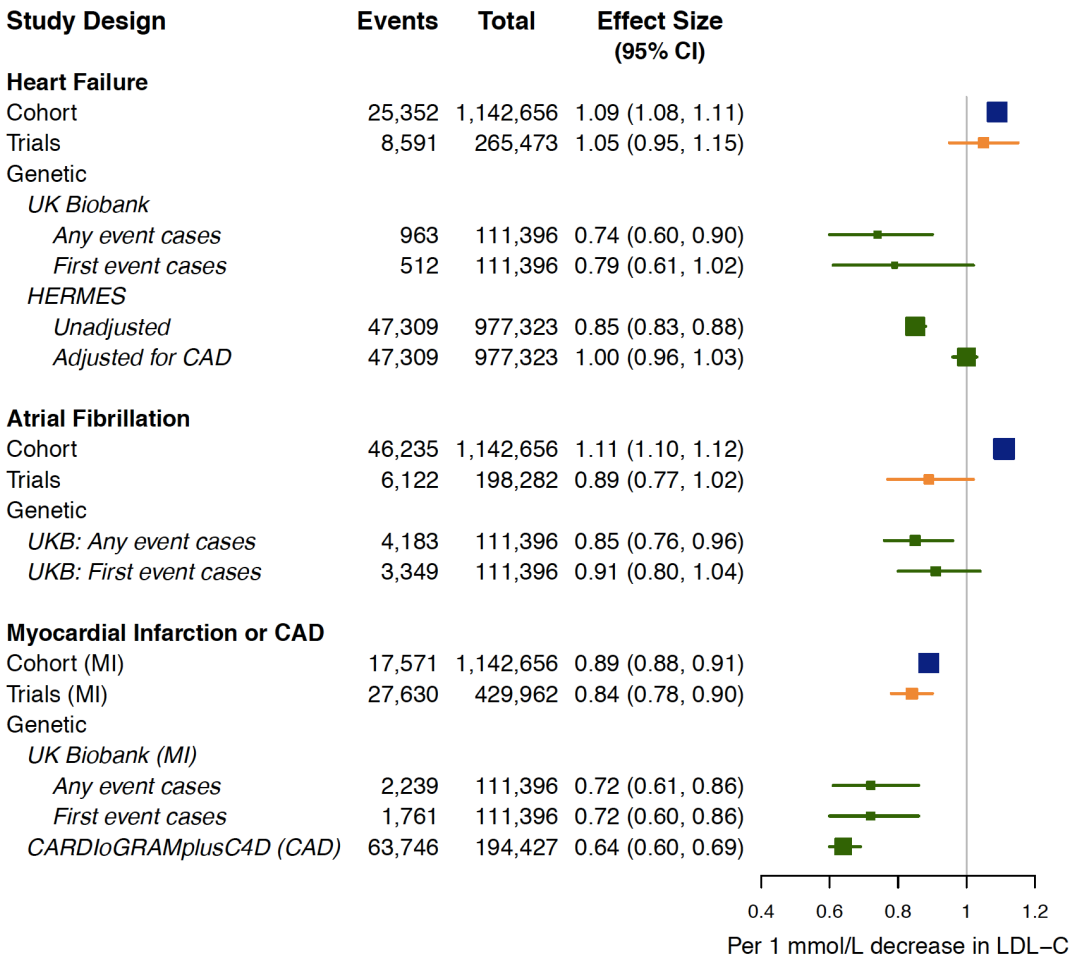


Figure 4-6 Comparison of the estimated associations between LDL-C and risk of HF, AF, and MI (or CAD) from cohort (hazard ratio), RCTs (risk ratio), and MR studies (odds ratio).

Note: The size of the box varied according to the data's variance (inverse variance).

4.8 Chapter Supplementary

Table S 4-1 The STROBE and RECORD statement

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 167	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	1.1-1.3: Page 167
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 168		
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 170		
Methods					
Study Design	4	Present key elements of study design early in the paper	Page 170		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 171		
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Supplementary appendices	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided.	6.1: Supplementary appendices

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
		Case-control study - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case		RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	6.2: Page 166, Supplementary appendices 6.3: Supplementary appendices
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Page 171-172, Supplementary appendices	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Page 171-172, Supplementary appendices
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 171-172		
Bias	9	Describe any efforts to address potential sources of bias	Page 173, Supplementary appendices		
Study size	10	Explain how the study size was arrived at	Supplementary appendices		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 173		

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	a)-b): Page 173 c): Supplementary appendices		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population. RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	12.1: Page 171 12.2: Supplementary appendices
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	Page 171 (Referenced to CALIBER portal, which includes extensive information.) Also, Referenced to a paper explaining the CALIBER program (S Denaxas et al, Int J Epidemiology)
Results					

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	a)-c): Supplementary appendices	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	Supplementary appendices
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	a)-c): Page 187 Chapter supplementary page 202 (Table S 4-2)		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures	Chapter supplementary page (Figure S 4-2)		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	a)-b): Page 189 (Figure 4-1) c): not relevant		

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Page 190 (Figure 4-2)		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Page 175-177		
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 183-185	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	Page 183-185
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 183-186		
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 184		
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Not relevant		
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	Not relevant

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.*Checklist is protected under Creative Commons Attribution (CC BY) license.

Table S 4-2 Full participant characteristics of the population-based EHR cohorts (n=1,142,656)

Baseline	LDL cholesterol at baseline (mmol/L)								P-values
	< 1.81	1.81-2.32	2.33-2.83	2.84-3.35	3.36-3.87	3.88-4.39	> 4.39	Total	
N	53,675	111,184	195,311	246,965	226,313	159,400	149,808	1,142,656	
Male	25,984 (48.4%)	49,796 (44.8%)	89,246 (45.7%)	118,073 (47.8%)	109,670 (48.5%)	76,533 (48.0%)	67,324 (44.9%)	536,626 (47.0%)	P < 0.001 P_{trend} 0.003
Female	27,691 (51.6%)	61,388 (55.2%)	106,065 (54.3%)	128,892 (52.2%)	116,643 (51.5%)	82,867 (52.0%)	82,484 (55.1%)	606,030 (53.0%)	
Age (year)	56.0 (15.0)	54.4 (14.5)	54.4 (13.8)	55.3 (13.2)	56.2 (12.7)	56.9 (12.3)	57.5 (12.1)	55.8 (13.2)	P < 0.001 P_{trend}<0.001
Ethnicity									
Caucasian	29,279 (85.4%)	57,345 (85.3%)	98,756 (86.2%)	126,080 (87.6%)	116,593 (89.6%)	83,485 (91.3%)	78,879 (93.1%)	590,417 (88.6%)	P < 0.001 P_{trend} < 0.001[$]
South Asian	2,474 (7.2%)	4,772 (7.1%)	7,815 (6.8%)	8,956 (6.2%)	6,746 (5.2%)	3,894 (4.3%)	2,696 (3.2%)	37,353 (5.6%)	
African	1,724 (5.0%)	3,322 (4.9%)	5,265 (4.6%)	5,485 (3.8%)	4,028 (3.1%)	2,373 (2.6%)	1,810 (2.1%)	24,007 (3.6%)	
Other	791 (2.3%)	1,750 (2.6%)	2,793 (2.4%)	3,358 (2.3%)	2,694 (2.1%)	1,689 (1.8%)	1,342 (1.6%)	14,417 (2.2%)	
Missing	19,407 (36.2%)	43,995 (39.6%)	80,682 (41.3%)	103,086 (41.7%)	96,252 (42.5%)	67,959 (42.6%)	65,081 (43.4%)	476,462 (41.7%)	
Health behaviors, physical and laboratory measurements at baseline									
Smoking									

Baseline	LDL cholesterol at baseline (mmol/L)								P-values
	< 1.81	**1.81-2.32**	**2.33-2.83**	**2.84-3.35**	**3.36-3.87**	**3.88-4.39**	**> 4.39**	**Total**	
Non-smokers	29,607 (55.4%)	64,750 (58.6%)	114,911 (59.1%)	145,408 (59.2%)	132,787 (59.0%)	91,939 (58.0%)	84,940 (57.1%)	664,342 (58.5%)	P < 0.001 P_{trend}< 0.001[$$]
Ex-smokers	14,429 (27.0%)	28,038 (25.4%)	48,963 (25.2%)	62,266 (25.4%)	57,144 (25.4%)	41,042 (25.9%)	38,214 (25.7%)	290,096 (25.5%)	
Current smokers	9,388 (17.6%)	17,771 (16.1%)	30,433 (15.7%)	37,903 (15.4%)	35,023 (15.6%)	25,404 (16.0%)	25,545 (17.2%)	181,467 (16.0%)	
Missing	251 (0.5%)	625 (0.6%)	1,004 (0.5%)	1,388 (0.6%)	1,359 (0.6%)	1,015 (0.6%)	1,109 (0.7%)	6,751 (0.6%)	
Body mass index (kg/m^2)	27.8 (6.3)	27.4 (6.0)	27.6 (5.9)	27.8 (5.7)	27.9 (5.4)	28.0 (5.2)	27.9 (5.0)	27.8 (5.6)	P < 0.001
Missing	2,069 (3.9%)	4,804 (4.3%)	8,550 (4.4%)	11,127 (4.5%)	10,102 (4.5%)	6,981 (4.4%)	6,924 (4.6%)	50,557 (4.4%)	P_{trend} < 0.001
Systolic blood pressure (mmHg)	133.4 (16.3)	133.0 (16.8)	134.0 (16.8)	135.5 (16.6)	136.7 (16.4)	137.7 (16.4)	138.5 (16.7)	135.8 (16.7)	P < 0.001 P_{trend} < 0.001
Missing	525 (1.0%)	1,345 (1.2%)	2,280 (1.2%)	2,631 (1.1%)	2,142 (0.9%)	1,313 (0.8%)	1,020 (0.7%)	11,256 (1.0%)	
Diastolic blood pressure (mmHg)	78.6 (9.1)	79.2 (9.3)	80.2 (9.3)	81.2 (9.2)	81.8 (9.1)	82.3 (9.1)	82.6 (9.1)	81.2 (9.3)	P < 0.001 P_{trend} < 0.001
Missing	525 (1.0%)	1,345 (1.2%)	2,280 (1.2%)	2,631 (1.1%)	2,142 (0.9%)	1,313 (0.8%)	1,020 (0.7%)	11,256 (1.0%)	
eGFR (mL/min/1.73m^2)	83.4 (31.1)	81.6 (24.5)	81.7 (21.1)	82.5 (19.7)	83.1 (18.8)	83.7 (18.8)	83.8 (18.6)	82.8 (20.7)	P < 0.001
Missing	1,183 (2.2%)	3,197 (2.9%)	5,977 (3.1%)	7,901 (3.2%)	7,370 (3.3%)	5,359 (3.4%)	5,414 (3.6%)	36,401 (3.2%)	P_{trend} < 0.001
CRP (mg/L)									

Baseline	LDL cholesterol at baseline (mmol/L)								P-values
	< 1.81	**1.81-2.32**	**2.33-2.83**	**2.84-3.35**	**3.36-3.87**	**3.88-4.39**	**> 4.39**	**Total**	
Mean (SD)	10.7 (25.6)	9.6 (23.2)	9.2 (21.7)	9.2 (21.4)	9.1 (21.4)	9.1 (21.1)	9.3 (21.5)	9.3 (21.8)	
Median (IQR)	4.0 (2.0-8.0)	4.0 (1.9-7.6)	4.0 (2.0-7.4)	4.0 (2.0-7.5)	4.0 (2.0-7.5)	4.0 (2.0-7.5)	4.0 (2.0-7.7)	4.0 (2.0-7.6)	P < 0.001 P_{trend} < 0.001
Missing	29,691 (55.3%)	61,355 (55.2%)	108,278 (55.4%)	137,786 (55.8%)	126,456 (55.9%)	89,634 (56.2%)	85,109 (56.8%)	638,309 (55.9%)	
LDL cholesterol (mmol/L)	1.5 (0.3)	2.1 (0.1)	2.6 (0.1)	3.1 (0.1)	3.6 (0.1)	4.1 (0.1)	4.9 (0.5)	3.3 (0.9)	P < 0.001 P_{trend} < 0.001
HDL cholesterol (mmol/L)	1.5 (0.6)	1.5 (0.5)	1.5 (0.5)	1.5 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.5 (0.4)	P < 0.001 P_{trend} < 0.001
Missing	118 (0.2%)	207 (0.2%)	331 (0.2%)	639 (0.3%)	712 (0.3%)	642 (0.4%)	769 (0.5%)	3,418 (0.3%)	
Total cholesterol (mmol/L)	3.8 (0.7)	4.3 (0.6)	4.7 (0.5)	5.2 (0.5)	5.7 (0.5)	6.2 (0.5)	7.0 (0.7)	5.5 (1.0)	P < 0.001
Missing	3,597 (6.7%)	7,022 (6.3%)	11,884 (6.1%)	14,706 (6.0%)	12,802 (5.7%)	8,770 (5.5%)	7,575 (5.1%)	66,356 (5.8%)	P_{trend} < 0.001
Triglyceride (mmol/L)									
Mean (SD)	1.6 (1.3)	1.4 (0.9)	1.4 (0.8)	1.4 (0.8)	1.5 (0.8)	1.6 (0.8)	1.7 (0.8)	1.5 (0.8)	
Median (IQR)	1.2 (0.8–2.0)	1.1 (0.8–1.7)	1.1 (0.8-1.7)	1.2 (0.9-1.8)	1.3 (1.0-1.9)	1.4 (1.1-2.0)	1.6 (1.2-2.1)	1.3 (0.9-1.9)	P < 0.001 P_{trend} < 0.001
Missing	300 (0.6%)	677 (0.6%)	1,227 (0.6%)	1,923 (0.8%)	2,137 (0.9%)	1,614 (1.0%)	1,916 (1.3%)	9,794 (0.9%)	
Health conditions at baseline									
Diabetes type 1	345	486	616	506	282	143	154	2,532	P < 0.001

Baseline	LDL cholesterol at baseline (mmol/L)								P-values
	< 1.81	**1.81-2.32**	**2.33-2.83**	**2.84-3.35**	**3.36-3.87**	**3.88-4.39**	**> 4.39**	**Total**	
	(0.6%)	(0.4%)	(0.3%)	(0.2%)	(0.1%)	(0.1%)	(0.1%)	(0.2%)	P_{trend} < 0.001[#]
Diabetes type 2	8,942 (16.7%)	10,769 (9.7%)	12,782 (6.5%)	11,258 (4.6%)	7,107 (3.1%)	3,982 (2.5%)	3,052 (2.0%)	57,892 (5.1%)	P < 0.001 P_{trend}< 0.001[##]
Chronic kidney disease	4,080 (7.6%)	5,603 (5.0%)	7,567 (3.9%)	8,385 (3.4%)	6,975 (3.1%)	4,629 (2.9%)	4,348 (2.9%)	41,587 (3.6%)	P < 0.001 P_{trend} < 0.001
Diagnosed cancer	2,344 (4.4%)	4,227 (3.8%)	6,858 (3.5%)	8,274 (3.4%)	7,541 (3.3%)	5,171 (3.2%)	4,870 (3.3%)	39,285 (3.4%)	P < 0.001 P_{trend} < 0.001
Definite COPD	1,529 (2.8%)	2,524 (2.3%)	3,753 (1.9%)	4,263 (1.7%)	3,583 (1.6%)	2,496 (1.6%)	2,244 (1.5%)	20,392 (1.8%)	P < 0.001 P_{trend} < 0.001
Medication prescriptions									
Statins (at baseline)	21,977 (40.9%)	28,348 (25.5%)	31,694 (16.2%)	28,161 (11.4%)	21,313 (9.4%)	15,057 (9.4%)	19,574 (13.1%)	166,124 (14.5%)	P < 0.001 P_{trend} < 0.001
Statins (during follow-up)	24,060 (44.8%)	35,926 (32.3%)	52,605 (26.9%)	65,908 (26.7%)	66,646 (29.4%)	56,064 (35.2%)	69,525 (46.4%)	370,734 (32.4%)	P < 0.001 P_{trend} < 0.001
Other lipid-lowering drugs	1,203 (2.2%)	1,174 (1.1%)	1,590 (0.8%)	1,687 (0.7%)	1,412 (0.6%)	916 (0.6%)	1,078 (0.7%)	9,060 (0.8%)	P < 0.001 P_{trend} < 0.001
Antihypertensive drugs	23,120 (43.1%)	38,645 (34.8%)	60,108 (30.8%)	73,326 (29.7%)	65,695 (29.0%)	45,849 (28.8%)	42,858 (28.6%)	349,601 (30.6%)	P < 0.001 P_{trend} < 0.001
Antiplatelet drugs	9,293 (17.3%)	12,019 (10.8%)	14,272 (7.3%)	13,782 (5.6%)	10,659 (4.7%)	6,843 (4.3%)	6,487 (4.3%)	73,355 (6.4%)	P < 0.001 P_{trend} < 0.001

Values are presented as numbers (percentage) or mean (standard deviation) or median (interquartile range) as appropriate.

Corresponding values for LDL-C are: 1.81 mmol/L = 70 mg/dL; 2.33 mmol/L = 90 mg/dL, 2.84 mmol/L = 110 mg/dL, 3.36 mmol/L = 130 mg/dL, 3.88 mmol/L = 150 mg/dL, 4.39 mmol/L = 170 mg/dL.

To convert mmol/L of HDL, total cholesterol and triglyceride to mg/dL, multiply by the factor of 38.67, 38.67, and 88.57, respectively.

$P trend for Caucasian vs other ethnicity $$P trend for non-smokers vs others (ex-smokers and current smokers)

#P trend for type 1 diabetes vs no diabetes ##P trend for type 2 diabetes vs no diabetes

Abbreviations: CRP; C-Reactive Protein, eGFR; estimated glomerular filtration rate, COPD; chronic obstructive pulmonary disease, HDL; high density lipoprotein, LDL; low density lipoprotein.

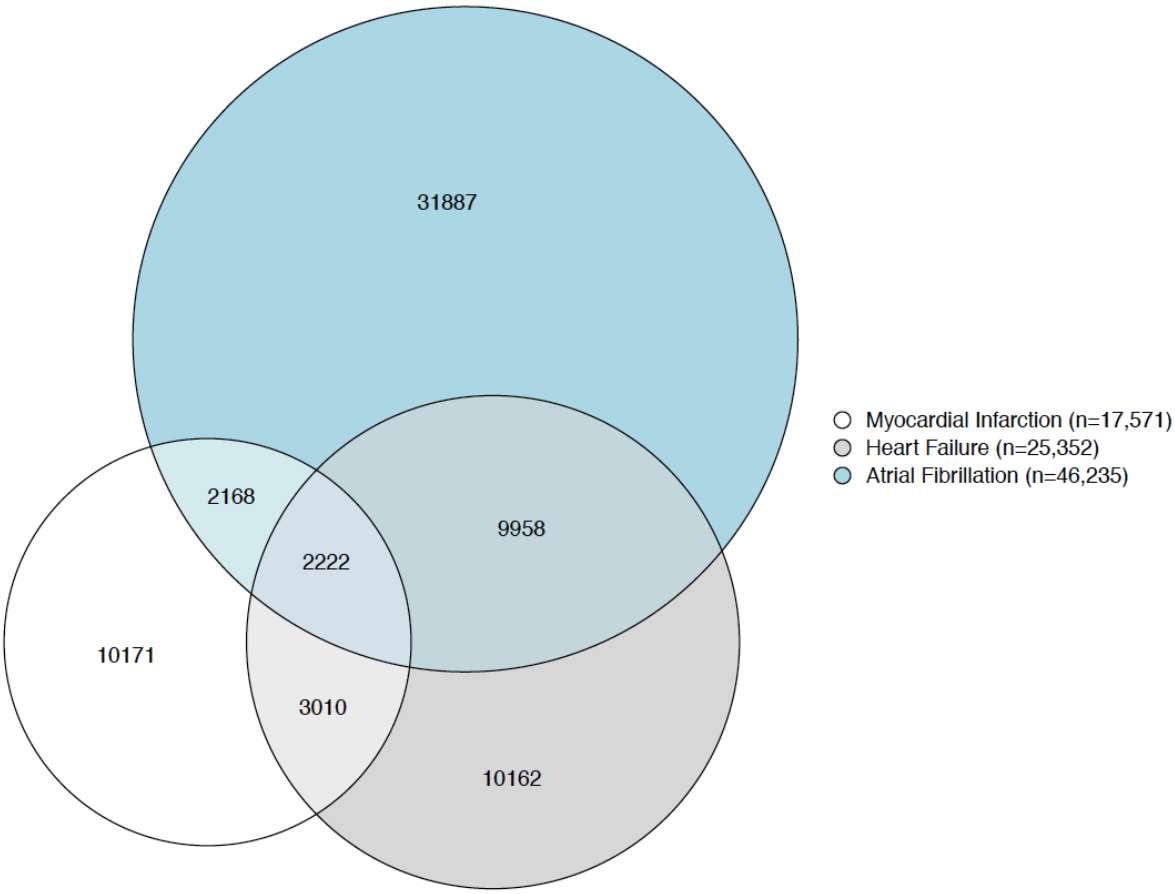


Figure S 4-1 Venn diagram for incident MI, HF, and AF in 1,142,656 CALIBER cohorts

Note: Median follow-up time for HF was 5.1 years (IQR: 2.4 to 8.4 years), for AF was 5.0 years (IQR: 2.3 to 8.3 years), and for MI was 5.1 years (IQR: 2.4 to 8.5 years)

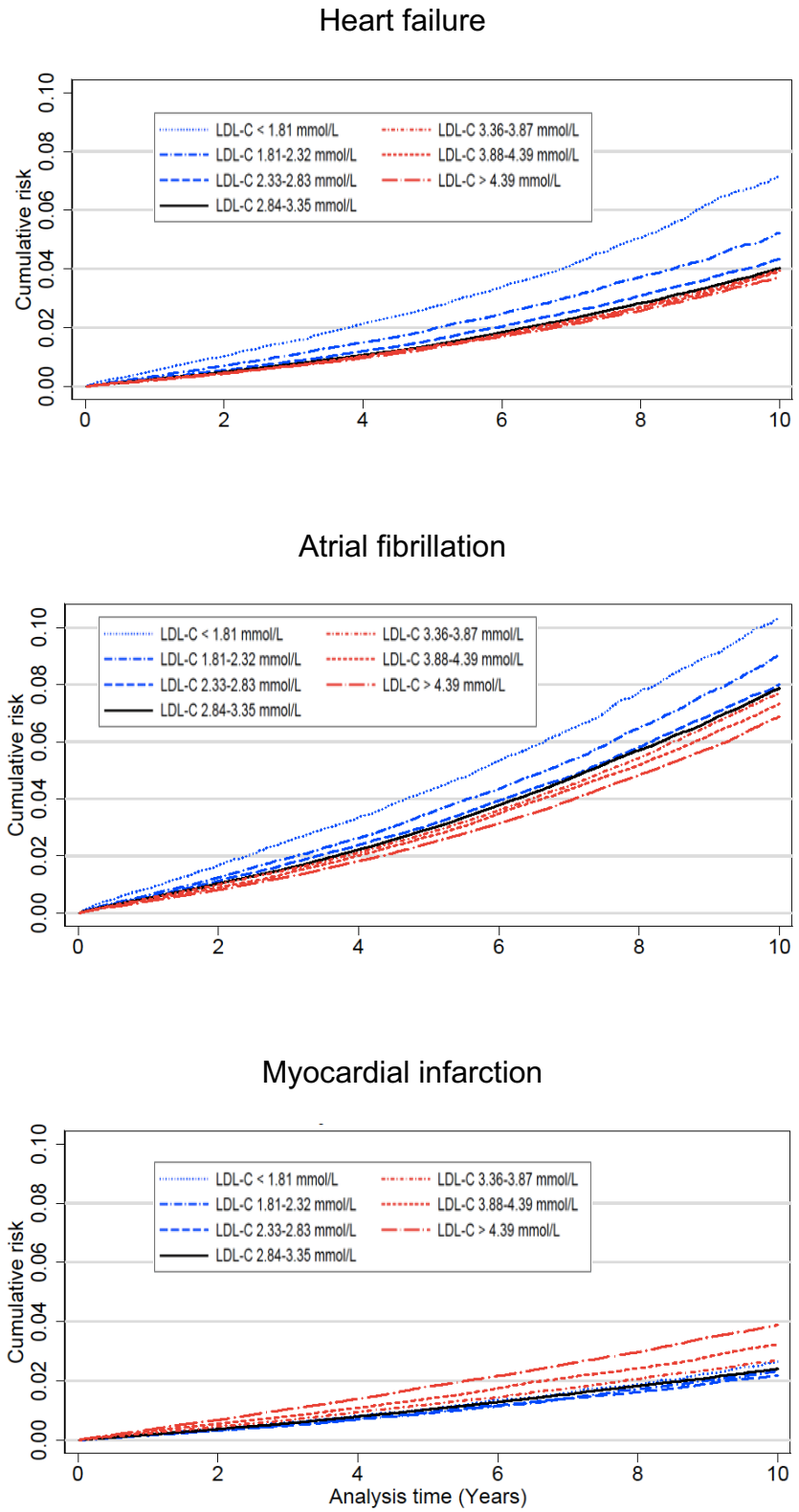


Figure S 4-2 Cumulative incidence of HF and AF by baseline LDL-cholesterol

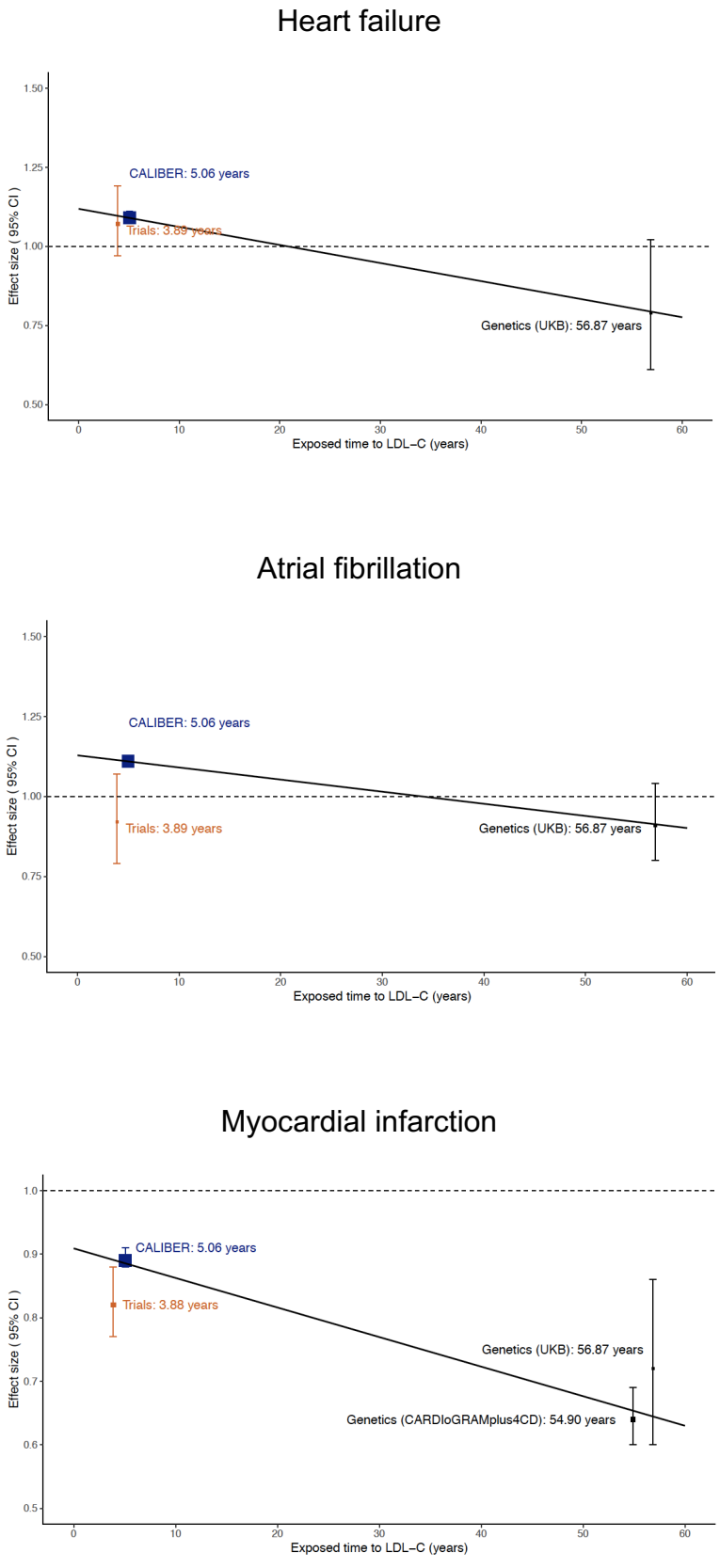


Figure S 4-3 Effect size per LDL-C exposure time in each study design

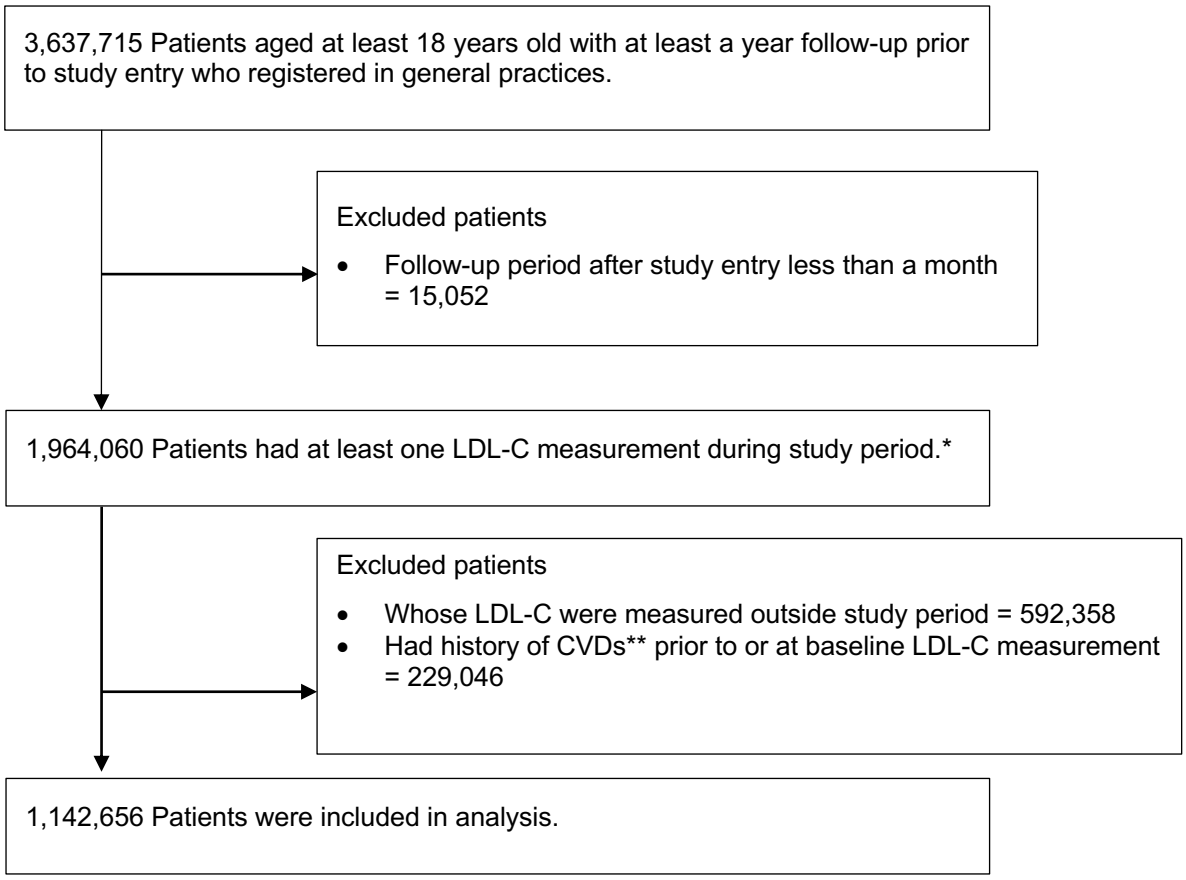


Figure S 4-4 Study flow diagram

Note: *Study period is between 1st Jan 1997 and 27th Jun 2016.

**CVDs include i) coronary artery disease (i.e., myocardial infarction, unstable angina, and stable angina), ii) stroke (i.e., haemorrhagic stroke, ischaemic stroke, and unclassified stroke), iii) transient ischaemic attack, iv) heart failure, v) atrial fibrillation, vi) abdominal aortic aneurysm, vii) peripheral arterial disease, and viii) sudden cardiac arrest.

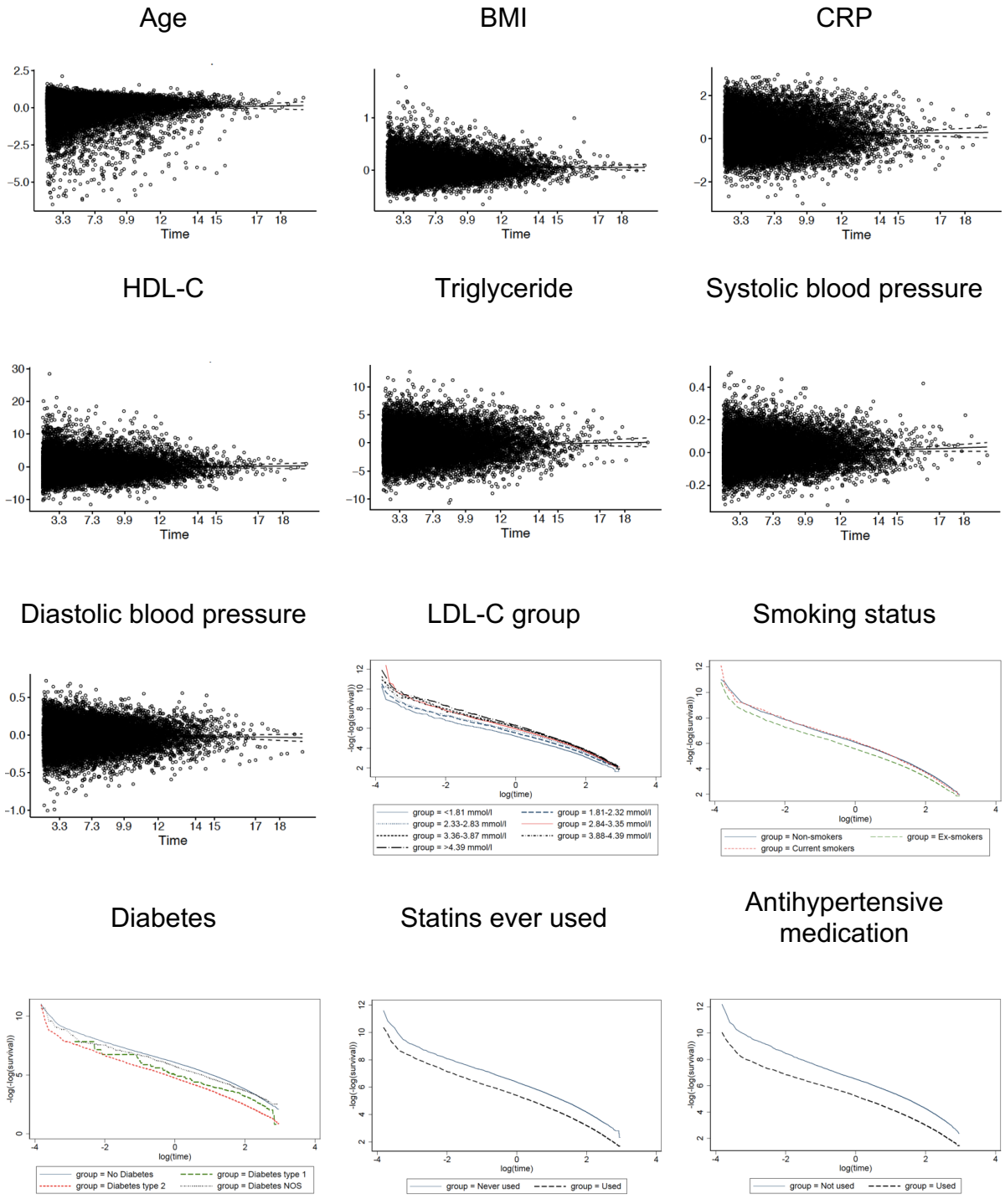


Figure S 4-5 Schoenfeld residual and proportional hazard plots of HF outcome

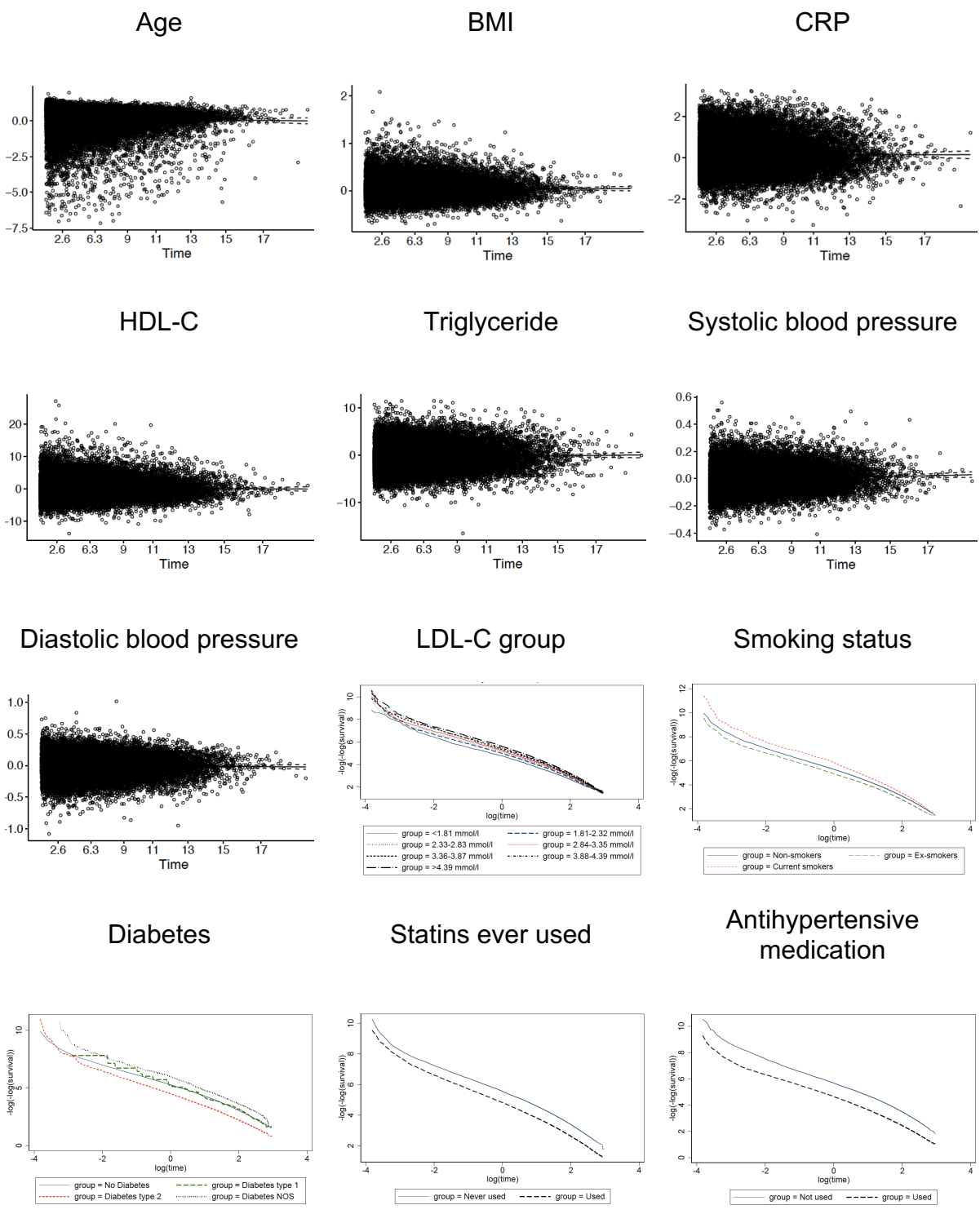


Figure S 4-6 Schoenfeld residual and proportional hazard plots of AF outcome

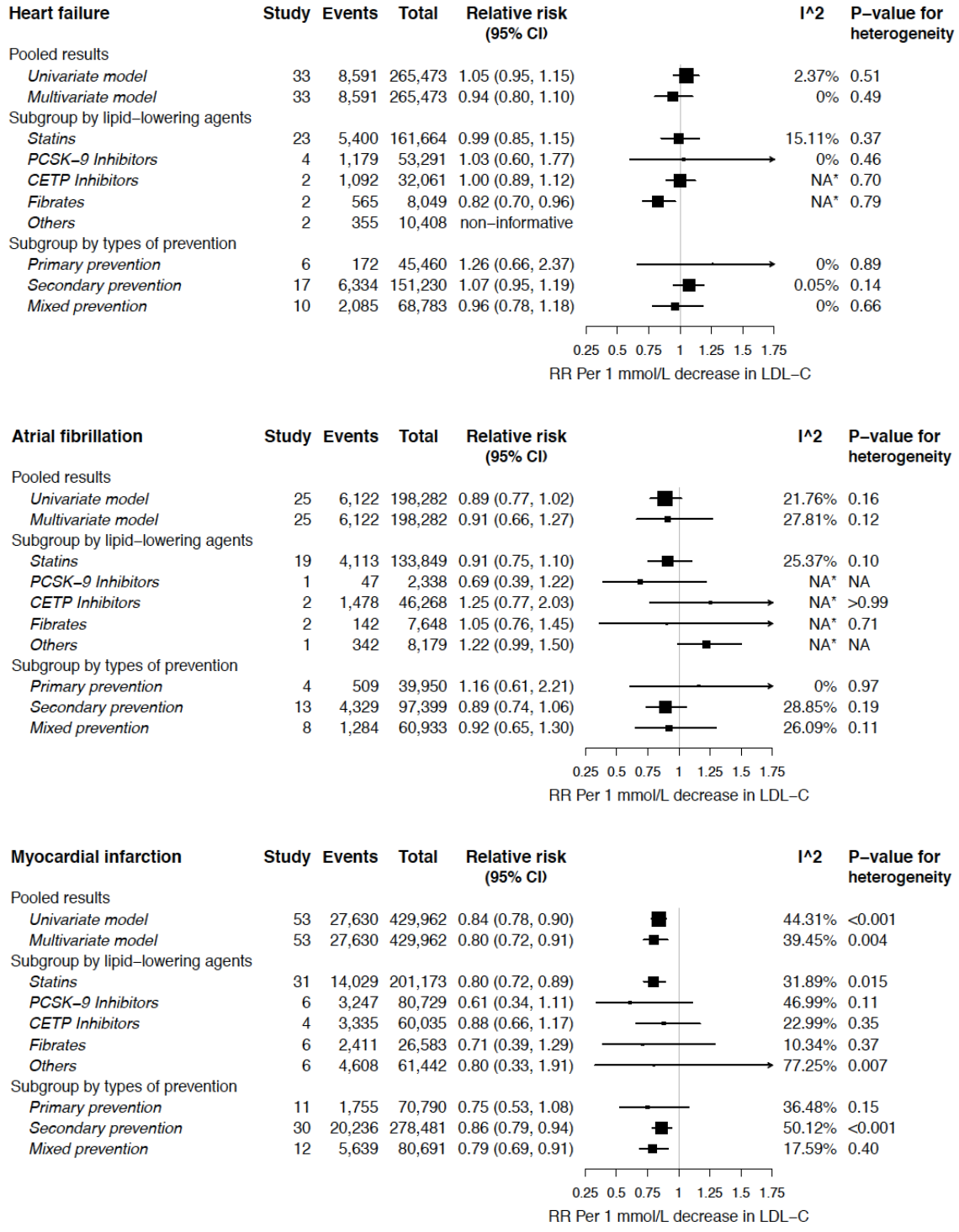
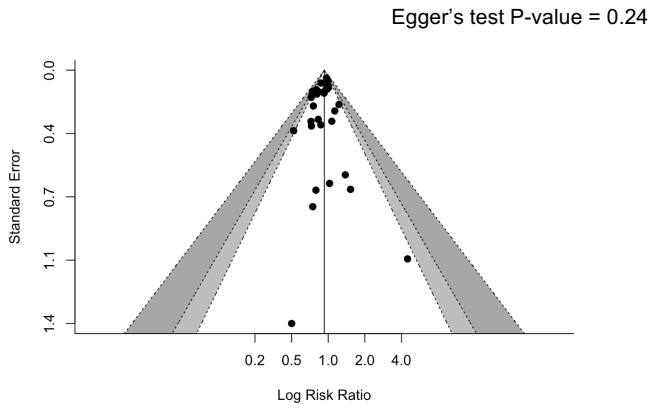


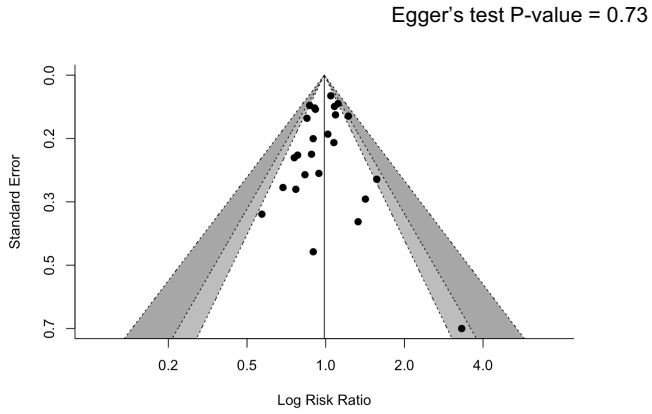
Figure S 4-7 Subgroup analysis of meta-regression

Note: * fixed effect models

Heart Failure



Atrial Fibrillation



Myocardial Infarction

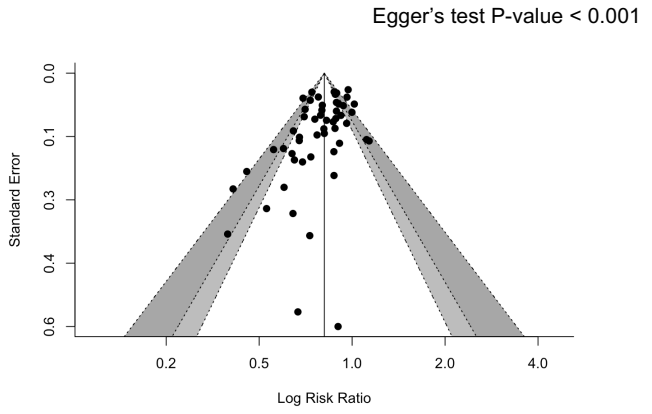
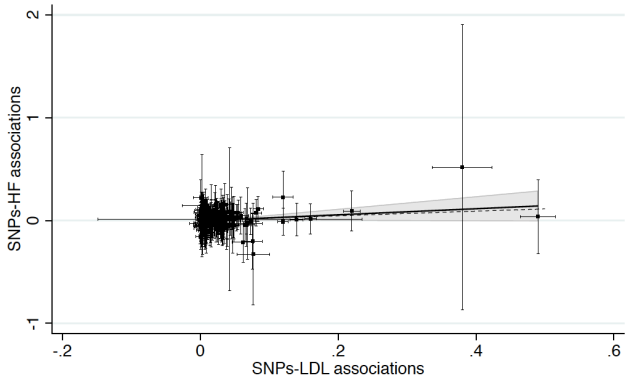
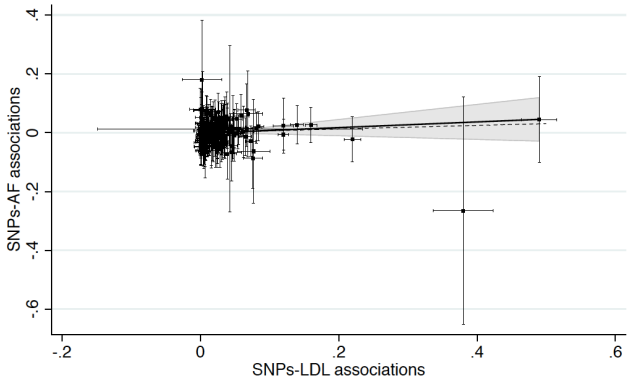


Figure S 4-8 Funnel plots of RCTs on HF (top), AF (middle), and MI (bottom) outcomes

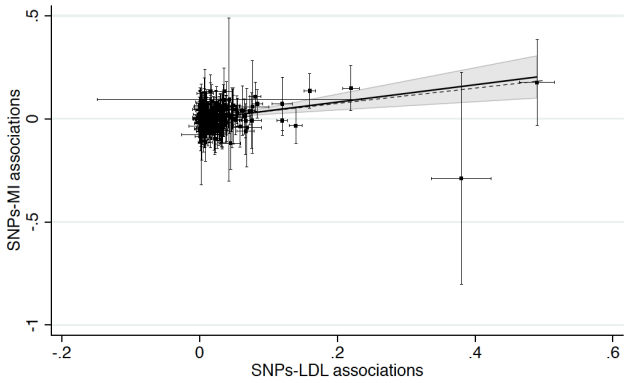
Heart failure (181 SNPs)



Atrial fibrillation (181 SNPs)



Myocardial infarction (181 SNPs)



Note: Each dot represents each study. Shaded area of white, grey, and dark grey indicates region of 90%, 95%, and 99% confidence interval, respectively.

Figure S 4-9 Scatter plots of genotype-LDL associations versus genotype-outcome associations

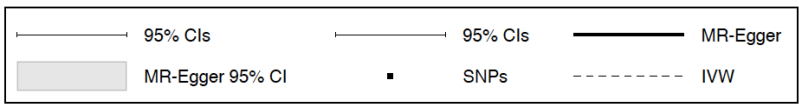


Table S 4-3 Testing for horizontal pleiotropy

Outcomes	Egger-intercept coefficients (p-values)	Q statistics, degree of freedom (p-values)
HF #181 SNPs (UKB)	-4.19×10^{-3} (0.54)	120.57, 180 (0.98)
HF #143 SNPs (HERMES)	8.74×10^{-4} (0.68)	Not available
AF #181 SNPs (UKB)	-2.12×10^{-3} (0.48)	175.35, 180 (0.12)
MI #181 SNPs (UKB)	-2.86×10^{-3} (0.49)	199.54, 180 (0.009)
CAD #177 SNPs (CARDIOGRAMplusC4D)	-3.84×10^{-3} (0.25)	281.29, 176 (<0.001)

Note: Significant Egger p-values might suggest potential directional horizontal pleiotropy. Significant Q statistics p-values might suggest either directional or balanced pleiotropy.

Table S 4-4 Power calculation of MR at two-sided alpha of 0.05

Parameter	HF	AF	MI (UKB)	CAD (CARDIOGRAMplusC4D)
Number of cases*	512	3,349	1,761	63,746
Number of controls*	110,884	108,047	109,635	130,681
Odds ratio to be detected	0.79	0.91	0.72	0.64
R^2 (SNPs LDL-C)	0.076	0.076	0.076	0.076
Calculated power	28.40%	27.59%	94.07%	> 99%

Note: *Number of cases and controls derived from SNPs-outcome consortium. Power was calculated based on the method explained in Hermani et al[223] and in supplementary appendices.

Table S 4-5 Correlation between instrumental variable effect and exposure effect

Outcomes	Correlation coefficients (p-values)
Heart failure #181 SNPs	-5.2×10^{-3} (0.94)
Atrial fibrillation #181 SNPs	-0.7×10^{-3} (0.99)
Myocardial infarction #181 SNPs	-1.0×10^{-3} (0.99)
Coronary artery disease #177 SNPs	0.091 (0.29)

Note: Highly correlated coefficients and significant p-values might suggest the invalidity of InSIDE (Instrumental Strength Independent of Direct Effect) assumption.

Table S 4-6 Features comparison of the evidence

Evidence	Baseline profile	Account for intercurrent MI?	Outcome analysis
Cohort	Free of CVDs	Yes	Incident
Trial	Free of CVDs	No	Incident & Prevalent
	High CV risks		
	Established CVDs		Recurrent
Genetic	Established CVDs	No (UK Biobank) Yes (HERMES)	Prevalent

Abbreviations: CVD; Cardiovascular disease, HERMES; Heart failure molecular epidemiology for therapeutic targets, MI; Myocardial infarction

CHAPTER 5 HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AS A CAUSE OF HEART FAILURE AND ATRIAL FIBRILLATION: COHORT, TRIAL, AND GENETIC EVIDENCE

5.1 Key messages

What is already known?

- Observational evidence strongly suggested that higher HDL-C levels play a protective role in myocardial infarction (MI), but their effects on heart failure (HF) and atrial fibrillation (AF) have not been widely investigated.
- Most randomised controlled trials (RCTs) failed to show a significant reduction in the risk of MI of HDL-C raising agents, such as niacin and CETP inhibitors.
- Mendelian randomisation (MR) did not support a causal relationship between HDL-C and the risk of coronary artery disease (CAD) or myocardial infarction (MI). Besides, MR results from AFGen consortium did not show the association between HDL-C gene score and the risk of AF.

What does this study add?

- We observed a significant U-shape association between HDL-C levels and the incidence of HF from the EHR data of 1.3 million people across England.
- Although meta-regression of RCTs showed that increased HDL-C levels might be related to an increased risk of HF (a direct association) and genetic evidence (MR) from UK Biobank (UKB) showed a null association, these even supported the observed U-shape association in the cohort study.

- The results from the biggest GWAS on HF (HERMES), which indicated that the association between HDL-C and HF was significantly mediated by coronary artery disease (CAD), further strengthen the findings from the cohort study.
- Observational findings showed a weak U-shape association of HDL-C with the risk of AF, whereas we found no association in terms of per SD increase in HDL-C levels. Both trial and genetic evidence did not support the causal association between HDL-C and the risk of AF.

5.2 Abstract

Objective: To evaluate the causal relevance of high-density lipoprotein cholesterol (HDL-C) to the risk of heart failure (HF) and atrial fibrillation (AF).
Design, setting, and participants: We compared the evidence from three study designs: 1) a population-based cohort study of people who were free of diagnosed CVDs at baseline using the linked electronic health records (EHRs: CALIBER platform); 2) Meta-regression of randomised controlled trials of lipid-lowering agents; and 3) Mendelian randomisation (MR) using summary-genetic data from GLGC, UK Biobank (UKB), HERMES, and the CARDIoGRAMplusC4D consortium.
Main exposure: High-density lipoprotein cholesterol (HDL-C)
Main outcome measures: HF and AF
Results: During the mean follow-up of 5 years (IQR 2-9 years) between 1st January 1997 and 30th June 2016, we identified new 29,876 HF and 54,201 AF cases amongst 1,338,276 participants from CALIBER who were free from CVDs at study entry. For HF and HDL-C, we observed an apparent U-shape association, regardless of gender, which was significantly modified by intercurrent CVDs. However, no association was observed with per SD increase in HDL-C levels (HR: 0.99 [95%CI 0.98 to 1.01]). Meanwhile, a meta-regression of RCTs showed a direct association between HDL-C and the risk of HF (RR: 1.13 [95%CI 1.01 to 1.26]), whereas genetic evidence from UKB showed no association (OR per 1 SD [0.4 mmol/L], with a genetically determined increase in HDL-C being 1.24 [95%CI 0.91 to 1.69]). Also, the MR results from HERMES suggested the role of CAD as a mediator of the

association between HDL-C and HF. For AF and HDL-C, we found a weak U-shape association. However, no association was observed with per SD increase in HDL-C (HR 1.00 [95%CI 0.94 to 1.64]), which was consistent with the results from the meta-regression of trials (RR 1.01 [95%CI 0.91 to 1.12]) and those from MR (OR 1.24 [95%CI 0.83 to 1.12]).

Conclusion: Observational evidence suggests that HDL-C might be nonlinearly associated with the risk of HF, which was further supported by the findings from meta-regression and MR. We also found a weak U-shape association between HDL-C and the risk of AF; however, it was not supported by trial and genetic evidence. Therefore, the role of HDL-C on the incidence of HF requires further investigation.

5.3 Introduction

5.3.1 Clinical Importance

High-density lipoprotein cholesterol (HDL-C) has been widely investigated for the causal relevance to myocardial infarction (MI). Although evidence from observational studies points to the protective effect of HDL-C against the risk of MI,[95] the most recent clinical guideline, whose conclusion was drawn from trials and genetic evidence, suggests otherwise.[36] Despite the fact that the role of HDL-C in the risk reduction of MI has become less interesting to clinicians over time, HDL-C still provides a significant value to the risk prediction of MI and cardiovascular disease (CVD), as most of CVD risk prediction scores used in clinical practice regard HDL-C as an important risk predictor (see Chapter 7).

Upon the arrival of CETP inhibitors, which can profoundly increase HDL-C levels by up to 128%,[81] a new indication should be sought, since results from trials failed to show cardiovascular benefits and even revealed potential harm. However, Sofat *et al*. have suggested that the adverse hypertensive effect of one of CETP inhibitors – Torcetrapib - is likely to be related to its unique chemical structure rather than class effect.[224] This was later confirmed by major trials of other CETP inhibitors (anacetrapib[140] and dalcetrapib[138]) in which participants in the active group of both trials did not show a clinically

significant increase (0.6-0.7 mmHg increased) in systolic blood pressure at the final visit. This warrants the safety of CETP inhibitors for the use in any forthcoming new indications.

Heart failure (HF) and atrial fibrillation (AF) have become increasingly prevalent globally, but no specific recommendation for primary preventive has been made, especially in healthy populations.[1,5] Because both diseases often co-exist and share risk factors and pathophysiological mechanisms, many new underlying mechanisms of HF and AF have been proposed.[8,42] Amongst those, oxidative stress and inflammation are widely discussed in the literature.[68,69,71,73,78,108] These can lead to cellular growth and fibroblastic activation resulting in cardiac structural remodelling and conduction abnormality.[225,226] HDL-C exerts a well-known pleiotropic effect on anti-inflammation, anti-oxidative stress, and anti-fibrosis.[39] Therefore, HDL-C might play a role in the origin of HF and AF, and its association with both common cardiac diseases should be examined.

5.3.2 Uncertainty

Several observational studies exploring the relationship between HDL-C and HF and AF revealed inconsistent results. Most of the community-based cohort studies did not find any association between HDL-C and incident HF,[74,77,227] whereas some had shown an inverse association in both males and females,[71,112] gender-specific basis,[108] or only subgroups of populations.[78] It is difficult to generalise the findings. Regarding the observational studies on incident AF, again, most of the previous studies failed to show the link with HDL-C levels,[32,70,73,74,76] although some suggested that increased HDL-C levels might be associated with a lower risk of AF.[31,69]

However, the association of HDL-C with clinical outcomes might not be simple and straightforward. Observational studies consistently revealed the U-shape association between HDL-C and the all-cause and cause-specific mortality in both genders,[228–230] and this might be extrapolated to other outcomes. Therefore, additional studies with a large sample size and a wide range of HDL-C levels are required so that a nonlinear trend of the association, if any, can be captured.

5.3.3 Objectives

In this study, we provide three new pieces of evidence from observational, trial, and genetic (Mendelian randomisation) studies to examine the association between HDL-C and the risk of HF and AF. We used MI (and CAD) as a control to validate whether our cohorts and methodology used throughout this study could reproduce results from the existing evidence. There are three specific objectives in this study: (1) to conduct a large observational study examining incident HF and AF in the same cohorts based on EHRs across a wide range of HDL-C levels (i.e., HDL-C 0.1 mmol/L [3.9 mg/dL] to 5.1 mmol/L [197.2 mg/dL]) and to further evaluate the role of intercurrent cardiovascular diseases (CVDs) on the association; (2) to examine whether the change in the risk of HF and AF is associated with the difference in HDL-C levels between the active and the control groups over the follow-up period in randomised controlled trials of lipid-lowering agents; (3) to investigate whether the genetic evidence using the Mendelian randomisation (MR) approach supports findings from both cohort and trial evidence or indicates otherwise.

5.4 Methods

5.4.1 Population-based cohort study

In the cohort part of this study, we used a longitudinal design from which participants were followed-up over the period between 1st January 1997 and 30th June 2016. Exposure and outcomes were ascertained through the linked EHRs among general practices (GPs), hospital admission records, and the national death registry (NOS) of England. Report on the cohort section had followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[195,196] and the REporting of studies Conducted using Observational Routinely-collected health Data (RECORD)[197] recommendations (see Table S 5-1).

Data sources and studied population

The studied populations were taken from the CALIBER (ClinicAl research using LInked Bespoke studies and Electronic health Records) platform as have been described in Chapter 4. In this study, we initially included 3.6 million

patients from 387 GPs across England from the CPRD database. We identified all patients aged 18 years or older who registered between 1st January 1997 and 30th June 2016 and had been followed-up with their GPs for at least one year. Individuals who had a history of CVDs at the baseline TG measurement were excluded.

Approval of this study was granted by the Independent Scientific Advisory Committee (ISAC) of the Medicines and Healthcare products Regulatory Agency (protocol number 12_153RARMnAR).

Exposure: HDL-C as an EHR phenotype

We used ambulatory care high-density lipoprotein cholesterol (HDL-C) measurement sampled in clinics and hospital out-patients and electronically recorded in the primary care. The raw data included a total of 7,673,826 measurements of plasma and serum samples (multiple records per patient), and plasma levels (3.2 % of all records) were multiplied by a factor of 1.03 to convert to serum levels before analysis.[198] We excluded outlier values (i.e., HDL-C < 0.1 [4 mg/dL] or $\geqslant$ 5 [193 mg/dL] mmol/L) from our analysis (1.2 % of all records). For individuals who had more than one HDL-C measurement within a year (25% of all patients), we used a yearly-averaged value, and this further refers to the term 'baseline HDL-C'. The earliest date of HDL-C measurement was used as the starting point of patient follow-up. For those with more than one measurement on a given day (0.05 % of all HDL-C records), the values were aggregated by taking the mean.

Covariates

Baseline covariates taken from the closest record to the baseline date (within a one-year interval) were selected based on their association with HDL-C, HF, and AF from previous studies.[74,178,199,200] These included age, socioeconomic status (i.e., index of multiple deprivations), smoking, body mass index, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), C-reactive protein, diabetes, use of antihypertensive medications, and use of statins. Any missing value of covariates was imputed using multiple imputations by chained equations (MICE) (supplementary appendices). A complete list of codes used to identify

all covariates in this study can be found from the CALIBER portal at https://www.caliberresearch.org/portal.

Follow-ups for incident HF, AF, and MI

Endpoints, including HF, AF, and MI were evaluated based on diagnostic codes (ICD-10 and Read codes), which include hospitalised- (HES), and non-hospitalised (CPRD) cases. Intercurrent CVDs were defined as any CVD events, including 1) coronary artery disease (i.e., myocardial infarction, unstable angina, and stable angina), 2) stroke (i.e., haemorrhagic stroke, ischaemic stroke, and unclassified stroke), 3) transient ischaemic attack, 4) abdominal aortic aneurysm, 5) peripheral arterial disease, 6) sudden cardiac arrest, 7) heart failure and 8) atrial fibrillation that occurred between the period after baseline TG measurement and before the first occurrence of the endpoints. The validity of HF,[178] AF,[179] and MI endpoints[175], as well as their definition, has been demonstrated previously in Chapter 3.

Statistical analysis

We used the Cox proportional hazard model to calculate the hazard ratio (HR) from the time of blood sampling HDL-C measurement to the time of the incident event, censoring (i.e., death or transfer out of practice), or end of the follow-up, whichever occurred first. All models were adjusted for baseline covariates and stratified by primary care practice and sex. We analysed baseline HDL-C as both continuous and categorical variables to avoid presuming a particular shape of the association.

For categorisation, since there is no established cut-off for HDL-C levels, we created seven strata of HDL-C levels based on the distribution of HDL-C levels in our data as follows: less than 0.91 mmol/L (35 mg/dL), 0.91-1.01 mmol/L (36-39 mg/dL), 1.02-1.27 mmol/L (40-49 mg/dL), 1.28-1.53 mmol/L (50-59 mg/dL), 1.54-1.78 mmol/L (60-69 mg/dL), 1.79-2.04 mmol/L (70-79 mg/dL), and 2.05 mmol/L (80 mg/dL) or higher. We chose the middle category (i.e., HDL-C 1.28-1.53 mmol/L) as a reference group to avoid the potential impact of an outlier, if any, on the overall shape of the association. The association of each endpoint with baseline HDL-C was reported as per continuous (per 1

standard deviation [0.4 mmol/L or 15.5 mg/dL] increase in HDL-C) or categorical HDL-C as described above.

In addition, we also reported gender-specific results due to the fact that HDL-C has a different threshold of an increased risk of atherosclerosis between male (<1.0 mmol/L) and female participants (<1.2 mmol/L).[201] Sensitivity analyses and test of the proportional hazards assumption were carried out and explained in the supplementary appendices. To compare our cohorts with the previous study,[229] we additionally included all-cause mortality as the outcome of interest (see Figure S12). All analyses in the cohort part had been done using STATA version 13 (MP version, StataCorp). A two-tailed P-value of < 0.05 was considered a statistically significant value. The Bonferroni method was used, as applicable, for multiple comparison adjustment.

5.4.2 Trial-level meta-analysis of randomised controlled trials

Study selection and outcome data

We included randomised controlled trials of lipid-modifying agents published up to July 2019. At first, since we considered our outcomes of interest (i.e., HF, AF, and MI) as hard outcomes, we included only major trials with at least 1,000 participants who were followed up for at least one year to perform a meta-analysis. This criterion was based on the Cholesterol Treatment Trialists (CTT) Collaboration. To further expand our search, we additionally included small trials (i.e., N<1,000) identified through systematic reviews of trials on fibrates[231,232] and niacin,[96] and through an additional search for CETP inhibitors (supplementary appendices). Depending on the availability of reported data, HF and MI were defined as both fatal and non-fatal cases. AF was defined as either reported atrial fibrillation or cardiac arrhythmia. All included studies were assessed for their quality using the Cochrane risk of bias tool, and the results were shown in Chapter 2.

Statistical analysis

We calculated relative risk (RR) of the outcome of interest per one mmol/L (38.67 mg/dL) increase in HDL-C levels between the active and the control groups at the end of the follow-up period (or the most extended period as

available) using the fixed-effect meta-regression method. Alternatively, we used the random effect method if there was any evidence of statistical heterogeneity (i.e., $I^2 > 75\%$ or P-value for heterogeneity < 0.10).[233]

For visualisation, we created bubble plots for the association between the relative risk of disease on the y-axis versus the absolute increase (in mmol/L) of HDL-C between the active and the control groups on the x-axis. Each bubble represents each trial, and its size is related to its inverse variance. Then the association between changes in HDL-C and the risk ratio of outcome was the beta-coefficient of slope from the linear equation derived from the estimated straight line. For trials with no events in an active or control arm, a nominal amount (0.5 cases) was added to the results for both trial groups.

The main results were derived from univariable meta-regression models. We also analysed multivariable meta-regression models and performed sensitivity analyses (supplementary appendices). In addition, we performed a meta-analysis of trials on HDL-C raising agents, such as CETP inhibitors and niacin, to further examine whether the association of changes in HDL-C, if any, depends upon the use of HDL-C raising agents. Potential publication bias was assessed by visualising a funnel plot of log RR (x-axis) and standard error (y-axis) of a model without a moderator (i.e., change in HDL-C levels) and by Egger's test P-value. All analyses in this part were done by using 'metafor' and the 'CALIBER datamanage' package in R version 3.3.2.

5.4.3 Mendelian randomisation (MR)

Data Sources

As in Chapter 4, we used summary-level genetic data of HDL-C from the Global Lipids Genetics Consortium (GLGC).[202] Summarised genetic data of coronary artery disease (CAD) was taken from the Coronary Artery Disease Genome-wide Replication and Meta-analysis plus Coronary Artery Disease Genetics (CARDIoGRAMplusC4D),[203] while those of HF, AF, and MI were taken from UK Biobank.[204] In addition, the results on HF were taken from the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES)

consortium.[205] Details of the consortia are provided in the supplementary section (supplementary appendices).

All data sets were limited to individuals of European ancestry, and beta-coefficients and standard errors were obtained for the per-allele association of each SNP with all exposures and outcomes from these data sources. No specific ethical approval was required for this study because we used publicly available summary statistics of genetic data in which the approval was already granted in the original studies. However, for the use of UK Biobank data, this study was approved by the committee with the application number 12113.

Selection of SNPs and MR Analyses

We used 185 lipid-associated SNPs initially identified by Willer *et al.* to generate a series of genetic instruments for HDL-C.[202] Since nearby SNPs might violate the independent assortment rules, all 185 SNPs were further pruned to a set of low linkage disequilibrium (pairwise $R^2 < 0.05$) within a window of 10,000 kb using the UKB10K LD reference. This process yielded a final set of 156 SNPs from UKB and 157 SNPs from CARDIoGRAMplusC4D. SNPs and effect allele frequency across consortia (e.g. GLGC and UKB) were checked to ensure that the same strand was used throughout the analysis.

We employed the two-sample MR approach with various sensitivity methods, including i) inverse variance weighted (IVW) MR, ii) MR-Egger, iii) weighted median MR, and iv) multivariate MR (MVMR) analyses to show the robustness of our findings and potential horizontal pleiotropy. We took the main results from the MVMR model since it takes into account the genetic variability of other lipid traits (i.e., LDL-C and TG). Details about testing the MR assumption and power calculation can be found in the supplementary appendices. All analyses in the MR part were performed using the 'TwoSampleMR' package in R version 3.3.2, and the 'mrrobust' package in STATA version 13 (MP version, StataCorp).

5.5 Results

5.5.1 Results from the observational study (CALIBER)

After the mean follow-up period of approximately five years (IQR 2 to 9 years) from a total of 1,338,276 individuals, we identified 29,876, 54,201, and 21,023 new cases of HF, AF, and MI, respectively (Figure S 5-3). At baseline, the study population had a mean age of 55.5 years, with the mean HDL-C being 1.50 (58 mg/dL) ± 0.40 mmol/L. From Table 5-1 and Table S 5-3, we observed that higher baseline HDL-C was associated with older age, a higher proportion of females with a profile of being healthier, such as non-smokers, lower BMI, blood pressure, TG, and CRP levels. However, higher HDL-C was also related to worsening renal function, in terms of eGFR, and higher prevalence of cancer and COPD but lower one of type 2 diabetes. Besides, there was a negative trend towards the use of lipid-lowering agents, antihypertensive agents, and antiplatelet agents as HDL-C levels increased. In addition, Table S 5-4 provided details of gender-specific characteristics of participants.

From Table S 5-2, we noticed the U-shape pattern of incident HF across HDL-C strata. In the lowest HDL-C group (i.e., HDL-C < 0.91 mmol/L), the incidence rate of HF was 4.49 per 1,000 person-years (95%CI 4.31, 4.68). The incidence decreased as HDL-C increased and was the lowest at 3.75 (3.67, 3.83) per 1,000 person-years in the middle group (i.e., HDL-C 1.28 – 1.53 mmol/L) before going upward to 4.23 (4.09, 4.39) in the highest HDL-C group (i.e., HDL-C > 2.04 mmol/L). In contrast, we noticed an upward trend of the incidence of AF across HDL-C strata starting from 6.53 (6.30, 6.76) per 1,000 person-years in the lowest strata to 8.34 (8.13, 8.56) per 1,000 person-years in the highest strata. Also, the patterns were found in both genders and more pronounced in the males than in the females.

Figure 5-1 showed the different patterns of the observed associations between HDL-C and incident HF, AF, and MI in the age- and sex-adjusted model (complete-cases) and in the fully adjusted model (multiply imputed covariates). For the association with the incident HF, the shape of the association significantly shifted from a mirrored J-shape to the U-shape upon full adjustment, which was similar in both genders (Figure S 5-4). However, the

association with the incident HF per 1 SD continuously increase in HDL-C was null (HR 0.99 [95%CI 0.98, 1.01]). Regarding the incidence of AF, a weak U-shape pattern of the association with HDL-C was observed, which did not significantly transform in a fully adjusted model. However, we found a sex-specific association in which a direct (positive) one was found amongst males (HR per 1 SD increase HDL-C was 1.03 [1.01, 1.05]), while an indirect (negative) one was found amongst females (HR was 0.98 [0.96, 0.99]), with the P-value of heterogeneity between groups < 0.001 (Figure S 5-4). Moreover, an inverse association was observed between HDL-C and incident MI, regardless of gender, which was slightly attenuated but remained statistically significant after full adjustment.

Subgroup analyses of HF and AF were given in Figure S 5-5 (overall association), Figure S 5-6 (HF, gender-specific), and Figure S 5-7 (AF, gender-specific). Overall, subgroup analyses showed robust results from the main findings. However, having any intercurrent CVDs might affect the association between HDL-C and the incident HF (P-value for heterogeneity between groups was 0.007), whereas gender (P-value < 0.001) and age group (P-value 0.001) stratified the association with the incident AF. Further subgroup analysis of categorical HDL-C did not profoundly deviate the results from the main findings (Table S 5-5 and Table S 5-6). Schoenfeld residual and proportional hazard plots of both outcomes did not show evidence of the violation of the proportional hazards assumption (Figure S 5-8 to Figure S 5-11).

Further adjusting for competing risk (i.e., death from other causes) did not change our findings (Figure 5-2). Interestingly, subtype analysis of HF had shown that there is a positive association between 1-SD increase in HDL-C levels and the risk of HF due to chronic respiratory disease (HR 1.32 [95%CI 1.18, 1.47]), whereas other subtypes of HF did not show a significant association with per SD change in HDL-C levels (Figure 5-2).

5.5.2 Results from meta-regression of randomised controlled trials

Amongst 52 RCTs that reported changes in HDL-C between the active and the control groups (Table S 2-10), 33 and 24 studies had reported HF (8,679 events) and AF (6,567) as their endpoints, respectively.

Meta-regression results, which was demonstrated in Figure 5-3, had shown that there was a positive association between increased HDL-C and the risk of HF (RR per 1 mmol/L increase in HDL-C was 1.13 [95%CI 1.01, 1.26] with I^2 = 19.9% and P-value for heterogeneity = 0.21). However, the strength of the association was attenuated towards null upon multivariable adjustment (RR 0.47 [95%CI 0.16, 1.40], I^2 = 21.8% and P-value for heterogeneity = 0.23, Figure S 5-13). Regarding AF, meta-regression results did not suggest any association between changes in HDL-C levels and the risk of AF (RR 1.01 [95%CI 0.91, 1.12], I^2 = 9.9%, P-value for heterogeneity = 0.22) in either the univariable or multivariable models.

Interestingly, we found that an increase in HDL-C levels was paradoxically associated with the increase in the risk of MI (RR 1.17 [95%CI 1.03, 1.33], I^2 61.5%, P-value for heterogeneity < 0.001). However, multivariable adjustment attenuated the positive association towards null (RR 0.94 [95%CI 0.71, 1.25], I^2 = 55.9%, P-value < 0.001, Figure S 5-14). Moreover, from Figure S 5-18, we observed that results between HDL-C and the risk of MI might be outweighed by seven trials of lipid-lowering agents in other groups, including Niacin trials (n=2), n-3 fatty acid trials (n=2), an ezetimibe trial (n=1), a cholestyramine trial (n=1), and a Bempedoic acid trial (n=1). This is because subgroup analysis showed that an increase in HDL-C due to lipid-lowering agents in other groups was strongly associated with an increased risk of MI (RR 3.31 [95%CI 1.54, 7.09], I^2 = 21.2%, P-value = 0.39).

From Figure S 5-15, there was no apparent evidence of publication bias for HF and AF outcomes, corresponding with the P-values from Egger's test of 0.70 and 0.47, respectively. However, the asymmetrical shape of the funnel plot of MI outcomes with a significant P-value from Egger's test (P=0.018) suggested potential publication bias in the MI outcomes.

5.5.3 Results from the genetic study

We extracted 156 SNPs from 512 cases, 3,349 cases, and 1,761 cases of the first diagnostic HF, AF, and MI in UK Biobank, respectively, and 157 SNPs from 63,746 CAD cases from the CARDIoGRAMplusC4D, which explained around 6.5% of the variability in HDL-C levels. In the HERMES consortium,

the results were derived from 110 SNPs, which were from 47,309 HF cases out of 977,323 individuals. Scatter plots for genotype-HDL-C associations and genotype-outcome associations are given in Figure S 5-19.

From Figure 5-4, we found that there was no association between one SD-genetically determined increase in HDL-C and the risk of HF from UKB (OR from MVMR model was 1.24 [95%CI 0.91, 1.69]), and the results were robust across all sensitivity MR models. Genetic evidence from HERMEs, on the other hand, showed an inverse association between one SD-genetically determined increase in HDL-C and the risk of HF (OR 0.93 [95%CI 0.91, 0.96]). However, the results became null after being adjusted for CAD (OR 0.98 [95%CI 0.95, 1.01]), suggesting that CAD might mediate the association between HDL-C and HF. In addition, no genetic evidence supported the causal association of genetically determined HDL-C levels with the risk of AF. Also, no evidence of horizontal pleiotropy was found in HF and AF outcomes (Table S 5-7).

Regarding MI and CAD outcomes, our genetic findings suggested that there was a horizontal pleiotropy between genetically determined HDL-C and the risk of MI and CAD, corresponding to the P-values from Egger's intercept of 0.010 and 0.008, respectively (Table S 5-7). This implied that the genetic instrument used as a proxy of HDL-C might be associated with MI and CAD through other pathways that were not related to HDL-C. Furthermore, the null findings from MVMR model suggested that the association between genetically determined HDL-C and the risk of MI and CAD were confounded by other lipid traits that were also regulated by the same genetic instrument.

When putting all the evidence together as shown in Figure 5-5, the observed U-shape association between HDL-C and the incident HF contradicted the direct association found from the meta-regression of RCTs, whereas genetic evidence from UKB did not support the causal association. Meanwhile, the genetic results from HERMES suggested that CAD might mediate the association between HDL-C and HF. For AF and HDL-C, we found a weak U-shape association. However, no association was observed with per continuous increase in HDL-C, which was supported by the results from the meta-

regression of trials and MR. In terms of the association between HDL-C and the risk of MI or CAD, our findings suggested that the inverse association observed from the cohort study might be confounded by other lipid traits or was a result of horizontal pleiotropy due to complex trait characteristics of HDL-C (i.e., genes that regulated HDL-C can also control other traits that might cause MI or CAD).

5.6 Discussion

In this study, we reported the first evaluation of the causal relevance of HDL-C in the two most common, but less-well studied, cardiac diseases globally: HF and AF. We compared higher resolution observational cohorts (more massive and ten times more participants than previous cohort studies), new meta-regression of trials on lipid-lowering agents, and new MR with the largest GWAS on HF outcomes (HERMES). We found a U-shape relationship of HDL-C with the risk of HF. However, the trial evidence suggested a direct association, whereas genetic evidence did not support a causal association, and HERMES suggested CAD as a mediator of the association. In addition, we reported a weak U-shape association between HDL-C and incident AF, which might be mediated by gender: the positive (direct) association observed amongst males while the negative (indirect) one amongst females. Nevertheless, trial and genetic evidence did not support these observational findings.

5.6.1 Heart failure - What is new about our observation?

This was the first study showing a U-shape association of HDL-C levels with incident HF (29,876 new cases). To date, only a few (much smaller) observational cohorts have reported the effect of HDL-C on the risk of HF, and their findings were mixed. Most of the studies showed a negative association,[71,78,106,108,109,112] while only two cohort studies showed no association.[72,77] In our analysis, intercurrent CVDs, but not MI, modified the association (P-value for heterogeneity between groups was 0.007). Amongst patients with intercurrent CVDs, there was no association between per continuous increase in HDL-C and the incident HF, whereas a direct

association was observed amongst those without intercurrent CVDs. Furthermore, we found that age group differences and intercurrent CVDs modified the association only amongst males, but not females (Figure S 5-6). Moreover, the strong positive association of HDL-C with HF due to chronic respiratory disease suggests other biological pathways linked between lipids and HF.

5.6.2 HDL-C and HF – How we extended knowledge from trials and genetic evidence?

Although meta-regression and genetic evidence suggested otherwise (i.e., meta-regression showed a direct association, whereas genetic evidence indicated no association), this might still support the U-shape association observed from the cohort study. The following three reasons justify why we were convinced that our observed U-shape association between HDL-C and incident HF was likely to be supported by trials and genetic evidence.

Firstly, it is worth noticing that in CETP inhibitor trials, such as ILLUMINATE[137] and ILLUSTRATE trials[135] in which reported HF events amongst the active group were higher than in the control group, their findings were partly consistent with our cohort findings. This is because the baseline HDL-C levels of participants in those trials were around 1.2-1.3 mmol/L (45-49 mg/dL), similar to our reference category in the cohort study (i.e., HDL-C of 1.28-1.53 mmol/L). After the follow-up period, patients in the active group had elevated levels of HDL-C, compared with the control group, by around 1.0-1.6 mmol/L (39-62 mg/dL). This seems to be concordant with our U-shape association observed in the cohort study, and this might explain why an increase in HDL-C was associated with a higher risk of HF from the meta-regression. Additionally, excluding CETP inhibitor trials (n=6) from the analysis shifted the univariable results to null (data not showed).

Secondly, the null genetic findings from UKB could be explained by two reasons. First, we did not have sufficient statistical power to detect the effect size. From Table S 5-8, we had less than 25% of power to detect OR of at least 1.24. Second, the two-sample MR method using summary statistics cannot well capture a nonlinear trend of the association, particularly the U-

shape pattern in which the sum effect can be null due to the neutralisation of the effect in each end. Moreover, the genetic evidence from HERMES, which suggested that CAD might explain the association between genetically determined HDL-C levels and the risk of HF, was partly consistent with our subgroup analysis in the cohort study, since we found that intercurrent CVDs might modify the association.

Lastly, a previous study using big data from representative samples (N>600,000) in Canada suggested the U-shape association of HDL-C with all-cause and cause-specific mortality,[229] while other cohort studies in Denmark[230] and Japan[228] also revealed similar findings. This might also extend to other outcomes. We additionally analysed all-cause mortality and our results were reproducible and comparable to previous findings (Figure S 5-12).

5.6.3 HDL-C and HF – Possible and alternative explanations

HDL-C may protect the heart from the development of HF by mechanisms beyond the reverse cholesterol transport mechanism. It has been proposed that HDL-C may exert anti-inflammatory and anti-oxidative effects that protect myocardium from being injured. Also, HDL-C may activate the nitric oxide-dependent vasodilatory pathway and enhance blood perfusion in the myocardium.[39] Although the insight of this complex U-shape association is still unclear, potential explanations of the association between an increased HDL-C and an adverse outcome are genetic mutations leading to very high HDL-C levels, such as loss of function in CETP genes[234], and the mutation of Scavenger receptor class B type 1 (*SCARB1*),[235] or the impairment of HDL-C function, such as cholesterol efflux capacity (CEC) at high HDL-C levels[236]. In other words, too high levels of HDL-C might mirror HDL-C dysfunctionality, which can lead to adverse outcomes.

For alternative explanations, there are four most common modifiable risk factors for HF, namely 1) hypertension; 2) diabetes (including insulin resistance); 3) metabolic syndrome; and 4) atherosclerotic disease (e.g. coronary, cerebral, and peripheral blood vessels).[3] At first, subgroup analysis showing that only patients without intercurrent CVDs followed the U-shape pattern with minimised potential bias due to atherosclerotic disease.

The association in the lower end (i.e., HDL-C < 0.91 mmol/L or 35 mg/dL) might be confounded by residual confounders as we noticed that patients in this group were likely to have an increased risk of HF. For instance, more people with diabetes, receiving antihypertensive medications, higher BMI, and higher levels of CRP (which represent higher degrees of inflammation). Meanwhile, the highest prevalence of cancer and COPD was found amongst patients in the higher end (i.e., HDL-C > 2.04 mmol/L or 79 mg/dL) which might partly explain the U-shaped pattern. Cancer patients might receive cardiotoxic chemotherapy, such as 5-fluorouracil, anthracyclines, and paclitaxel, where right-side heart failure (corpulmonale) is a common COPD complication. Moreover, further excluding the first four-year cases did not reveal potential reverse causation.

5.6.4 HDL-C and AF - What do the observation, trials, and genetic add?

We reported for the first time a weak U-shape association with AF. In addition, we reported gender-specific associations in which we found a direct association amongst males, whereas the opposite association was observed in females. We also reported that it was the age group that significantly modified the association.

To date, there is no previous meta-analysis of HDL-C levels *per se* on the risk of AF, and the evidence from trials did not reveal significant association. Additionally, genetic evidence showed robust findings, which did not support the causal association of HDL-C with the risk of AF. Our genetic findings were similar to the previous work (the AFGen consortium) from which the hazard ratio (HR) of AF per one gene score increase in HDL-C was 1.01 (95%CI 0.98, 1.03).[92] However, it is worth noticing that both ours and the previous work might have insufficient statistical power (<80%) to detect the effect size (Table S 5-8).

5.6.5 HDL-C and AF – Possible explanations

Three mechanisms might explain the association between HDL-C and AF: i) old age and the male gender, ii) thyroid dysfunction, and iii) inflammation.[237] Interestingly, when considering gender-specific characteristics (Table S 5-4),

we observed that some characteristics amongst male patients in the highest HDL-C group (i.e., >2.04 mmol/L or >79 mg/dL) might increase their risk of AF, such as increased CRP levels and an increased proportion of current smokers, compared with those in the lower HDL-C group. This might explain a positive association between HDL-C and the incident AF amongst males. In contrast, the reverse relationship found in females might be partially explained by the physiological properties of high HDL-C that exert an anti-inflammatory property. Additionally, because our study did not include thyroid function as a covariate, endocrine patterns such as low total cholesterol, low HDL-C, and low LDL-C amongst females in the very low HDL-C group might imply underlying hyperthyroidism.[238] Therefore, thyroid function might still confound our results.

5.6.6 Further insight into HDL-C and MI

We found a monotonic and robust inverse association between HDL-C and the incident MI in both age- and sex-adjusted and fully adjusted models. Our observational results are consistent with the previous report by the Emerging Risk Factors Collaboration (ERFC).[95] In contrast, our meta-regression suggested a direct association, which might be outweighed by the impact of a few trials, especially from trials of lipid-lowering agents in other groups (Figure S 5-14). In REDUCE-IT (n3-fatty acid)[139] and CLEAR Harmony trials (Bempedoic acid)[143], participants in the active group, whose HDL-C levels were decreased after the follow-up, had a lower risk of MI, compared with those in the control group. In some trials, such as the AIM-HIGH trial (niacin)[156], individuals showed a higher risk of MI in the active group who had increased levels of HDL-C, compared with those in the control group (Table S 2-11).

Importantly, our meta-regression results did not replicate the findings from previous work in which changes in HDL-levels were not associated with the risk of coronary heart disease.[96] This discrepancy can be explained by the different criteria of study selection. We mostly included major trials (n>1,000), whereas the previous work did not, and we did acknowledge the potential publication bias and significant heterogeneity of our meta-regression findings

on MI outcomes. However, adding more studies are likely to increase heterogeneity, which might not improve the validity of our results. Additionally, another reason for working on MI outcomes in our study was to allow a comparison across different study designs, and our meta-regression results seemed to support the observational cohort results.

Our genetic findings indicate that the genetic instrument of HDL-C might be associated with MI outcomes through other pathways that are not related to HDL-C levels. With the adjustment for other lipid traits, which attenuated the association towards null, our results were consistent with previous MR studies on HDL-C and the risk of MI.[164,239,240] Therefore, HDL-C was not causally relevant to the risk of MI, and this might explain why HDL-C-raising trials, such as trials on CETP inhibitors and niacin, failed to significantly reduce the risk of MI.

5.6.7 Strengths

This is the first study that comprehensively investigated the association and causation of HDL-C on the two most common CVDs using three different study designs (i.e., cohort, trial, and genetic). The strengths of our study are as follows: 1) Compared to prior cohort studies (Table 2-2, Table S 2-8, and Table S 2-9), we used a vast and representative cohort (N=1,338,276). This enabled us to sub-categorise our cohort into seven strata and to have very high statistical power (>90%) to detect a small effect or a nonlinear relationship. Also, having a substantial sample size allowed us to study HF, AF, and MI together and improves the ability to evaluate intercurrent diseases. Furthermore, this was the first time that we could examine an association of the disease with low HDL-C levels (i.e., HDL-C < 0.91 mmol/L or 35 mg/dL); 2) This was the first meta-analysis focusing on the role of HDL-C *per se,* regardless of lipid-lowering agents, on the risk of HF and AF; 3) A number of MR sensitivity analyses were applied in at least three data sources (i.e., UK Biobank, CARDIoGRAMplus4CD, HERMES) to ensure the validity and robustness of genetic findings; and 4) MR analysis on a large consortium (HERMES) secured generalisability, especially in European populations.

5.6.8 Limitations

As previously described in Chapter 4, using EHR might cause misclassification bias due to underestimation of the true incidence. However, in Chapter 3, we demonstrated the validity of using EHR phenotyping to define HF, AF, and MI outcomes. Therefore, this limitation should not be a major concern.

Specific to HDL-C, the main limitation of using EHR is unmeasured confounders. This is because of the incompleteness of data collection. As mentioned previously, we did not adjust our models for some factors that might be related to outcomes, such as baseline thyroid function. Moreover, we could not investigate the impact of HDL-C functions or subclasses of HDL particles (i.e., HDL_2 HDL_3) since these measures are not routinely used in clinical practice, which means no available data in EHR.

Regarding the limitations of our meta-regression approach, we acknowledged that the estimated relative risk per change in HDL-C was based on a linear model. Therefore, it might not well capture a non-linear trend. Moreover, using only summary-level data of trials further hindered detection, if any, of association patterns. Individual-level data of patients are required to overcome this. This limitation also applied to our MR design. The use of the two-sample MR approach limited our ability to perform nonlinear examination since we could not gain access to participants' genetic data.[241]

Potential bias from the observational study design are as follows:

Selection bias: patients who were recruited in my analysis are the individuals with lipid measurement. Therefore, only patients with an indication to measure blood lipids will be included. This would limit the generalisibility of my findings rather than artifact the results. Also, the selection bias can be arisen when there is a discrepancy in the quality of care at practice level. For example, the same patient might be eligible to have lipid measured in one GP but ineligible if he or she goes to another GP. To minimise the bias due to the variation of practice level, I have stratified all analysis by gender and practice level.

Misclassification and information bias:

- Extracted phenotypes of outcome are mainly based on signs and symptoms but not based on an objective confirmation, such as echocardiogram results. For instance, there were only 3% of HF cases who had codes for echocardiography that confirmed HF. Therefore, it is likely to include other health conditions that are mimic signs and symptoms of HF, such as chronic respiratory disease exacerbation as HF cases (i.e., false positive cases).
- Diagnosis taken from EHRs is based solely from one physician (not by adjudication committee), and this might vary according to level of expertise of physicians. Therefore, this prone to misclassification bias.

These two scenarios above would result in misclassification bias and increasing false positive cases. In other words, this would lead to decrease in specificity due to increasing false positive cases, which leads to type I error, and could inflating the observation. However, subgroup analysis of HF based solely on echocardiography codes (i.e., systolic and diastolic HF) had shown no associations with HDL-C, which are consistent with the main findings (Figure S). Therefore, the impact of misclassification bias can be less concerned.

Attrition bias (i.e., bias due to loss to follow-up or dropping out): In my study, there were 5% of studied populations who were censored due to death from other causes, and this might compete the outcome of interest. However, in age and sex-adjusted model, further adjusting for competing risk (Figure S) did not significantly deviate the findings.

5.6.9 Implications of findings

The current clinical guidelines have no specific recommendations for primary prevention of HF and AF, and our results suggested that HDL-C levels might be associated with the risk of HF in a U-shape fashion with partial support by trials and genetic evidence. Therefore, physicians might monitor patients who had high or low HDL-C levels for the presentation of HF, especially those with high risk.

Further research is required to address the potential nonlinear association observed between HDL-C and incident HF in our cohort study. In terms of RCT meta-analysis, we need individual-level data from each major RCT in order to capture changes in HDL-C between the active and the control groups with a better resolution. To address the nonlinearity in the context of MR, we can calculate the population-averaged causal effect, which is the average difference in the outcome providing that the exposure for every individual in the population is increased by a fixed amount. In practice, it is a reasonable estimation of nonlinear effect.[242] Alternatively, we can apply semiparametric approaches such as fractional polynomial or the piecewise linear method, as suggested in the literature.[243]

5.7 Conclusion

HDL-C might be nonlinearly associated with the risk of HF, which was partially supported by trials and genetic evidence. We also observed that gender mediated the association between HDL-C and the risk of AF, although trial and genetic evidence did not support a causal relationship. Therefore, the role of HDL-C on the incidence of HF still requires further investigation. Our study additionally indicated that HDL-C was not causally related to the risk of MI, and the observed inverse association might be a result of confounders rather than a real association.

Table 5-1 Observational cohort: participant characteristics of the population-based EHR cohort (n= 1,338,276)

Baseline characteristics	HDL cholesterol at baseline (mmol/L)				P-values
	< 0.91	1.28 - 1.53	> 2.04	Total	
N	91,396	357,828	125,653	1,338,276	
Female	22.7%	53.3%	81.8%	53.1%	P < 0.001
Age (year)	51.6 (12.7)	55.5 (13.2)	59.1 (13.2)	55.5 (13.3)	P < 0.001
White	85.1%	89.3%	93.0%	89.2%	P < 0.001
Missing*	40.2%	41.9%	44.7%	42.0%	
Health behaviors, physical and laboratory measurements at baseline					
Non-smokers	48.0%	58.8%	63.1%	58.1%	P < 0.001
Missing*	0.8%	0.6%	0.6%	0.6%	
Body mass index (kg/m^2)	30.3 (5.9)	27.9 (5.4)	24.5 (4.3)	27.7 (5.6)	P < 0.001
Missing	4.8%	4.6%	4.6%	4.6%	
Systolic blood pressure (mmHg)	135.8 (15.8)	135.9 (16.9)	135.7 (18.0)	135.8 (16.9)	P < 0.001
Missing	1.4%	1.1%	0.7%	1.0%	
Diastolic blood pressure (mmHg)	82.3 (9.3)	81.3 (9.3)	79.9 (9.3)	81.2 (9.3)	P < 0.001
Missing	1.4%	1.1%	0.7%	1.0%	
eGFR (mL/min/1.73m^2)	87.8 (27.0)	82.8 (20.2)	76.3 (18.4)	82.6 (21.0)	P < 0.001
Missing	4.2%	4.0%	4.5%	4.1%	
CRP (mg/L), median (IQR)	5.0 (2.2-10.0)	4.0 (2.0-8.0)	3.0 (1.2-6.2)	4.0 (2.0-8.0)	P < 0.001
Missing	57.9%	56.1%	53.1%	56.0%	
LDL cholesterol (mmol/L)	3.1 (1.0)	3.4 (0.9)	3.1 (0.9)	3.3 (0.9)	P < 0.001
Missing	24.4%	18.5%	19.7%	19.2%	
HDL cholesterol (mmol/L)	0.8 (0.1)	1.4 (0.1)	2.4 (0.3)	1.5 (0.4)	P < 0.001
Total cholesterol (mmol/L)	5.0 (1.1)	5.5 (1.0)	5.9 (1.0)	5.5 (1.0)	P < 0.001
Missing	6.2%	5.7%	5.8%	6.0%	
Triglyceride (mmol/L), median (IQR)	2.1 (1.5-3.0)	1.3 (1.0-1.8)	0.9 (0.7-1.1)	1.3 (0.9-1.9)	P < 0.001
Missing	14.4%	12.1%	14.1%	12.5%	
Health conditions at baseline					
Diabetes type 2	9.6%	4.2%	1.8%	4.8%	P < 0.001
Chronic kidney disease	3.6%	3.3%	3.3%	3.4%	P < 0.001
Cancer	2.9%	3.3%	4.1%	3.4%	P < 0.001
COPD	1.4%	1.7%	2.7%	1.8%	P < 0.001
Medication					
Statins (at baseline)	16.2%	12.3%	9.3%	12.6%	P < 0.001
Statins (at follow-up)	37.5%	30.8%	23.2%	31.0%	P < 0.001
Other lipid-lowering drugs	1.4%	0.6%	0.5%	0.7%	P < 0.001
Antihypertensive drugs	29.9%	29.0%	27.2%	28.9%	P < 0.001
Antiplatelet drugs	7.2%	5.8%	5.3%	6.0%	P < 0.001

Note: Values are presented as percentage, mean (standard deviation) or median (interquartile range) as appropriate. *Percentages of missing category were separately calculated from complete cases. Corresponding values for HDL-C are: 0.91 mmol/L = 35.19 mg/dL; 1.01 mmol/L = 39.06 mg/dL, 1.27 mmol/L = 49.11 mg/dL, 1.53 mmol/L = 59.17 mg/dL, 1.78 mmol/L = 68.83 mg/dL, 2.04 mmol/L = 78.89 mg/dL. To convert mmol/L of LDL-C, total cholesterol and triglyceride to mg/dL, multiply by the factor of 38.67, 38.67, and

88.57, respectively. **Abbreviations:** CRP; C-Reactive Protein, eGFR; estimated glomerular filtration rate, COPD; chronic obstructive pulmonary disease, HDL; high density lipoprotein, LDL; low density lipoprotein.

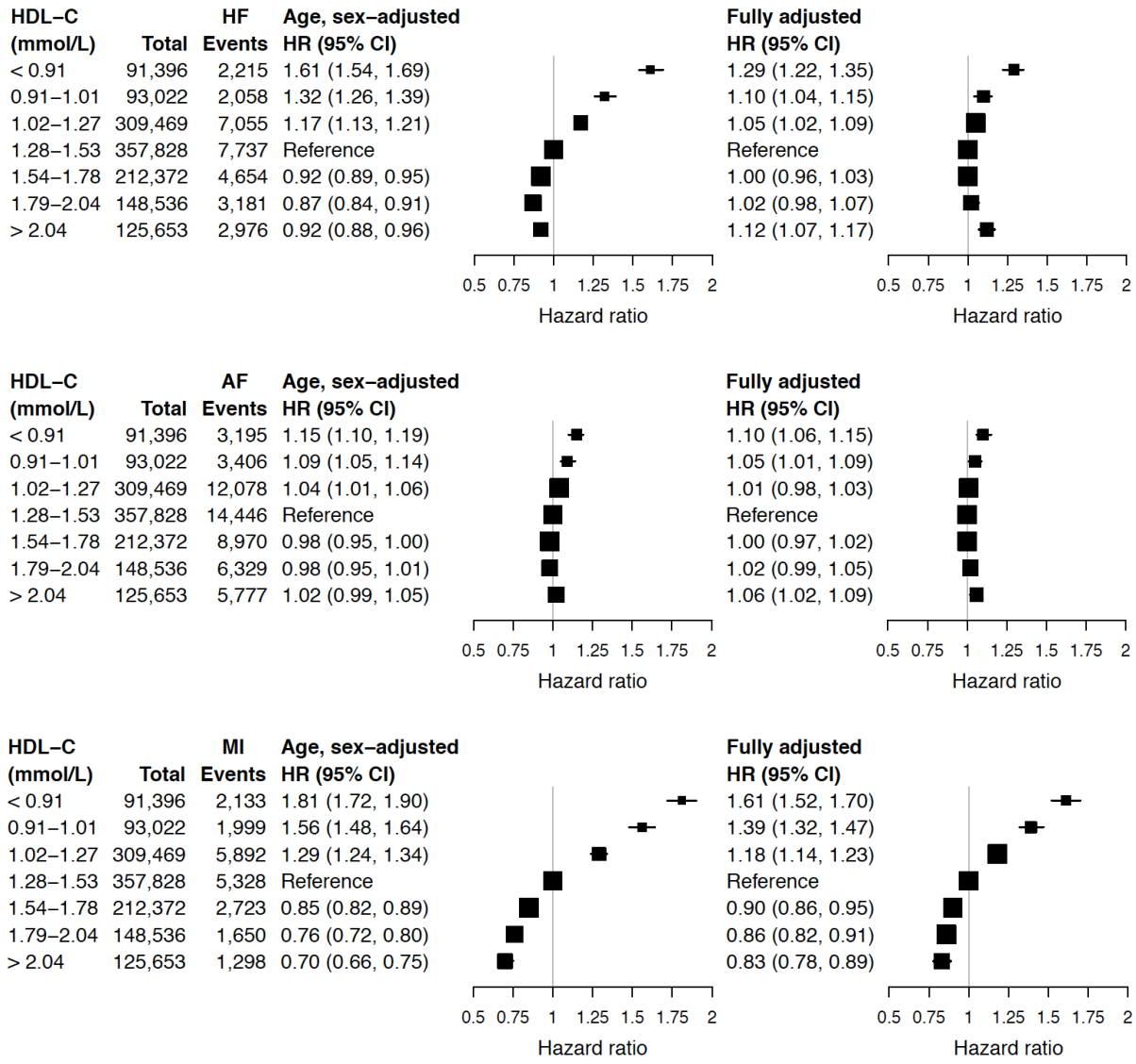
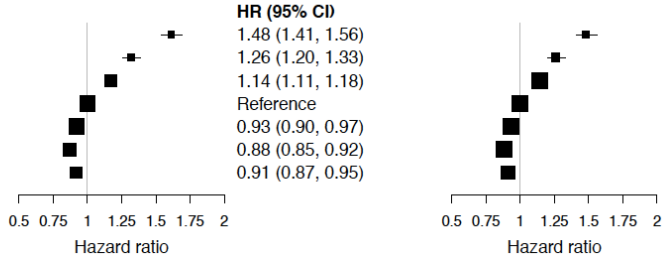


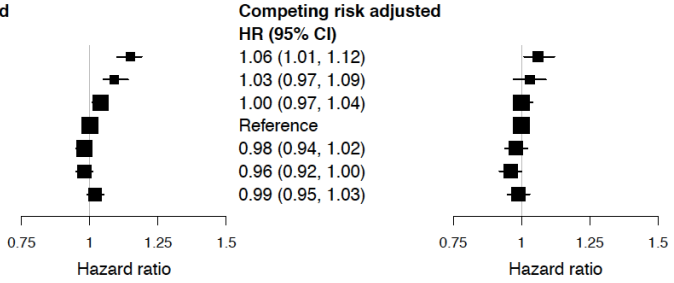
Figure 5-1 Observational cohort: The associations of different levels of HDL-C and incident HF (total 7,607,199 person-years), AF (total 7,513,759 person-years), and MI (total 7,609,784 person-years) among 1,338,276 individuals without diagnosed CVD at baseline over the median follow-up period of 5 years (interquartile range: 2 to 9 years).

Note: Fully adjusted models were stratified for gender and primary care practice and adjusted for age, socioeconomic status, smoking, body mass index, systolic blood pressure, diastolic blood pressure, high-density lipoprotein cholesterol, triglyceride, C-reactive protein, diabetes, use of blood pressure lowering drugs and use of statins. The size of the boxes varies based on inverse variance of the data in each category. Corresponding values for HDL-C are: 0.91 mmol/L = 35 mg/dL; 1.01 mmol/L = 39 mg/dL, 1.27 mmol/L = 49 mg/dL, 1.53 mmol/L = 59 mg/dL, 1.78 mmol/L = 69 mg/dL, 2.04 mmol/L = 79 mg/dL

HDL–C (mmol/L)	Total	HF Events	Age, sex–adjusted HR (95% CI)	Competing risk adjusted HR (95% CI)
< 0.91	91,396	2,215	1.61 (1.54, 1.69)	1.48 (1.41, 1.56)
0.91–1.01	93,022	2,058	1.32 (1.26, 1.39)	1.26 (1.20, 1.33)
1.02–1.27	309,469	7,055	1.17 (1.13, 1.21)	1.14 (1.11, 1.18)
1.28–1.53	357,828	7,737	Reference	Reference
1.54–1.78	212,372	4,654	0.92 (0.89, 0.95)	0.93 (0.90, 0.97)
1.79–2.04	148,536	3,181	0.87 (0.84, 0.91)	0.88 (0.85, 0.92)
> 2.04	125,653	2,976	0.92 (0.88, 0.96)	0.91 (0.87, 0.95)



HDL–C (mmol/L)	Total	AF Events	Age, sex–adjusted HR (95% CI)	Competing risk adjusted HR (95% CI)
< 0.91	91,396	3,195	1.15 (1.10, 1.19)	1.06 (1.01, 1.12)
0.91–1.01	93,022	3,406	1.09 (1.05, 1.14)	1.03 (0.97, 1.09)
1.02–1.27	309,469	12,078	1.04 (1.01, 1.06)	1.00 (0.97, 1.04)
1.28–1.53	357,828	14,446	Reference	Reference
1.54–1.78	212,372	8,970	0.98 (0.95, 1.00)	0.98 (0.94, 1.02)
1.79–2.04	148,536	6,329	0.98 (0.95, 1.01)	0.96 (0.92, 1.00)
> 2.04	125,653	5,777	1.02 (0.99, 1.05)	0.99 (0.95, 1.03)



HF Subtype	Events	HR (95% CI) per one SD increase in HDL–C
Overall HF	30,348	0.99 (0.98, 1.01)
Systolic HF	685	0.97 (0.88, 1.07)
Diastolic HF	161	0.98 (0.80, 1.20)
HF due to chronic respiratory disease	362	1.32 (1.18, 1.47)
Hypertensive HF	220	1.12 (0.94, 1.32)
Other HF	28,917	0.99 (0.97, 1.00)
AF Subtype		
Overall AF	54,201	1.01 (1.00, 1.02)
Paroxysmal AF	5,026	1.03 (1.00, 1.07)
Persistent/Permanent AF	66	0.91 (0.66, 1.25)
Atrial flutter	1,356	1.11 (1.04, 1.19)
Other AF	47,753	1.00 (0.99, 1.01)

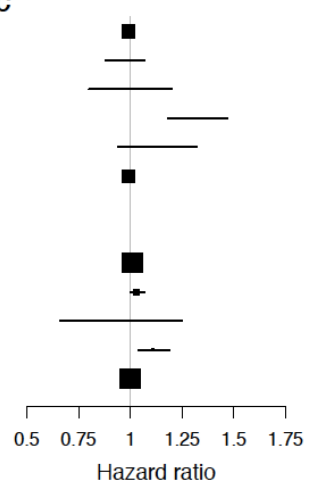


Figure 5-2 Competing risk adjustment and subtype analysis

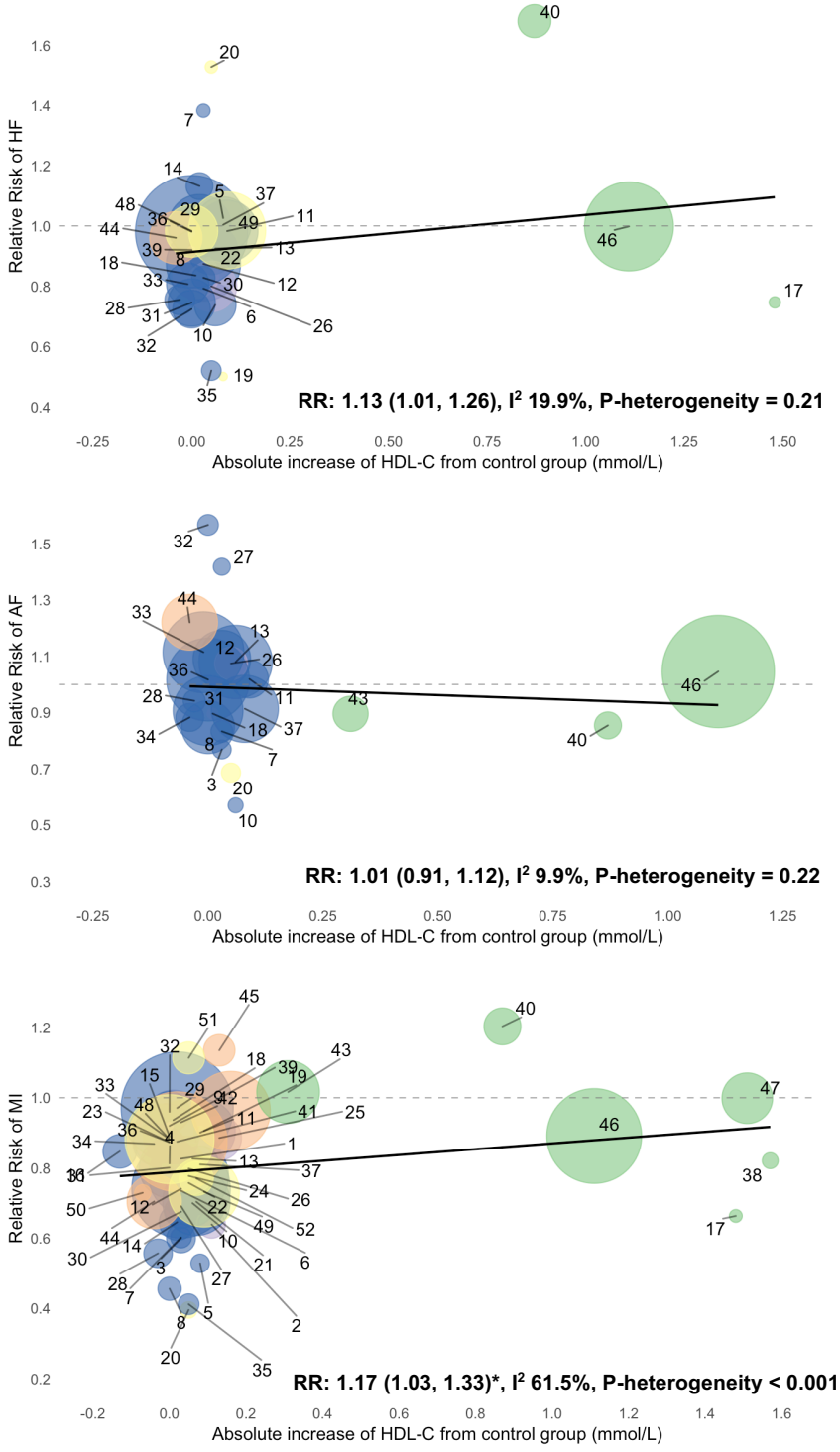


Figure 5-3 RCTs of lipid-lowering agents: Univariable meta-regression of the risk of HF (33 trials: 8,679 events), AF (24 trials: 6,567 events), and MI (52 trials: 27,336 events).

CETP inhibitors Fibrates Others PCSK-9 Inhibitors Statins

Note: Numbers designated in plots represent study identification (Supplementary Table S11) and the size of bubbles was proportional (weighted) to inverse-variance. *Random effect model.

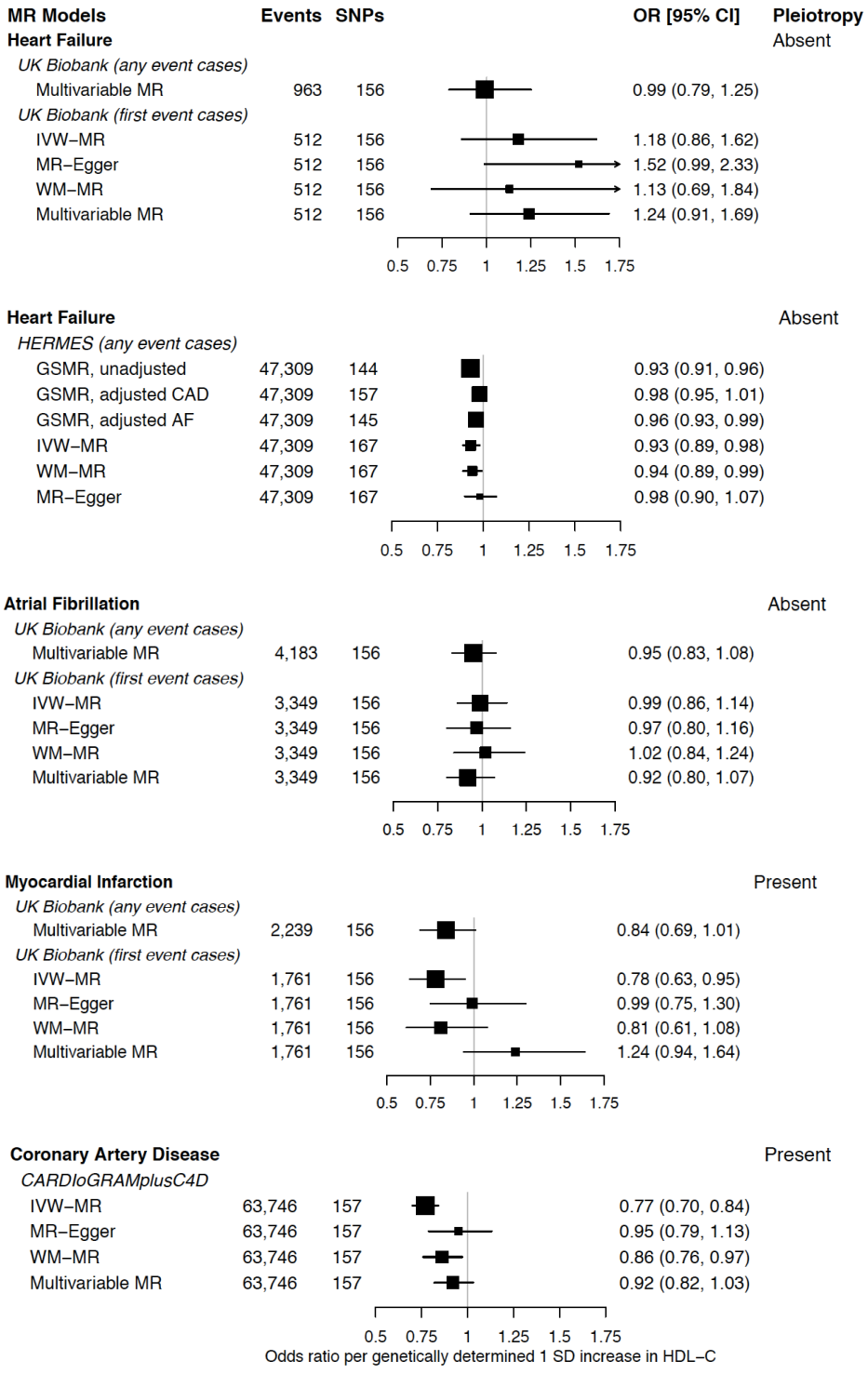


Figure 5-4 Genetic (MR) study of associations between genetically determined 1 SD (0.41 mmol/L or 15.85 mg/dL) increased HDL-C and the risk of HF, AF, MI, and CAD.

Abbreviations: CARDIoGRAMplusC4D; Coronary Artery Disease Genome-wide Replication and Meta-analysis plus Coronary Artery Disease Genetics, CI; Confidence interval, HERMES;

Heart Failure Molecular Epidemiology for Therapeutic Targets Consortium, IVW; Inverse variance weighted, MR; Mendelian randomisation, OR; Odds ratio, SNPs; Single nucleotide polymorphisms, WM; Weighted median.

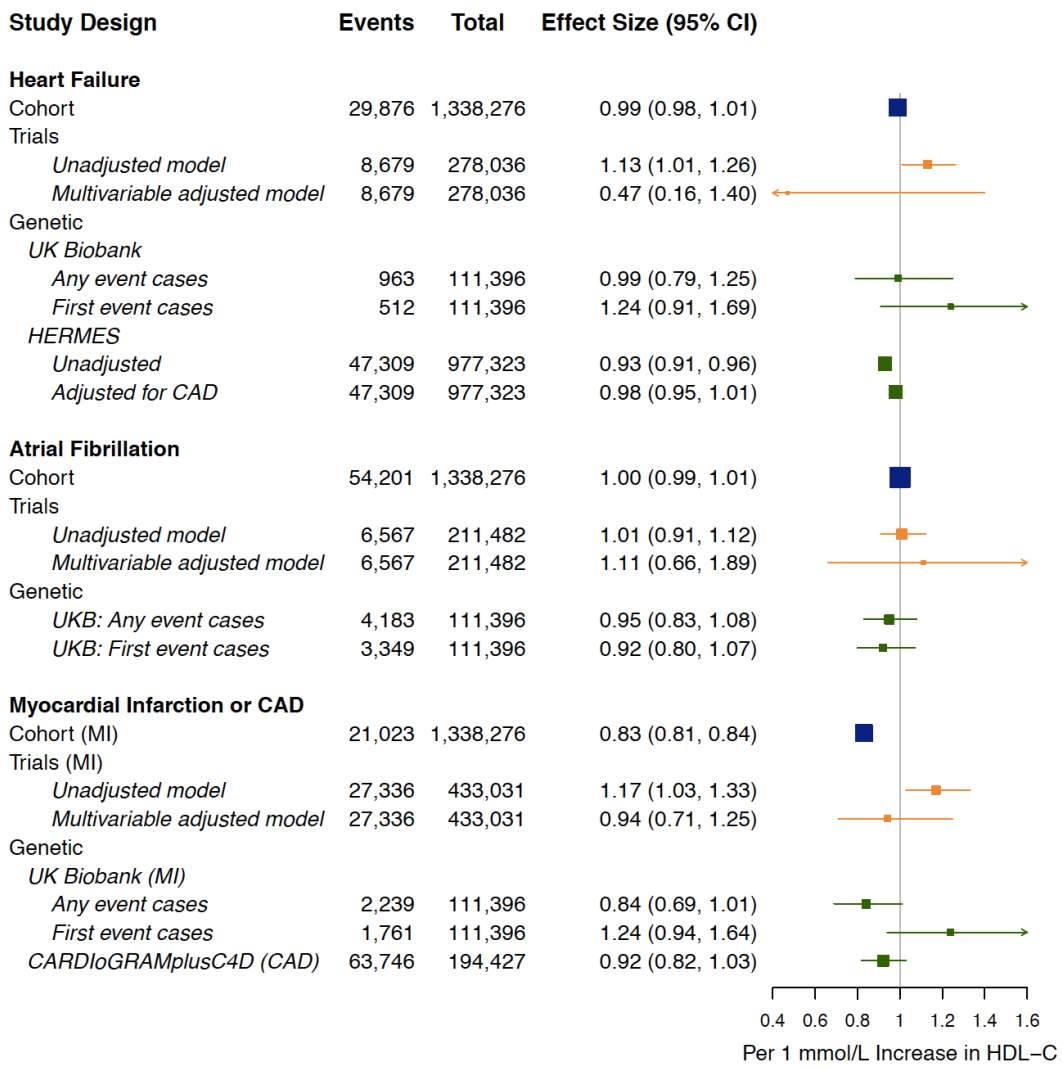


Figure 5-5 Comparison of the estimated associations between HDL-C and risk of HF, AF, and MI (or CAD) from cohort (hazard ratio), RCTs (risk ratio), and MR study (odds ratio).

5.8 Chapter Supplementary

Table S 5-1 STROBE and RECORD checklist

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 220-221	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	1.1) – 1.3) Page 220-221
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 221-222		
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 223		
Methods					
Study Design	4	Present key elements of study design early in the paper	Page 223		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 223-224		
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants	a) Page 223-224, Supplementary appendices b) Not applicable	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided.	6.1) Page 224, Supplementary appendices 6.2) Page 225 6.3) Supplementary appendices

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
		(b) Cohort study - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case		RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Page 224-225, Supplementary appendices	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Page 224-225, Supplementary appendices, Also refer to CALIBER portal
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 224-225		
Bias	9	Describe any efforts to address potential sources of bias	Page 225-226		
Study size	10	Explain how the study size was arrived at	Supplementary appendices		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 225-226		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	a) Page 225-226 b-c) Supplementary appendices d) Not applicable e) Supplementary appendices		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.	12.1) Page 223 12.2) Page 224 (Exposure: HDL-C as an EHR phenotype)

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
				RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	12.3) Page 223, Refer to CALIBER portal, which included extensive information. Also, refer to a paper explaining the CALIBER platform (S Denaxas et al. Int J Epidemiol)
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	Chapter supplementary Figure S 5-3	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	Chapter supplementary Figure S 5-2
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	a)-b) Table 5-1 b) Table 5-1		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures	Table S 5-2		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	a)-b) Figure 5-1 c) Not applicable		

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
		(b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period			
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Figure S 5-4 to S 5-7, Table S 5-5 to S 5-6		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Page 233-234		
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 239-240	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	Page 239-240
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 239-241		
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 239		
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Not relevant		
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	Not relevant

Note *Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.

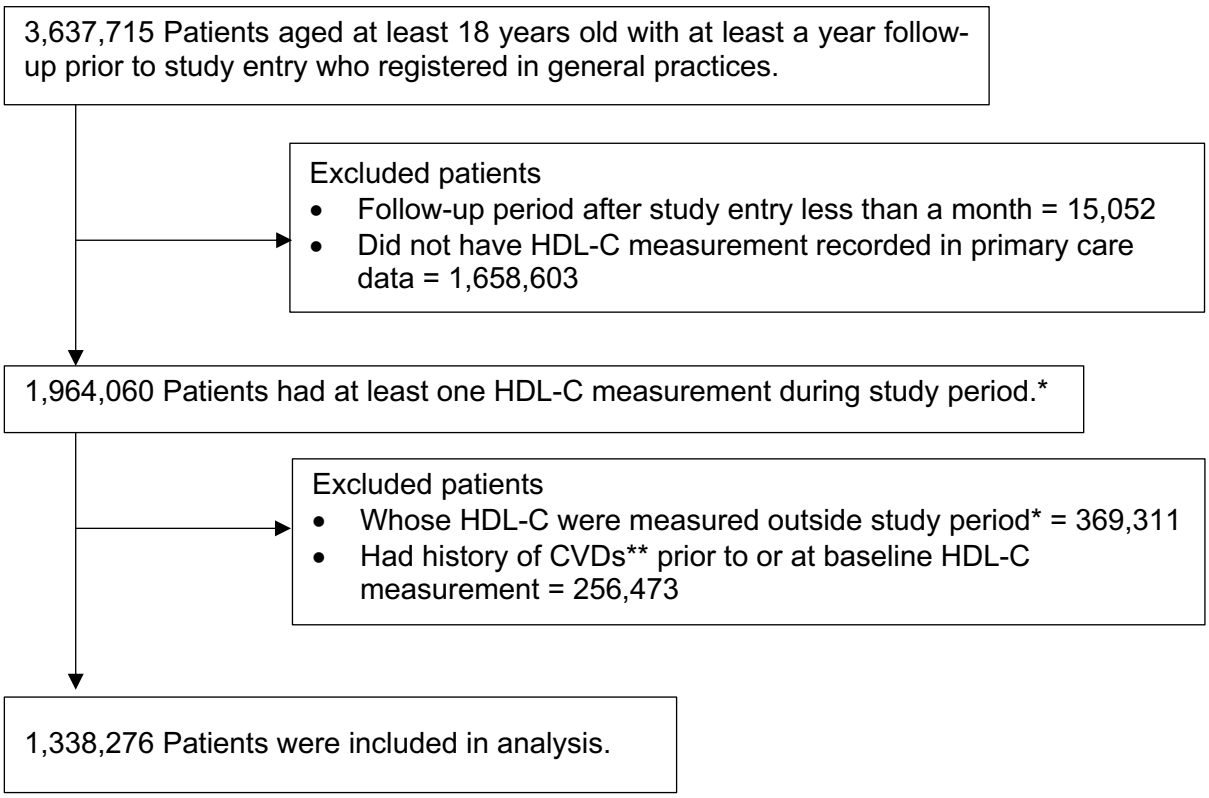


Figure S 5-1 Study flow diagram

Note: *Study period is between 1st Jan 1997 and 30th Jun 2016. **CVDs include 1) coronary artery disease (i.e., myocardial infarction, unstable angina, and stable angina), 2) stroke (i.e., haemorrhagic stroke, ischaemic stroke, and unclassified stroke), 3) transient ischaemic attack, 4) heart failure, 5) atrial fibrillation, 6) abdominal aortic aneurysm, 7) peripheral arterial disease, and 8) sudden cardiac arrest.

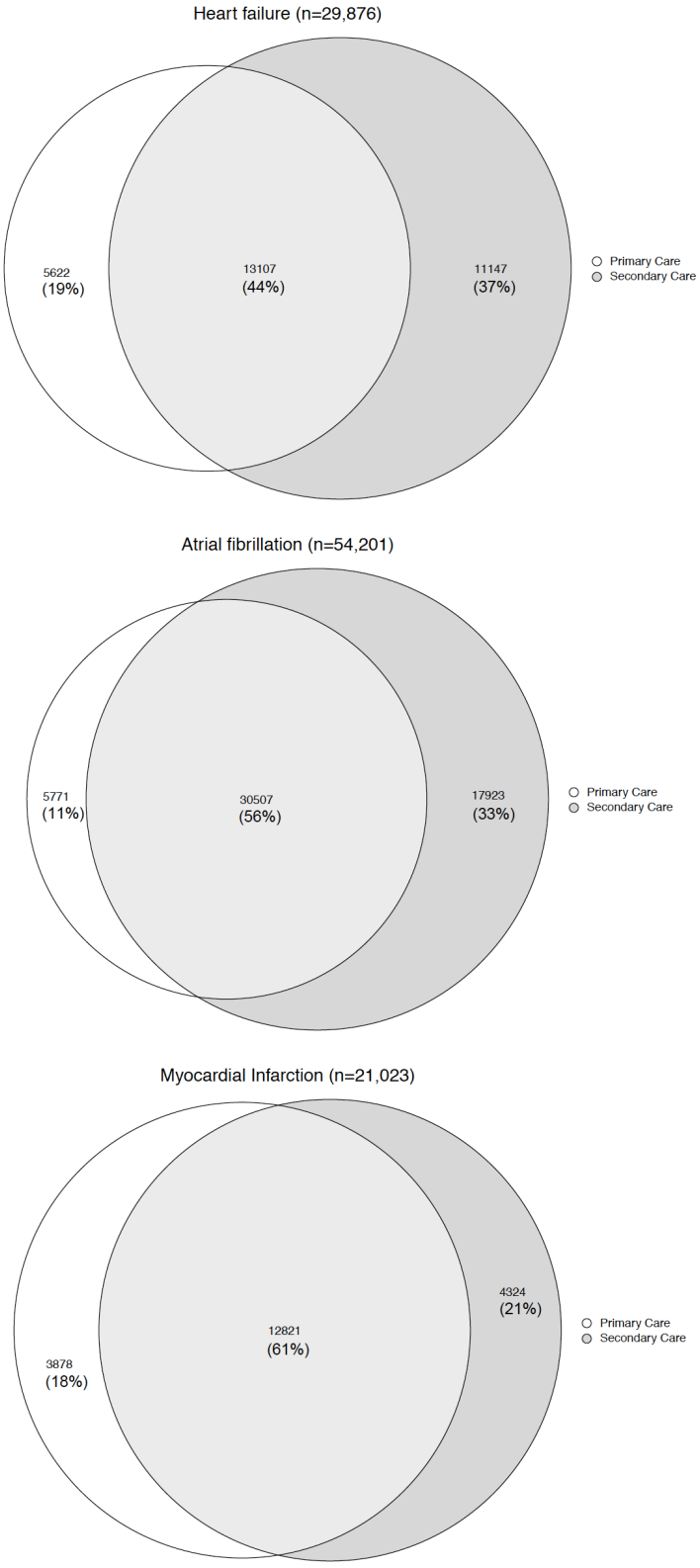


Figure S 5-2 Venn diagrams to illustrate the linkage process of HF (top) AF (middle) and MI (bottom)

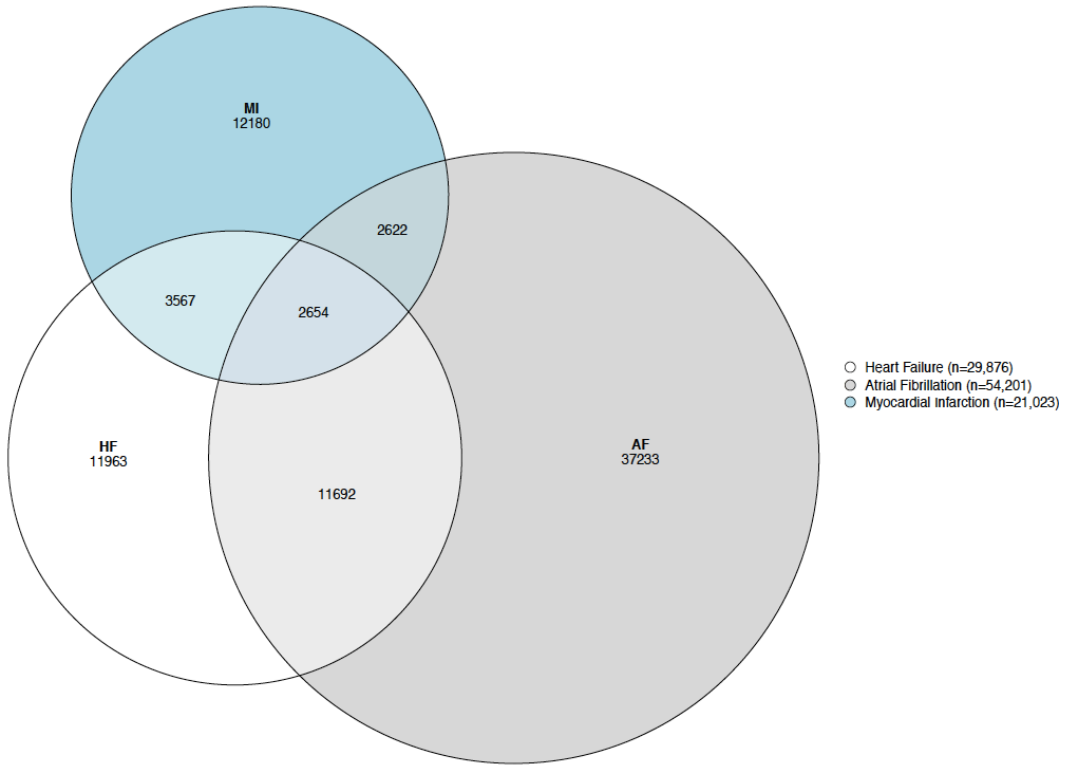


Figure S 5-3 A Venn diagram for incident MI, HF, and AF in 1,338,276 CALIBER cohorts

Note: Median follow-up time was 5 years (IQR: 2 to 9 years)

Table S 5-2 Incidence rate of HF and AF by HDL-C levels

HDL-C levels (mmol/L)	< 0.91	0.91 – 1.01	1.02 – 1.27	1.28 – 1.53	1.54 – 1.78	1.79 – 2.04	> 2.04	Total
Total								
HF events	2,215	2,058	7,055	7,737	4,654	3,181	2,976	29,876
Person-years (per 1,000)	493,482	515,079	1,763,625	2,063,296	1,222,406	846,512	702,797	7,607,199
Incidence rate (95%CI)	4.49	4.00	4.00	3.75	3.81	3.76	4.23	3.93
	(4.31, 4.68)	(3.83, 4.17)	(3.91, 4.09)	(3.67, 3.83)	(3.70, 3.92)	(3.63, 3.89)	(4.09, 4.39)	(3.88, 3.97)
AF events	3,195	3,406	12,078	14,446	8,970	6,329	5,777	54,201
Person-years	489,625	510,302	1,743,203	2,037,579	1,205,874	834,673	692,500	7,513,759
Incidence rate (95%CI)	6.53	6.67	6.93	7.09	7.44	7.58	8.34	7.21
	(6.30, 6,76)	(6.45, 6.90)	(6.81, 7.05)	(6.98, 7.21)	(7.29, 7.59)	(7.40, 7.77)	(8.13, 8.56)	(7.15, 7.27)
Male								
HF events	1,668	1,461	4,431	3,757	1,695	929	699	14,640
Person-years	379,722	371,774	1,097,056	968,601	414,381	212,535	126,262	3,570,330
Incidence rate (95%CI)	4.39	3.92	4.03	3.87	4.09	4.37	5.53	4.10
	(4.18, 4.60)	(3.73, 4.13)	(3.92, 4.15)	(3.75, 4.00)	(3.90, 4.28)	(4.09, 4.66)	(5.14, 5.96)	(4.03, 4.16)
AF events	2,533	2,586	8,011	7,589	3,521	2,003	1,394	27,637
Person-years (per 1,000)	376,367	367,759	1,082,658	953,816	407,135	208,768	123,518	3,520,020
Incidence rate (95%CI)	6,73	7.03	7.39	7.95	8.64	9.59	11.28	7.85
	(6.47, 6.99)	(6.76, 7.30)	(7.23, 7.56)	(7.77, 8.13)	(8.36, 8.93)	(9.18, 10.02)	(10.70, 11.89)	(7.75, 7.94)
Female								
HF events	547	597	2,624	3,980	2,959	2,252	2,277	15,236
Person-years (per 1,000)	113,760	143,305	666,570	1,094,696	808,025	633,977	576,535	4,036,869
Incidence rate (95%CI)	4.80	4.16	3.93	3.63	3.66	3.55	3.94	3.77
	(4.24, 5.22)	(3.84, 4.51)	(3.78, 4.09)	(3.52, 3.75)	(3.53, 3.79)	(3.40, 3.70)	(3.79, 4.11)	(3.71, 3.83)
AF events	662	820	4,067	6,857	5,449	4,326	4,383	26,564
Person-years	113,259	142,543	660,545	1,083,763	798,739	625,906	568,982	3,993,738
Incidence rate (95%CI)	5.84	5.75	6.15	6.32	6.82	6.91	7.70	6.65
	(5.41, 6.30)	(5.37, 6.16)	(5.97, 6.34)	(6.17, 6.47)	(6.64, 7.00)	(6.70, 7.12)	(7.47, 7.93)	(6.57, 6.73)

Abbreviations: AF; Atrial fibrillation, HF; Heart failure.

Table S 5-3 Full participant characteristics of the population-based EHR cohorts (n= 1,338,276)

Baseline	HDL cholesterol at baseline (mmol/L)								P-values
	< 0.91	**0.91-1.01**	**1.02-1.27**	**1.28-1.53**	**1.54-1.78**	**1.79-2.04**	**> 2.04**	**Total**	
N	91,396	93,022	309,469	357,828	212,372	148,536	125,653	1,338,276	
Male	70,666 (77.3%)	66,910 (71.9%)	192,057 (62.1%)	166,934 (46.7%)	71,323 (33.6%)	37,135 (25.0%)	22,856 (18.2%)	627,881 (46.9%)	P < 0.001 P_{trend} < 0.001
Female	20,730 (22.7%)	26,112 (28.1%)	117,412 (37.9%)	190,894 (53.3%)	141,049 (66.4%)	111,401 (75.0%)	102,797 (81.8%)	710,395 (53.1%)	
Age (year)	51.6 (12.7)	52.6 (12.7)	54.1 (13.0)	55.5 (13.2)	56.9 (13.3)	57.7 (13.4)	59.1 (13.2)	55.5 (13.3)	P < 0.001 P_{trend} < 0.001
Ethnicity									
Caucasian	46,537 (85.1%)	47,743 (86.2%)	161,251 (87.6%)	185,693 (89.3%)	110,353 (90.8%)	76,293 (92.1%)	64,600 (93.0%)	692,470 (89.2%)	P < 0.001 P_{trend} < 0.001[$]
South Asian	5,078 (9.3%)	4,494 (8.1%)	12,276 (6.7%)	10,603 (5.1%)	4,770 (3.9%)	2,523 (3.0%)	1,625 (2.3%)	41,369 (5.3%)	
African	1,540 (2.8%)	1,736 (3.1%)	6,254 (3.4%)	7,365 (3.5%)	4,269 (3.5%)	2,646 (3.2%)	2,230 (3.2%)	26,040 (3.4%)	
Other	1,505 (2.8%)	1,382 (2.5%)	4,389 (2.4%)	4,261 (2.0%)	2,153 (1.8%)	1,390 (1.7%)	984 (1.4%)	16,064 (2.1%)	
Missing	36,736 (40.2%)	37,667 (40.5%)	125,299 (40.5%)	149,906 (41.9%)	90,827 (42.8%)	65,684 (44.2%)	56,214 (44.7%)	562,333 (42.0%)	
Health behaviors, physical and laboratory measurements at baseline									
Smoking									
Non-smokers	43,490 (48.0%)	47,434 (51.4%)	168,932 (55.0%)	209,200 (58.8%)	130,275 (61.7%)	93,938 (63.6%)	78,900 (63.1%)	772,169 (58.1%)	P < 0.001 P_{trend}< 0.001[$$]
Ex-smokers	23,447 (25.9%)	24,441 (26.5%)	81,412 (26.5%)	90,860 (25.6%)	52,360 (24.8%)	35,569 (24.1%)	30,247 (24.2%)	338,336 (25.4%)	
Current smokers	23,694 (26.1%)	20,414 (22.1%)	57,066 (18.6%)	55,461 (15.6%)	28,471 (13.5%)	18,215 (12.3%)	15,794 (12.6%)	219,115 (16.5%)	
Missing	765 (0.8%)	733 (0.8%)	2,059 (0.7%)	2,307 (0.6%)	1,266 (0.6%)	814 (0.5%)	712 (0.6%)	8,656 (0.6%)	
Body mass index (kg/m^2)	30.3 (5.9)	29.9 (5.8)	29.2 (5.7)	27.9 (5.4)	26.7 (5.1)	25.7 (4.7)	24.5 (4.3)	27.7 (5.6)	P < 0.001 P_{trend} < 0.001
Missing	4,404 (4.8%)	4,415 (4.7%)	14,268 (4.6%)	16,531 (4.6%)	9,586 (4.5%)	6,593 (4.4%)	5,775 (4.6%)	61,572 (4.6%)	
Systolic blood pressure (mmHg)	135.8 (15.8)	136.1 (15.9)	136.3 (16.3)	135.9 (16.9)	135.5 (17.4)	135.0 (17.7)	135.7 (18.0)	135.8 (16.9)	P < 0.001 P_{trend} < 0.001
Missing	1,304 (1.4%)	1,161 (1.2%)	3,587 (1.2%)	3,862 (1.1%)	1,944 (0.9%)	1,178 (0.8%)	932 (0.7%)	13,968 (1.0%)	

Baseline	HDL cholesterol at baseline (mmol/L)								P-values
	< 0.91	**0.91-1.01**	**1.02-1.27**	**1.28-1.53**	**1.54-1.78**	**1.79-2.04**	**> 2.04**	**Total**	
Diastolic blood pressure (mmHg)	82.3 (9.3)	82.3 (9.3)	82.0 (9.3)	81.3 (9.3)	80.5 (9.3)	80.0 (9.3)	79.9 (9.3)	81.2 (9.3)	P < 0.001
Missing	1,304 (1.4%)	1,161 (1.2%)	3,587 (1.2%)	3,862 (1.1%)	1,944 (0.9%)	1,178 (0.8%)	932 (0.7%)	13,968 (1.0%)	P_{trend} < 0.001
eGFR (mL/min/1.73m^2)	87.8 (27.0)	87.2 (23.0)	85.7 (21.7)	82.8 (20.2)	80.3 (19.0)	78.3 (18.2)	76.3 (18.4)	82.6 (21.0)	P < 0.001
Missing	3,850 (4.2%)	3,608 (3.9%)	12,100 (3.9%)	14,355 (4.0%)	8,958 (4.2%)	6,523 (4.4%)	5,697 (4.5%)	55,091 (4.1%)	P_{trend} < 0.001
CRP (mg/L)									
Mean (SD)	11.7 (25.3)	10.5 (22.8)	9.9 (22.5)	9.3 (21.3)	8.8 (21.2)	8.5 (21.3)	8.5 (21.0)	9.4 (21.9)	
Median (IQR)	5.0 (2.2-10.0)	5.0 (2.0-9.0)	4.4 (2.0-8.0)	4.0 (2.0-8.0)	4.0 (2.0-7.0)	3.0 (1.5-6.6)	3.0 (1.2-6.2)	4.0 (2.0-8.0)	P < 0.001 P_{trend} < 0.001
Missing	52,904 (57.9%)	54,263 (58.3%)	177,628 (57.4%)	200,819 (56.1%)	116,450 (54.8%)	80,556 (54.2%)	66,701 (53.1%)	749,321 (56.0%)	
LDL cholesterol (mmol/L)	3.1 (1.0)	3.3 (0.9)	3.4 (0.9)	3.4 (0.9)	3.4 (0.9)	3.3 (0.9)	3.1 (0.9)	3.3 (0.9)	P < 0.001
Missing	22,326 (24.4%)	18,635 (20.0%)	57,009 (18.4%)	66,260 (18.5%)	39,216 (18.5%)	28,644 (19.3%)	24,708 (19.7%)	256,798 (19.2%)	P_{trend} < 0.001
HDL cholesterol (mmol/L)	0.8 (0.1)	1.0 (0.0)	1.2 (0.1)	1.4 (0.1)	1.6 (0.1)	1.9 (0.1)	2.4 (0.3)	1.5 (0.4)	P < 0.001 P_{trend} < 0.001
Total cholesterol (mmol/L)	5.0 (1.1)	5.2 (1.0)	5.4 (1.0)	5.5 (1.0)	5.6 (1.0)	5.6 (1.0)	5.9 (1.0)	5.5 (1.0)	P < 0.001
Missing	5,621 (6.2%)	5,887 (6.3%)	20,036 (6.5%)	20,440 (5.7%)	13,195 (6.2%)	7,878 (5.3%)	7,277 (5.8%)	80,334 (6.0%)	P_{trend} < 0.001
Triglyceride (mmol/L)									
Mean (SD)	2.5 (1.7)	2.1 (1.3)	1.8 (1.1)	1.5 (0.8)	1.3 (0.7)	1.1 (0.6)	1.0 (0.5)	1.6 (1.0)	
Median (IQR)	2.1 (1.5-3.0)	1.9 (1.3-2.6)	1.6 (1.2-2.2)	1.3 (1.0-1.8)	1.1 (0.8-1.5)	1.0 (0.8-1.3)	0.9 (0.7-1.1)	1.3 (0.9-1.9)	P < 0.001 P_{trend} < 0.001
Missing	13,121 (14.4%)	11,302 (12.1%)	35,647 (11.5%)	43,423 (12.1%)	26,606 (12.5%)	19,831 (13.4%)	17,669 (14.1%)	167,599 (12.5%)	
Health conditions									
Diabetes type 1	230 (0.3%)	223 (0.2%)	707 (0.2%)	724 (0.2%)	444 (0.2%)	356 (0.2%)	392 (0.3%)	3,076 (0.2%)	P < 0.001 P_{trend} = 0.802[#]
Diabetes type 2	8,812 (9.6%)	7,688 (8.3%)	20,706 (6.7%)	14,952 (4.2%)	6,540 (3.1%)	3,198 (2.2%)	2,274 (1.8%)	64,170 (4.8%)	P < 0.001 P_{trend}< 0.001[##]
Chronic kidney disease	3,304 (3.6%)	3,079 (3.9%)	10,329 (3.3%)	11,891 (3.3%)	7,304 (3.4%)	4,915 (3.3%)	4,200 (3.3%)	45,022 (3.4%)	P < 0.001 P_{trend} = 0.077
Cancer	2,649 (2.9%)	2,738 (2.9%)	9,709 (3.1%)	11,868 (3.3%)	7,729 (3.6%)	5,598 (3.8%)	5,125 (4.1%)	45,416 (3.4%)	P < 0.001 P_{trend} < 0.001
COPD	1,302 (1.4%)	1,224 (1.3%)	4,736 (1.5%)	5,948 (1.7%)	3,969 (1.9%)	2,996 (2.0%)	3,430 (2.7%)	23,605 (1.8%)	P < 0.001 P_{trend} < 0.001

Baseline	HDL cholesterol at baseline (mmol/L)								P-values
	< 0.91	0.91-1.01	1.02-1.27	1.28-1.53	1.54-1.78	1.79-2.04	> 2.04	Total	
Medications									
Statins (at baseline)	14,762 (16.2%)	14,478 (15.6%)	46,268 (15.0%)	43,982 (12.3%)	23,795 (11.2%)	14,189 (9.6%)	11,734 (9.3%)	169,208 (12.6%)	P < 0.001 P_{trend} < 0.001
Statins (during follow-up)	34,251 (37.5%)	34,253 (36.8%)	111,116 (35.9%)	110,250 (30.8%)	59,711 (28.1%)	36,236 (24.4%)	29,199 (23.2%)	415,016 (31.0%)	P < 0.001 P_{trend} < 0.001
Other lipid-lowering drugs	1,267 (1.4%)	840 (0.9%)	2,205 (0.7%)	1,969 (0.6%)	1,105 (0.5%)	709 (0.5%)	625 (0.5%)	8,720 (0.7%)	P < 0.001 P_{trend} < 0.001
Antihypertensive drugs	27,283 (29.9%)	27,820 (29.9%)	94,025 (30.4%)	103,884 (29.0%)	59,994 (28.2%)	39,816 (26.8%)	34,127 (27.2%)	386,949 (28.9%)	P < 0.001 P_{trend} < 0.001
Antiplatelet drugs	6,572 (7.2%)	6,307 (6.8%)	20,393 (6.6%)	20,712 (5.8%)	11,981 (5.6%)	7,667 (5.2%)	6,692 (5.3%)	80,324 (6.0%)	P < 0.001 P_{trend} < 0.001

Note: Values are presented as numbers (percentage) or mean (standard deviation) or median (interquartile range) as appropriate. Corresponding values for HDL-C are: 0.91 mmol/L = 35.19 mg/dL; 1.01 mmol/L = 39.06 mg/dL, 1.27 mmol/L = 49.11 mg/dL, 1.53 mmol/L = 59.17 mg/dL, 1.78 mmol/L = 68.83 mg/dL, 2.04 mmol/L = 78.89 mg/dL. To convert mmol/L of LDL-C, total cholesterol and triglyceride to mg/dL, multiply by the factor of 38.67, 38.67, and 88.57, respectively. $^{\$}$P trend for Caucasian vs other ethnicity $^{\$\$}$P trend for non-smokers vs others (ex-smokers and current smokers) $^{\#}$P trend for type 1 diabetes vs no diabetes $^{\#\#}$P trend for type 2 diabetes vs no diabetes

Abbreviations: CRP; C-Reactive Protein, eGFR; estimated glomerular filtration rate, COPD; chronic obstructive pulmonary disease, HDL; high density lipoprotein, LDL; low density lipoprotein.

Table S 5-4 Gender-specific characteristics of participants

Baseline	HDL cholesterol at baseline (mmol/L)						
	< 0.91	**0.91-1.01**	**1.02-1.27**	**1.28-1.53**	**1.54-1.78**	**1.79-2.04**	**> 2.04**
Male	70,666	66,910	192,057	166,934	71,323	37,135	22,856
Female	20,730	26,112	117,412	190,894	141,049	111,401	102,797
Age (year)							
Male	51.7 (12.4)	52.7 (12.3)	53.9 (12.5)	55.0 (12.6)	56.4 (12.8)	57.2 (12.8)	58.3 (12.8)
Female	51.4 (13.7)	52.4 (13.7)	54.4 (13.7)	55.9 (13.7)	57.2 (13.6)	57.9 (13.6)	59.3 (13.3)
Caucasian ethnicity							
Male	85.2%	86.8%	88.5%	90.7%	91.8%	92.7%	92.5%
Female	85.1%	85.0%	86.1%	88.2%	90.3%	91.9%	93.1%
Health behaviors, physical and laboratory measurements at baseline							
Current smokers							
Male	25.6%	21.6%	18.4%	16.4%	15.6%	17.2%	22.5%
Female	27.8%	23.4%	18.9%	14.9%	12.4%	10.7%	10.4%
Body mass index (kg/m^2)							
Male	29.8 (5.3)	29.3 (5.1)	28.5 (4.8)	27.3 (4.4)	26.3 (4.1)	25.5 (3.9)	24.7 (3.8)
Female	31.8 (7.4)	31.3 (7.1)	30.2 (6.7)	28.4 (6.1)	26.9 (5.5)	25.8 (4.9)	24.5 (4.4)
Systolic blood pressure (mmHg)							
Male	136.4 (15.4)	137.1 (15.4)	137.4 (15.5)	137.6 (15.8)	137.9 (16.0)	138.1 (16.4)	139.6 (16.7)
Female	133.5 (17.0)	133.8 (17.1)	134.4 (17.3)	134.3 (17.7)	134.2 (17.9)	134.0 (18.0)	134.8 (18.2)
Diastolic blood pressure (mmHg)							
Male	82.7 (9.3)	82.9 (9.3)	82.9 (9.2)	82.5 (9.3)	82.1 (9.3)	81.9 (9.5)	82.2 (9.5)
Female	80.7 (9.3)	80.8 (9.2)	80.6 (9.1)	80.2 (9.2)	79.7 (9.2)	79.3 (9.2)	79.3 (9.2)
eGFR (mL/min/1.73m^2)							
Male	91.7 (25.2)	92.3 (22.0)	92.4 (20.8)	91.8 (19.4)	91.2 (19.1)	90.0 (19.6)	87.8 (22.9)
Female	74.6 (28.7)	74.3 (20.4)	75.0 (18.7)	75.0 (17.4)	74.9 (16.4)	74.4 (15.9)	73.8 (16.1)
CRP (mg/L), median (interquartile range)							

Baseline	HDL cholesterol at baseline (mmol/L)						
	< 0.91	**0.91-1.01**	**1.02-1.27**	**1.28-1.53**	**1.54-1.78**	**1.79-2.04**	**> 2.04**
Male	5.0 (2.0-9.0)	4.0 (2.0-8.0)	4.0 (2.0-8.0)	3.8 (2.0-7.8)	3.4 (1.5-7.5)	3.0 (1.4-8.0)	3.7 (1.5-8.0)
Female	5.5 (3.0-11.0)	5.0 (3.0-10.0)	5.0 (2.3-9.0)	4.0 (2.0-8.0)	4.0 (2.0-7.0)	3.0 (1.5-6.2)	3.0 (1.1-6.0)
LDL cholesterol (mmol/L)							
Male	3.1 (0.9)	3.3 (0.9)	3.4 (0.9)	3.4 (0.9)	3.3 (0.9)	3.2 (0.9)	2.9 (0.9)
Female	3.2 (1.0)	3.3 (1.0)	3.4 (1.0)	3.4 (0.9)	3.4 (0.9)	3.3 (0.9)	3.1 (0.9)
HDL cholesterol (mmol/L)							
Male	0.8 (0.1)	1.0 (0.1)	1.1 (0.1)	1.4 (0.1)	1.6 (0.1)	1.9 (0.1)	2.4 (0.4)
Female	0.8 (0.1)	1.0 (0.1)	1.2 (0.1)	1.4 (0.1)	1.6 (0.1)	1.9 (0.1)	2.4 (0.3)
Total cholesterol (mmol/L)							
Male	5.0 (1.1)	5.2 (1.0)	5.4 (1.0)	5.5 (1.0)	5.5 (1.0)	5.6 (1.0)	5.7 (1.0)
Female	5.0 (1.2)	5.2 (1.1)	5.4 (1.1)	5.5 (1.1)	5.6 (1.0)	5.7 (1.0)	5.9 (1.0)
Triglyceride (mmol/L), median (interquartile range)							
Male	2.2 (1.5-3.1)	1.9 (1.4-2.6)	1.6 (1.2-2.2)	1.3 (1.0-1.8)	1.1 (0.8-1.5)	1.0 (0.8-1.3)	0.9 (0.7-1.2)
Female	1.9 (1.3-2.8)	1.8 (1.3-2.5)	1.6 (1.1-2.2)	1.3 (1.0-1.8)	1.1 (0.9-1.5)	1.0 (0.8-1.3)	0.9 (0.7-1.1)
Health conditions							
Diabetes type 2							
Male	9.2%	7.7%	6.1%	3.9%	3.2%	2.6%	2.9%
Female	11.3%	9.8%	7.6%	4.4%	3.0%	2.0%	1.6%
Chronic kidney disease							
Male	3.2%	2.8%	2.7%	2.4%	2.4%	2.3%	2.5%
Female	5.0%	4.5%	4.4%	4.1%	4.0%	3.7%	3.5%
Diagnosed cancer							
Male	2.8%	2.9%	3.1%	3.4%	3.8%	4.2%	4.6%
Female	3.3%	3.1%	3.3%	3.3%	3.5%	3.6%	4.0%
Definite COPD							
Male	1.3%	1.2%	1.5%	1.8%	2.3%	2.7%	4.5%
Female	1.7%	1.5%	1.6%	1.6%	1.7%	1.8%	2.3%

Baseline	HDL cholesterol at baseline (mmol/L)						
	< 0.91	**0.91-1.01**	**1.02-1.27**	**1.28-1.53**	**1.54-1.78**	**1.79-2.04**	**> 2.04**
Medications							
Statins (at baseline)							
Male	16.5%	15.8%	15.2%	12.8%	12.2%	10.8%	10.9%
Female	15.0%	14.9%	14.5%	11.8%	10.7%	9.1%	9.0%
Statins (during follow-up)							
Male	38.1%	37.7%	37.0%	32.8%	30.8%	28.1%	27.1%
Female	35.2%	34.6%	34.0%	29.0%	26.7%	23.2%	22.4%
Other lipid-lowering drugs							
Male	1.4%	0.9%	0.7%	0.5%	0.5%	0.4%	0.5%
Female	1.2%	1.0%	0.7%	0.6%	0.5%	0.5%	0.5%
Antihypertensive drugs							
Male	29.1%	28.9%	28.9%	27.4%	26.9%	26.8%	28.9%
Female	32.6%	32.4%	32.7%	30.5%	28.9%	26.8%	26.8%
Antiplatelet drugs							
Male	7.3%	6.9%	6.6%	5.8%	5.8%	5.4%	6.2%
Female	6.9%	6.5%	6.6%	5.7%	5.6%	5.1%	5.1%

Note: Values are presented as percentage or mean (standard deviation) unless specified elsewhere.

Corresponding values for HDL-C are: 0.91 mmol/L = 35.19 mg/dL; 1.01 mmol/L = 39.06 mg/dL, 1.27 mmol/L = 49.11 mg/dL, 1.53 mmol/L = 59.17 mg/dL, 1.78 mmol/L = 68.83 mg/dL, 2.04 mmol/L = 78.89 mg/dL. To convert mmol/L of LDL-C, total cholesterol and triglyceride to mg/dL, multiply by the factor of 38.67, 38.67, and 88.57, respectively.

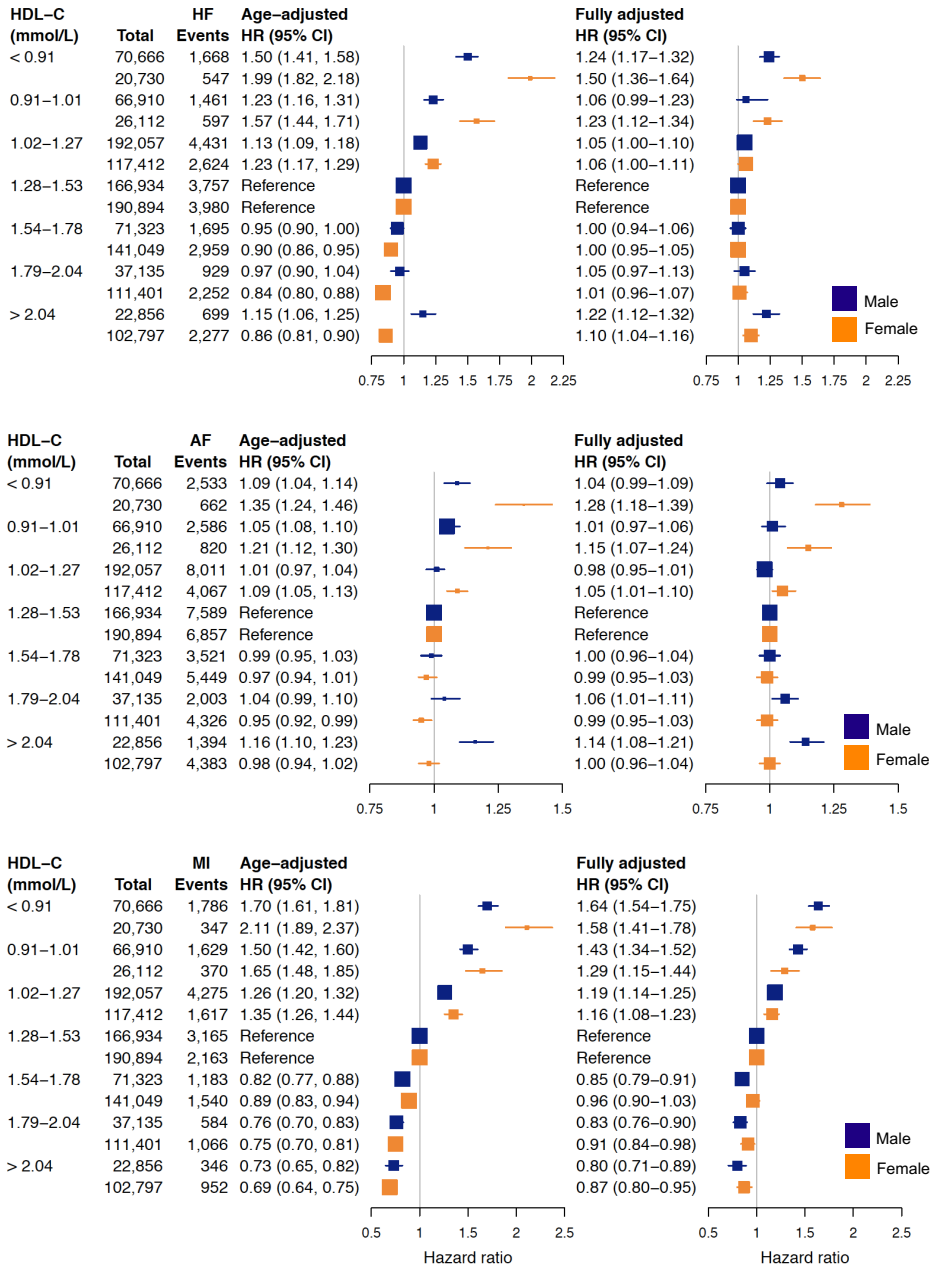


Figure S 5-4 Gender-specific associations by different levels of HDL-C

Note: HF (total 3,570,330 person-years [male] and 4,036,869 person-years [female]), AF (total 3,520,020 person-years [male] and 3,993,738 person-years [female]), and MI (total 3,561,222 person-years [male] and 4,048,562 person-years [female]) among 1,338,276 individuals without diagnosed CVD at baseline after the median follow-up of 5 years (interquartile range: 2-9 years).

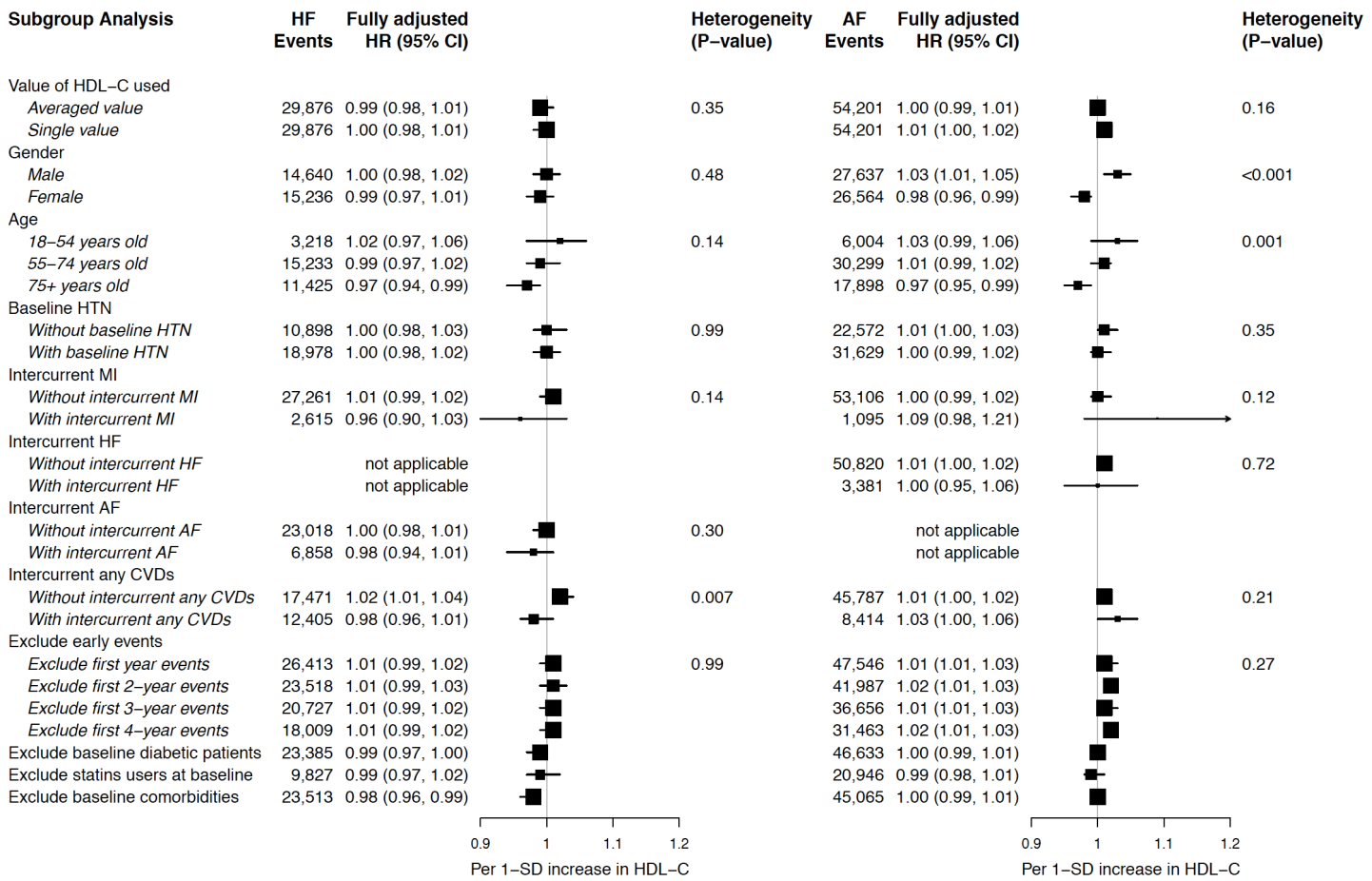


Figure S 5-5 Subgroup analyses of the associations between HDL-C and incident HF and AF (no gender-specific)

Note: All models are stratified for primary care practice and adjusted for age, body mass index, smoking, systolic blood pressure, diabetes, socioeconomic status, low-density lipoprotein cholesterol, triglycerides, use of blood pressure lowering drugs and use of statins, and TG was logarithmic transformation. Baseline comorbidities include having diagnosed with cancer, kidney disease, and COPD at baseline LDL-C measurement. Any CVDs included acute MI, coronary revascularisation, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, peripheral arterial disease, ventricular fibrillation, heart failure (excluded in HF outcome), and atrial fibrillation (excluded in AF outcome).

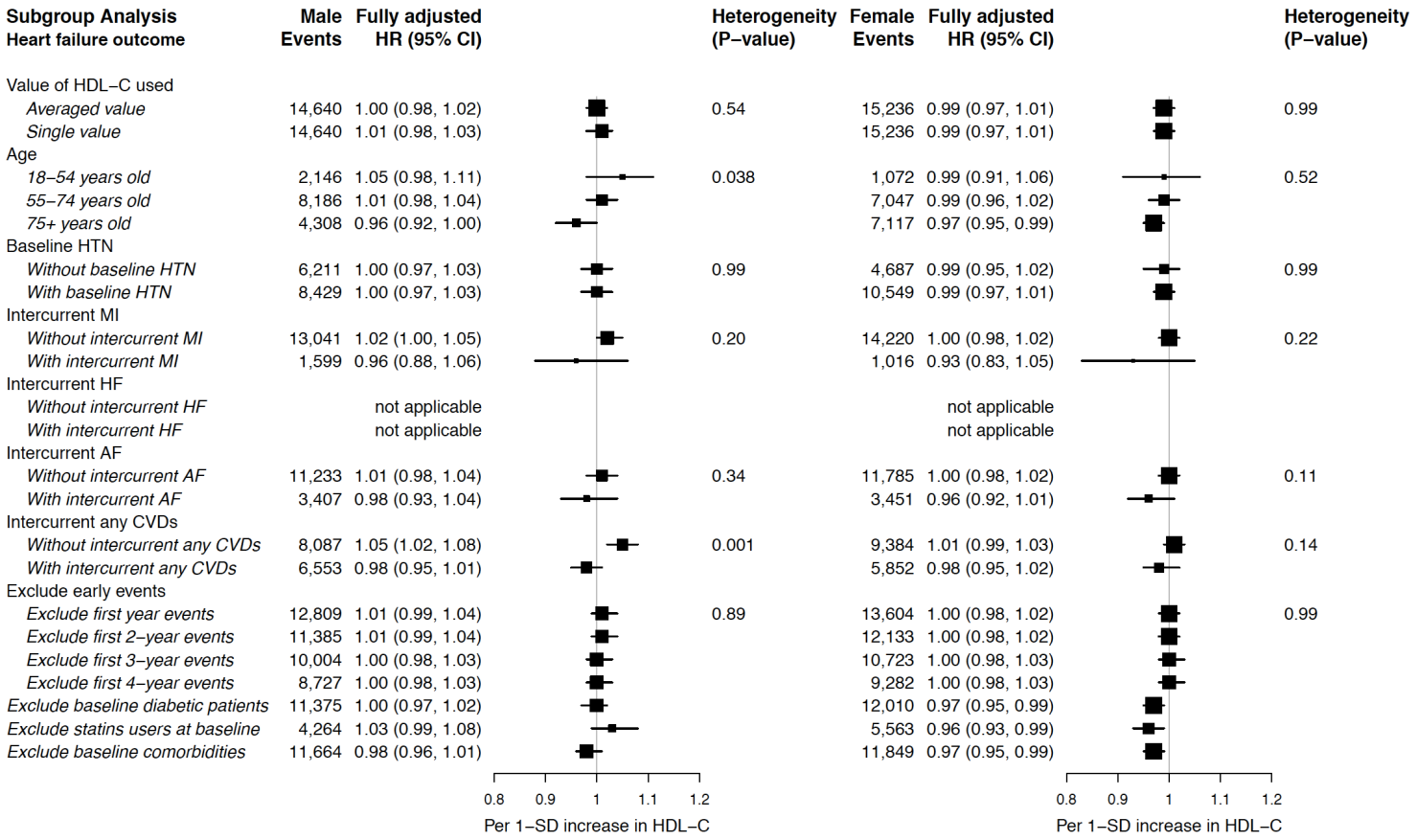


Figure S 5-6 Subgroup analyses of the gender-specific associations between HDL-C and incident HF

Note: All models are stratified for primary care practice and adjusted for age, body mass index, smoking, systolic blood pressure, diabetes, socioeconomic status, low-density lipoprotein cholesterol, triglycerides, use of blood pressure lowering drugs and use of statins, and TG was logarithmic transformation. Baseline comorbidities include having diagnosed with cancer, kidney disease, and COPD at baseline LDL-C measurement. Any CVDs included acute MI, coronary revascularisation, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, peripheral arterial disease, ventricular fibrillation, heart failure (excluded in HF outcome), and atrial fibrillation (excluded in AF outcome).

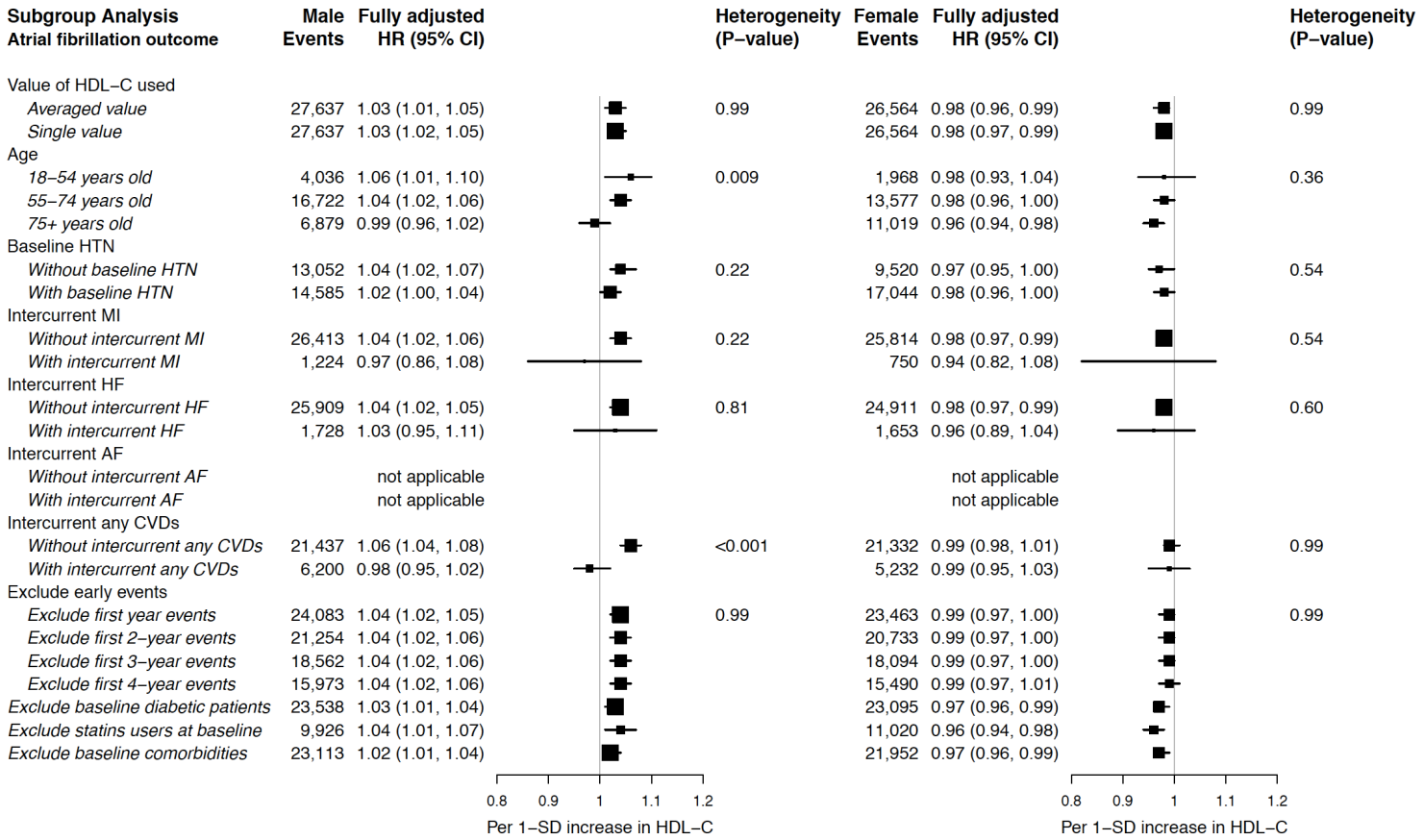


Figure S 5-7 Subgroup analyses of the gender-specific associations between HDL-C and incident AF

Note: All models are stratified for primary care practice and adjusted for age, body mass index, smoking, systolic blood pressure, diabetes, socioeconomic status, low-density lipoprotein cholesterol, triglycerides, use of blood pressure lowering drugs and use of statins, and TG was logarithmic transformation. Baseline

comorbidities include having diagnosed with cancer, kidney disease, and COPD at baseline LDL-C measurement. Any CVDs included acute MI, coronary revascularisation, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, peripheral arterial disease, ventricular fibrillation, heart failure (excluded in HF outcome), and atrial fibrillation (excluded in AF outcome).

Table S 5-5 Subgroup analysis of categorical HDL-C levels and incident HF in selected characteristics among males

HDL-C levels (mmol/L)	Time to diagnosed HF				Intercurrent any CVDs			
	Only include first-4-year cases		Only include after-4-year cases		With		Without	
	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)
<0.91	947 (39,150)	**1.17 (1.08-1.27)**	721 (31,516)	**1.21 (1.10-1.34)**	746	1.08 (0.97-1.20)	922 (69,920)	**1.16 (1.07-1.26)**
0.91-1.01	891 (38,394)	1.05 (0.97-1.14)	570 (28,516)	1.01 (0.92-1.12)	681	1.09 (0.99-1.21)	780 (66,229)	0.98 (0.90-1.06)
1.02-1.27	2,639 (112,756)	1.01 (0.96-1.07)	1,792 (79,301)	1.07 (1.00-1.15)	2,054	1.05 (0.97-1.13)	2,377 (190,003)	0.99 (0.94-1.06)
1.28-1.53	2,304 (99,675)	1.00 (Reference)	1,453 (67,259)	1.00 (Reference)	1,656	1.00 (Reference)	2,101 (165,278)	1.00 (Reference)
1.54-1.78	1,016 (42,453)	1.00 (0.93-1.08)	679 (28,870)	1.00 (0.91-1.10)	743	1.05 (0.96-1.16)	952 (70,580)	1.00 (0.93-1.08)
1.79-2.04	544 (21,835)	1.04 (0.95-1.15)	385 (15,300)	1.00 (0.89-1.13)	396	1.06 (0.93-1.20)	533 (36,739)	1.07 (0.97-1.18)
>2.04	386 (12,951)	**1.18 (1.05-1.32)**	313 (9,905)	1.19 (1.04-1.35)	277	0.95 (0.82-1.09)	422 (22,579)	**1.31 (1.17-1.45)**
Total	8,727 (367,214)		5,913 (260,667)		6,553 (6,553)		8,087 (621,328)	

Note: Embolden figures represent statistically significant P-value (Bonferroni adjusted P-value < 0.05). All models were adjusted for age, age squared, index of multiple deprivation, BMI, smoking status, systolic and diastolic blood pressure, LDL-C, ln TG, ln C-reactive protein, diabetes, using antihypertensive medications, and using statins.

Table S 5-6 Subgroup analysis of categorical HDL-C levels and incident HF in selected characteristics among females

HDL-C levels (mmol/L)	Time to diagnosed HF				Age at baseline			
	Only include first-4-year cases		Only include after-4-year cases		Less than 75 years old		75 years old or more	
	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)
<0.91	240 (8,975)	**1.61 (1.39-1.86)**	307 (11,755)	**1.35 (1.19-1.53)**	406 (19,399)	**1.47 (1.31-1.64)**	141 (1,331)	**1.31 (1.10-1.57)**
0.91-1.01	257 (11,390)	1.19 (1.03-1.36)	340 (14,722)	1.11 (0.99-1.25)	394 (24,319)	1.13 (1.01-1.26)	203 (1,793)	**1.33 (1.14-1.54)**
1.02-1.27	1,037 (48,733)	1.03 (0.95-1.12)	1,587 (68,679)	1.00 (0.94-1.07)	1,675 (107,827)	1.05 (0.99-1.12)	949 (9,585)	1.03 (0.95-1.11)
1.28-1.53	1,476 (77,878)	1.00 (Reference)	2,504 (113,016)	1.00 (Reference)	2,212 (172,647)	1.00 (Reference)	1,768 (18,247)	1.00 (Reference)
1.54-1.78	1,124 (58,053)	1.00 (0.92-1.08)	1,835 (82,996)	0.99 (0.93-1.06)	1,455 (125,398)	1.00 (0.93-1.07)	1,504 (15,651)	0.99 (0.93-1.07)
1.79-2.04	891 (46,110)	1.07 (0.98-1.16)	1,361 (65,291)	1.01 (0.94-1.08)	997 (97,962)	1.00 (0.93-1.08)	1,255 (13,439)	1.01 (0.93-1.09)
>2.04	929 (43,854)	1.09 (1.00-1.20)	1,348 (58,943)	1.10 (1.02-1.18)	980 (88,608)	**1.17 (1.08-1.27)**	1,297 (14,189)	1.01 (0.93-1.09)
Total	5,954 (294,993)		9,282 (415,402)		8,119 (636,160)		7,117 (74,235)	
HDL-C levels (mmol/L)	Diabetes mellitus (DM) at baseline				Co-morbidity at baseline			
	DM patients		Non-DM patients		Comorbid patients		Non-comorbid patients	
	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)
<0.91	227 (3,958)	**1.24 (1.06-1.46)**	320 (16,772)	**1.56 (1.38-1.75)**	128 (1,935)	1.27 (1.04-1.54)	419 (18,795)	**1.53 (1.37-1.70)**
0.91-1.01	225 (4,314)	1.03 (0.88-1.20)	372 (21,798)	**1.28 (1.15-1.43)**	141 (2,263)	1.28 (1.06-1.54)	456 (23,849)	**1.20 (1.09-1.33)**
1.02-1.27	863 (14,825)	1.00 (0.90-1.10)	1,761 (102,587)	1.07 (1.01-1.13)	592 (10,257)	1.03 (0.92-1.15)	2,032 (107,155)	1.06 (1.00-1.12)
1.28-1.53	869 (14,199)	1.00 (Reference)	3,111 (176,695)	1.00 (Reference)	845 (16,271)	1.00 (Reference)	3,135 (174,623)	1.00 (Reference)
1.54-1.78	507 (7,482)	1.10 (0.98-1.23)	2,452 (133,567)	0.97 (0.92-1.02)	627 (12,313)	0.99 (0.89-1.11)	2,332 (128,736)	1.00 (0.94-1.05)
1.79-2.04	301 (4,263)	1.18 (1.03-1.36)	1,951 (107,138)	0.98 (0.93-1.04)	511 (9,555)	1.09 (0.97-1.22)	1,741 (101,846)	0.99 (0.93-1.05)
>2.04	234 (3,415)	**1.26 (1.07-1.47)**	2,043 (99,382)	1.05 (0.98-1.11)	543 (9,561)	**1.23 (1.10-1.39)**	1,734 (93,236)	1.05 (0.98-1.12)
Total	3,226 (52,456)		12,010 (657,939)		3,387 (62,155)		11,849 (648,240)	
HDL-C levels (mmol/L)	Use of statins at baseline							
	Statins users		Non-statins users					
	Events (Total)	HR (95%CI)	Events (Total)	HR (95%CI)				
<0.91	368 (7,452)	**1.32 (1.18-1.49)**	179 (13,278)	**1.81 (1.53-2.13)**				
0.91-1.01	428 (9,230)	**1.17 (1.06-1.30)**	169 (16,882)	**1.29 (1.10-1.53)**				
1.02-1.27	1,843 (40,648)	1.03 (0.97-1.10)	781 (76,764)	1.10 (1.00-1.20)				
1.28-1.53	2,610 (56,396)	1.00 (Reference)	1,370 (134,498)	1.00 (Reference)				
1.54-1.78	1,850 (38,403)	1.01 (0.95-1.08)	1,109 (102,646)	0.97 (0.89-1.05)				
1.79-2.04	1,304 (26,312)	1.04 (0.97-1.11)	948 (85,089)	1.00 (0.91-1.08)				
>2.04	1,270 (23,576)	**1.14 (1.06-1.23)**	1,007 (79,221)	1.04 (0.95-1.13)				
Total	9,673 (202,017)		5,563 (508,378)					

Note: Embolden figures represent statistically significant P-value (Bonferroni adjusted P-value < 0.05). All models were adjusted for age, age squared, index of multiple deprivation, BMI, smoking status, systolic and diastolic blood pressure, LDL-C, ln TG, ln C-reactive protein, diabetes, using antihypertensive medications, and using statins

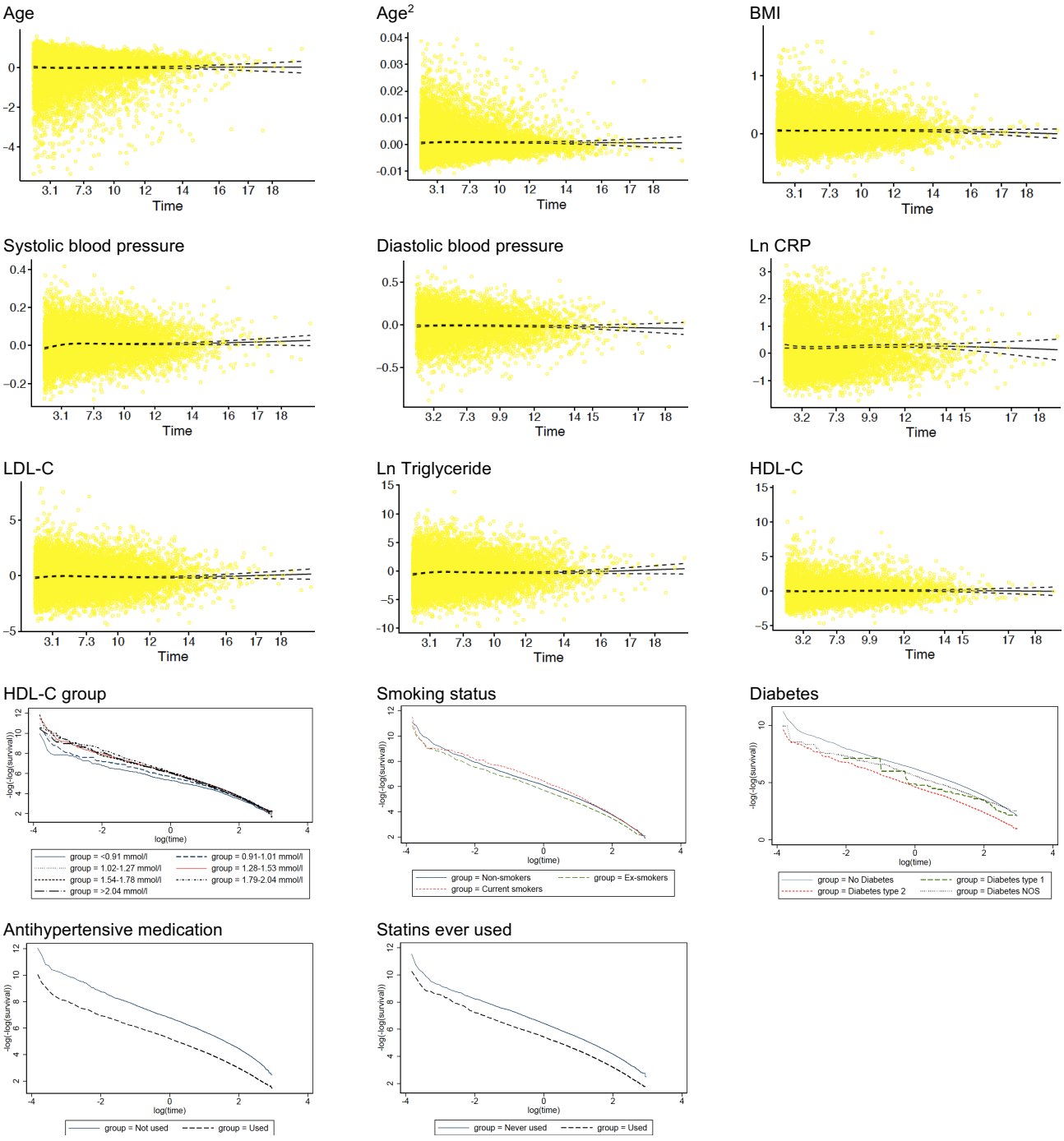


Figure S 5-8 Schoenfeld residual and proportional hazard plots on HF outcome in males

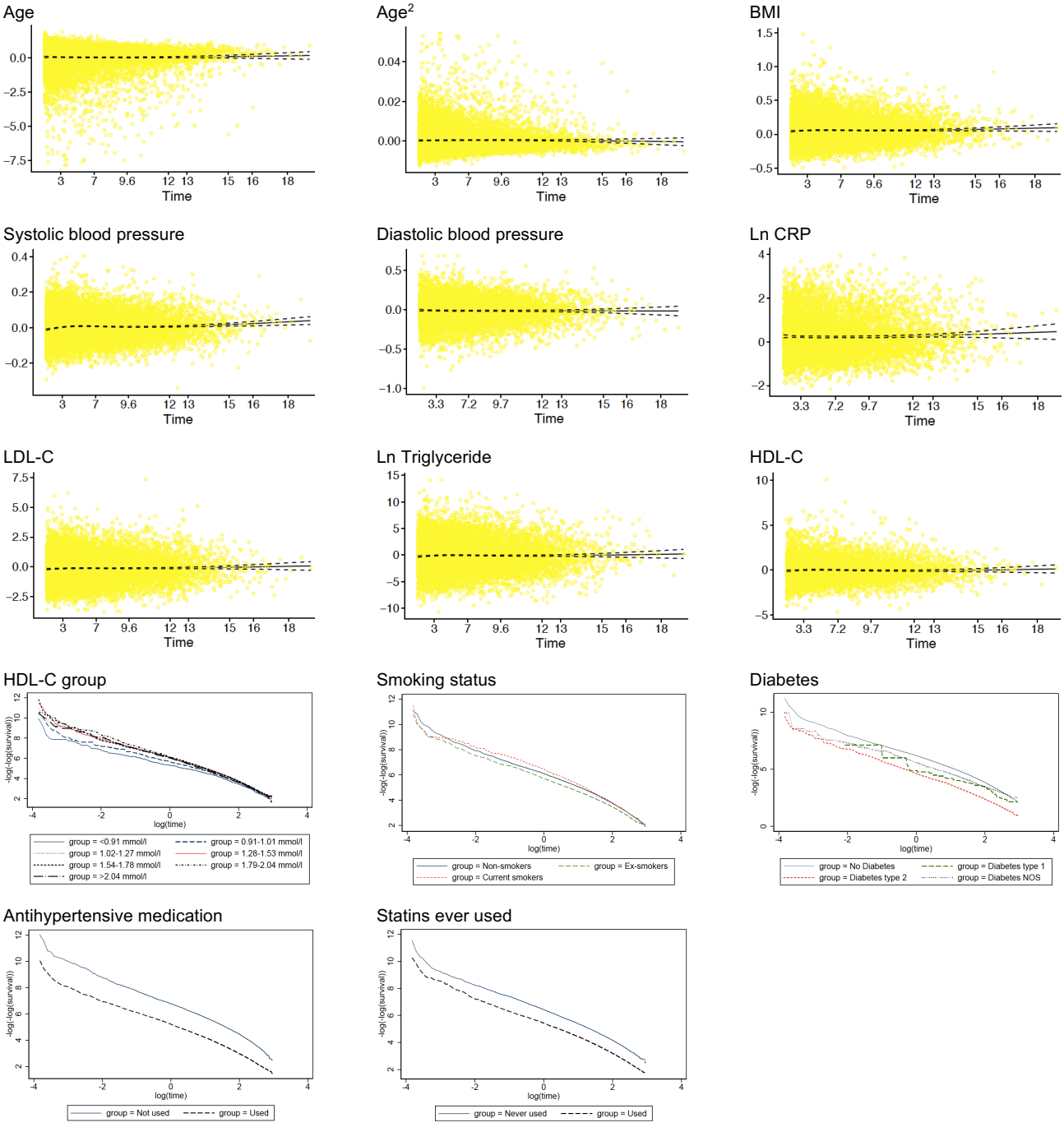


Figure S 5-9 Schoenfeld residual and proportional hazard plots on HF outcome in females

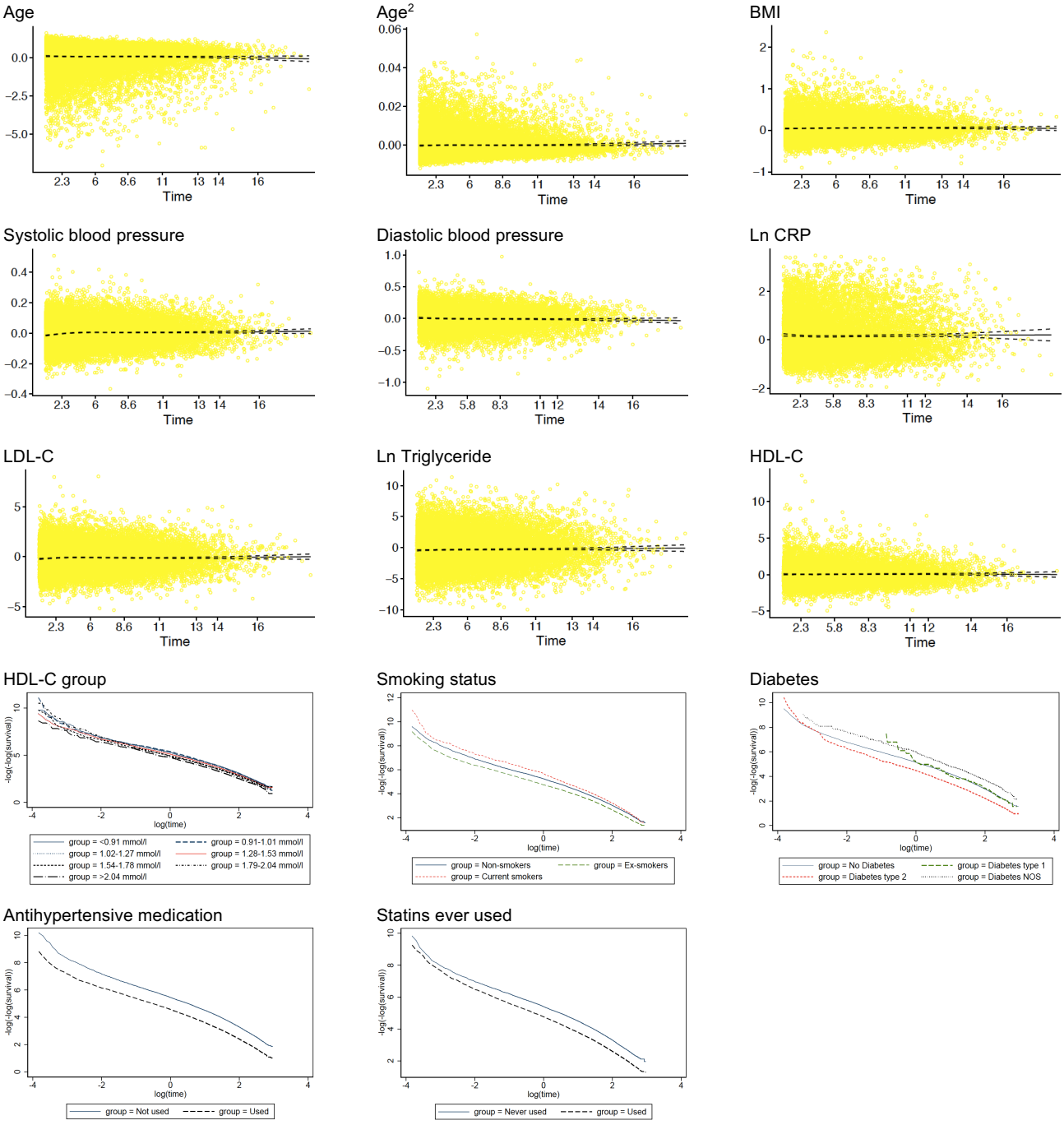


Figure S 5-10 Schoenfeld residual and proportional hazard plots on AF outcome in males

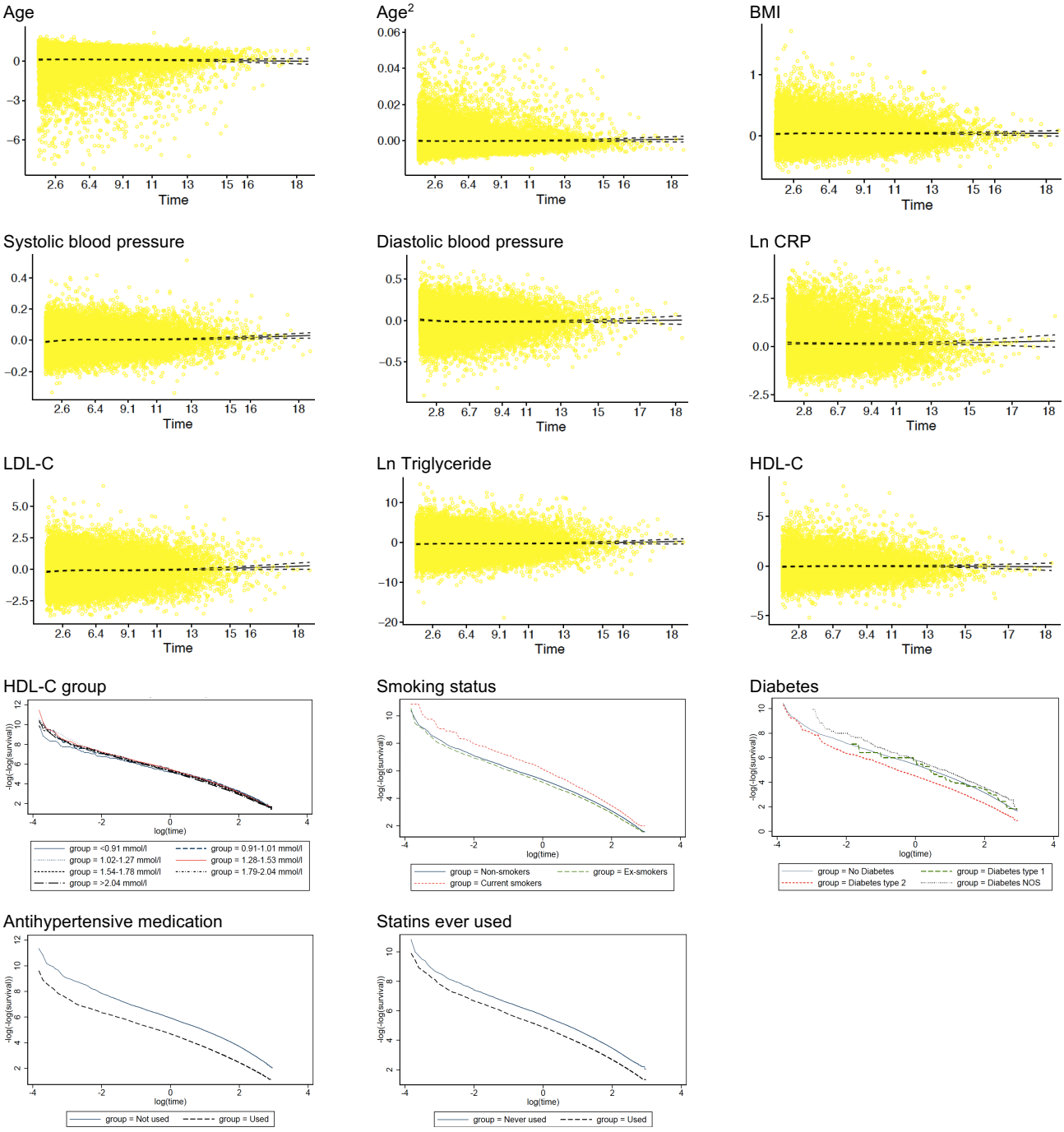
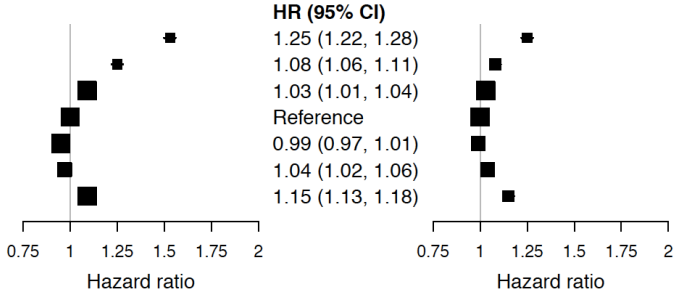


Figure S 5-11 Schoenfeld residual and proportional hazard plots on AF outcome in females

No gender-specific hazard ratio (HR)

HDL–C (mmol/L)	Total	Events	Age, sex–adjusted HR (95% CI)	Fully adjusted HR (95% CI)
< 0.91	91,396	10,805	1.53 (1.50, 1.56)	1.25 (1.22, 1.28)
0.91–1.01	93,022	9,999	1.25 (1.22, 1.28)	1.08 (1.06, 1.11)
1.02–1.27	309,469	33,896	1.09 (1.08, 1.11)	1.03 (1.01, 1.04)
1.28–1.53	357,828	39,880	Reference	Reference
1.54–1.78	212,372	24,948	0.95 (0.94, 0.97)	0.99 (0.97, 1.01)
1.79–2.04	148,536	18,336	0.97 (0.95, 0.99)	1.04 (1.02, 1.06)
> 2.04	125,653	18,284	1.09 (1.07, 1.10)	1.15 (1.13, 1.18)



Gender-specific hazard ratio (HR)

HDL–C (mmol/L)	Total	Events	Age–adjusted HR (95% CI)	Fully adjusted HR (95% CI)
< 0.91	70,666	1,668	1.45 (1.41, 1.49)	1.21 (1.18, 1.25)
	20,730	547	1.83 (1.76, 1.91)	1.44 (1.38, 1.50)
0.91–1.01	66,910	1,461	1.17 (1.14, 1.21)	1.05 (1.02, 1.08)
	26,112	597	1.47 (1.41, 1.52)	1.22 (1.17, 1.27)
1.02–1.27	192,057	4,431	1.05 (1.03, 1.07)	1.00 (0.98, 1.03)
	117,412	2,624	1.18 (1.15, 1.20)	1.08 (1.06, 1.10)
1.28–1.53	166,934	3,757	Reference	Reference
	190,894	3,980	Reference	Reference
1.54–1.78	71,323	1,695	1.02 (0.99, 1.04)	1.03 (1.01, 1.06)
	141,049	2,959	0.91 (0.89, 0.93)	0.97 (0.94, 0.99)
1.79–2.04	37,135	929	1.11 (1.08, 1.15)	1.12 (1.08, 1.15)
	111,401	2,252	0.92 (0.90, 0.94)	1.00 (0.98, 1.03)
> 2.04	22,856	699	1.49 (1.44, 1.54)	1.39 (1.34, 1.43)
	102,797	2,277	0.98 (0.96, 1.00)	1.09 (1.06, 1.11)

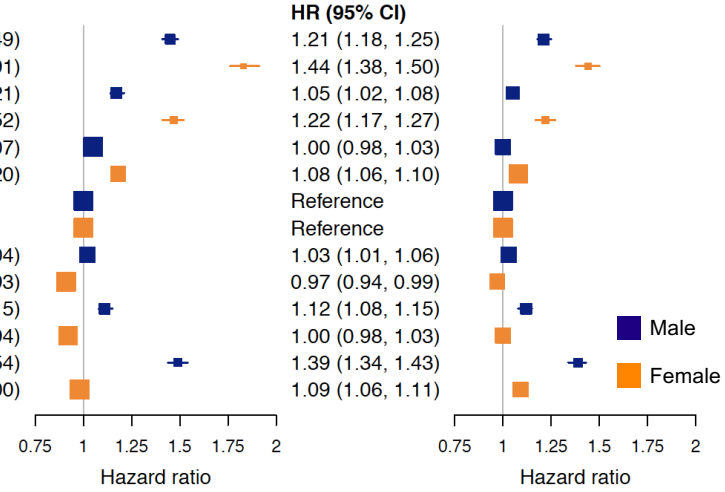


Figure S 5-12 The association between HDL-C levels and all-cause mortality in CALIBER (n=1,338,276)

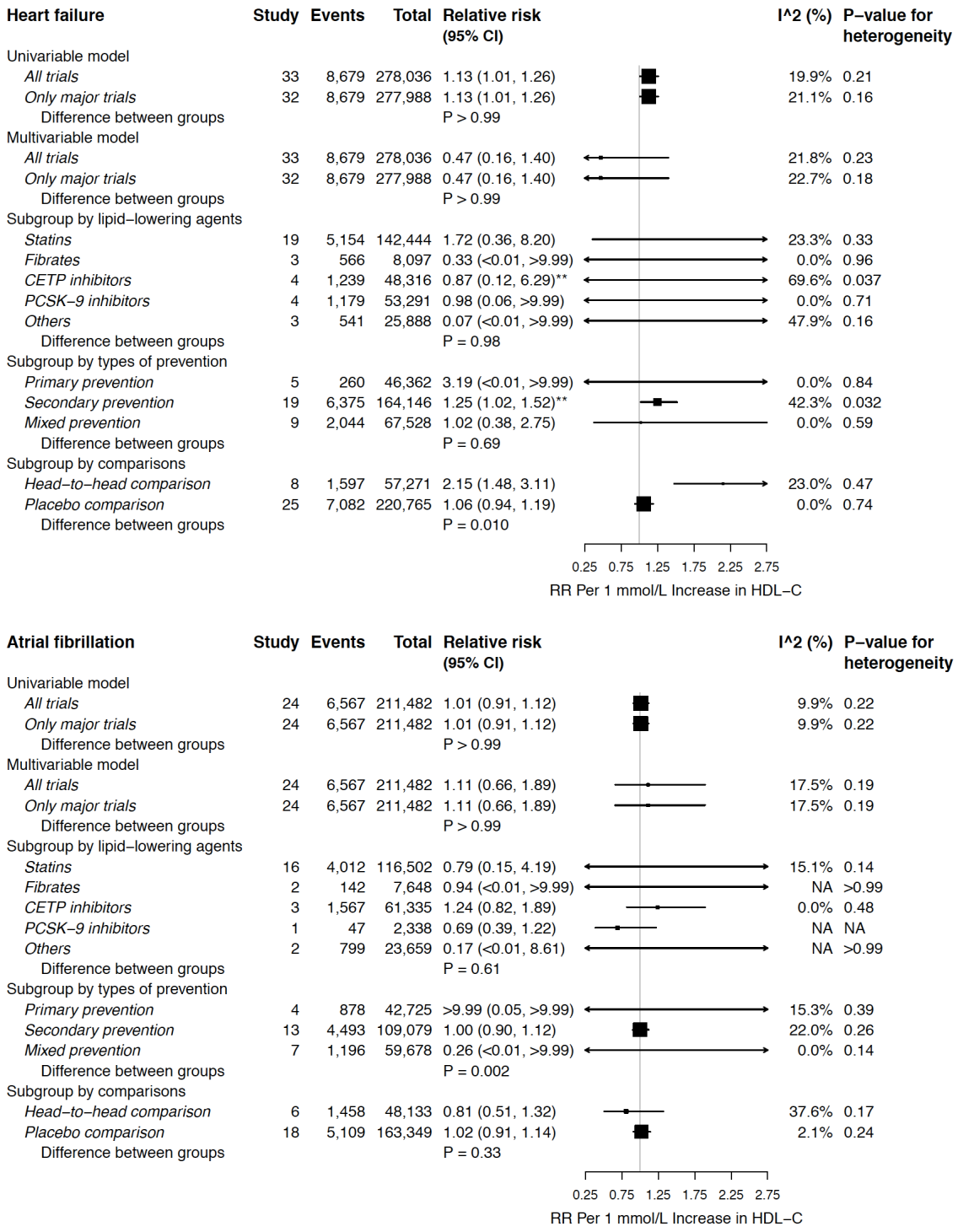


Figure S 5-13 Sensitivity and subgroup analysis of meta-regression of the change in HDL-C levels and the risk of HF and AF

Note: **Random effect meta-regression, Others included Niacin trials (n=2), Omega-3 fatty acid trials (n=2), Cholestyramine trial (n=1), Bompedoic acid trial (n=1), and Ezetimibe trials (n=1)

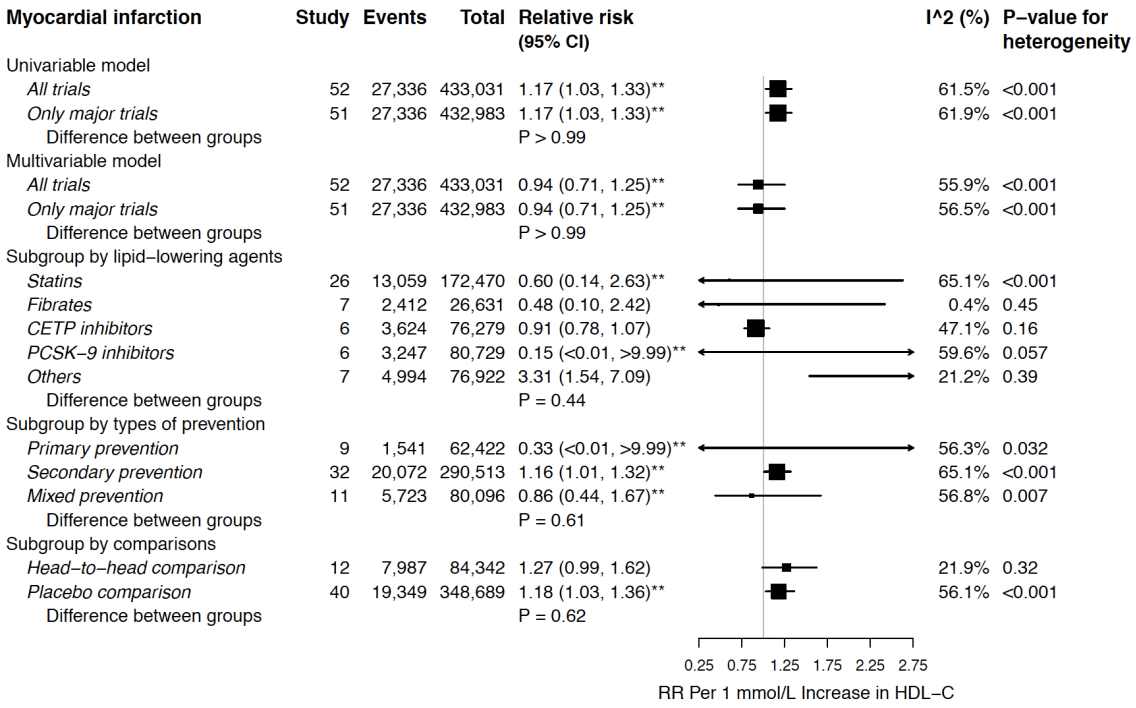


Figure S 5-14 Subgroup analysis of meta-regression between change in HDL-C and the risk of MI

Note: **Random effect meta-regression. Others included Niacin trials (n=2), Omega-3 fatty acid trials (n=2), Cholestyramine trial (n=1), Bompedoic acid trial (n=1), and Ezetimibe trials (n=1)

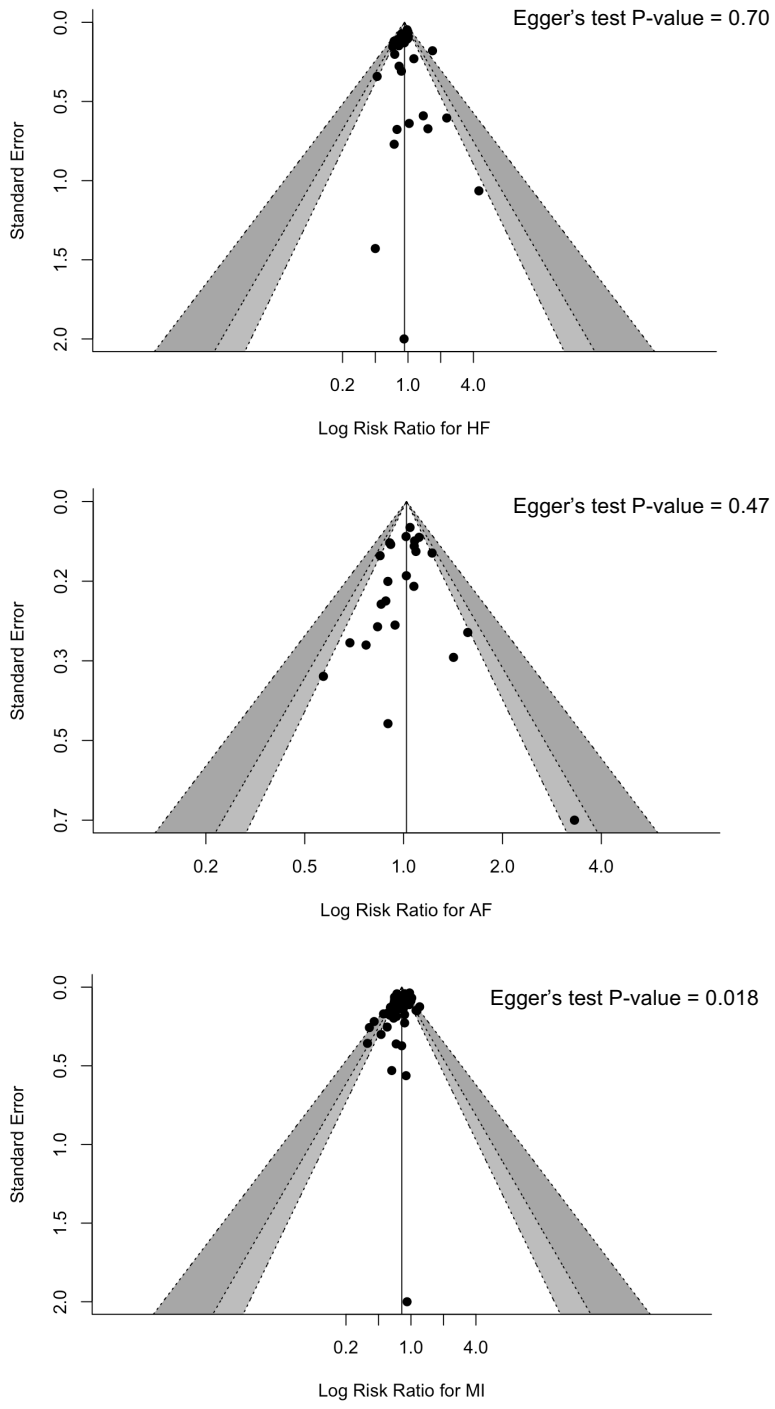


Figure S 5-15 Funnel plot of RCTs on HF (top), AF (middle), and MI (bottom) outcomes

Note: grey shade and dark grey shade represent areas of 95 and 99 % confidence interval, respectively.

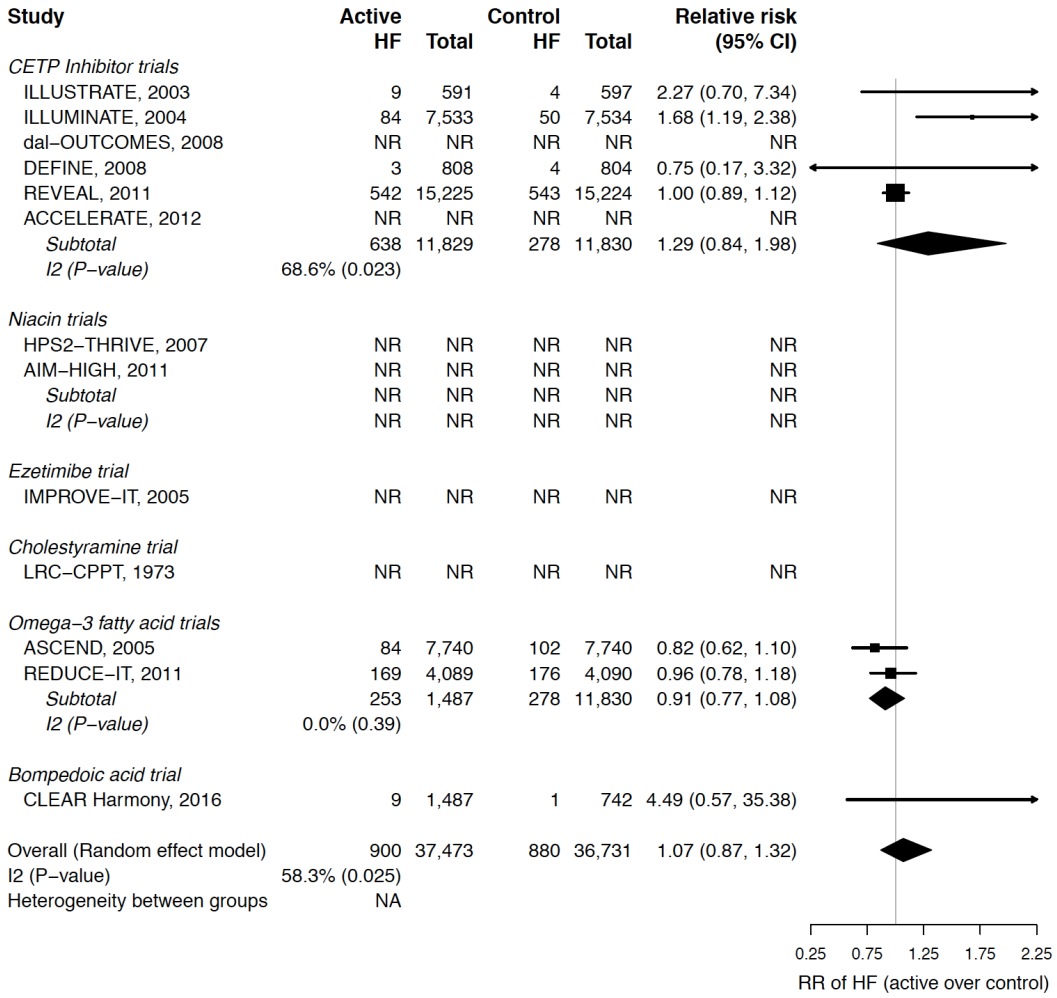


Figure S 5-16 Meta-analysis of trials on HDL-C raising drugs (7 trials) on the risk of HF

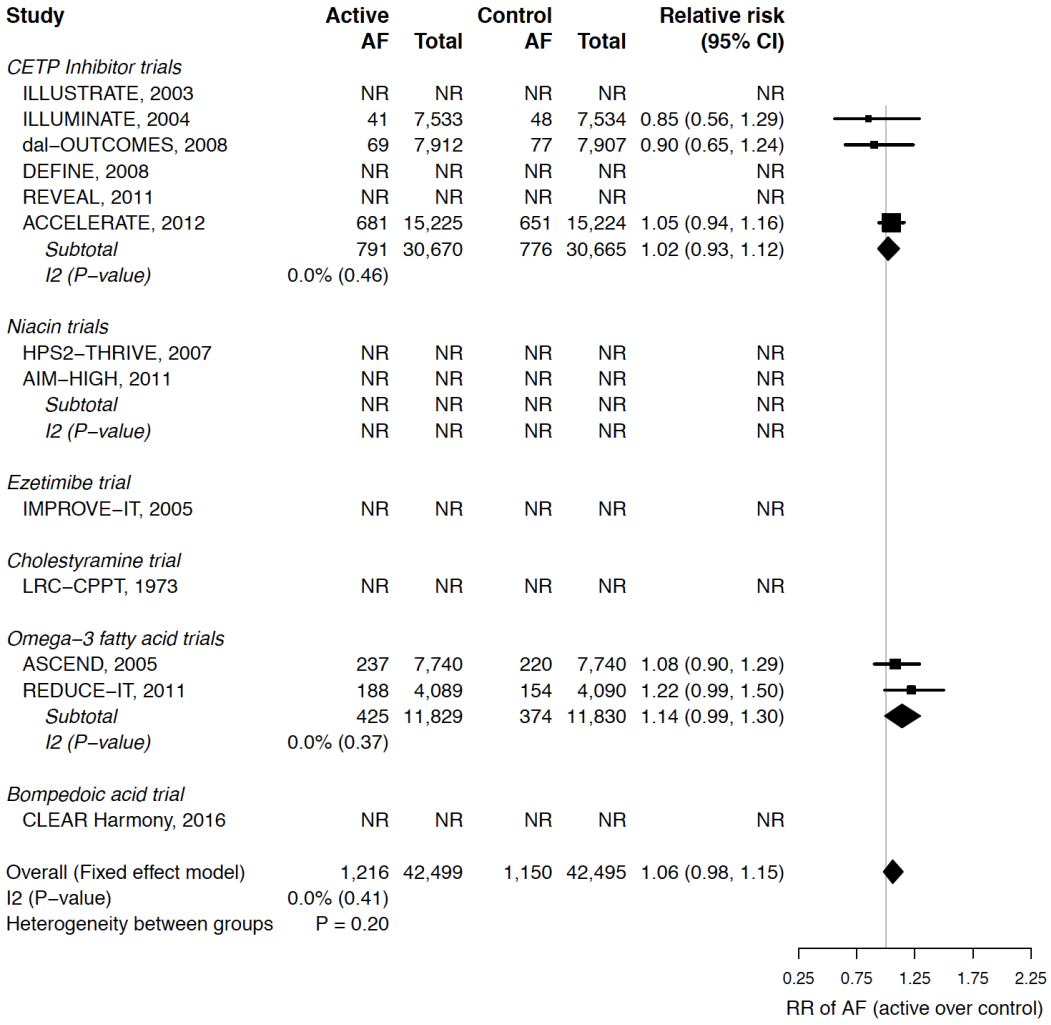


Figure S 5-17 Meta-analysis of trials on HDL-C raising drugs (5 trials) on the risk of AF

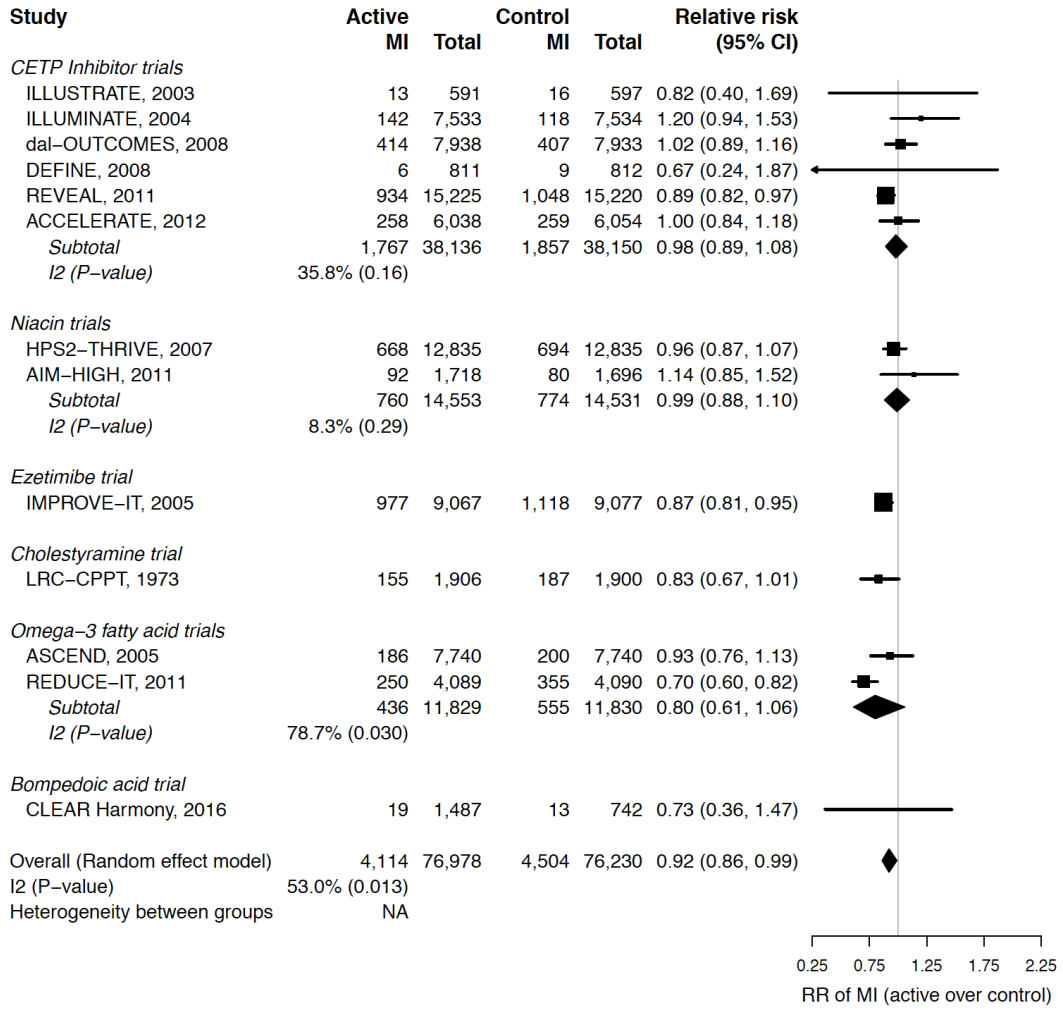
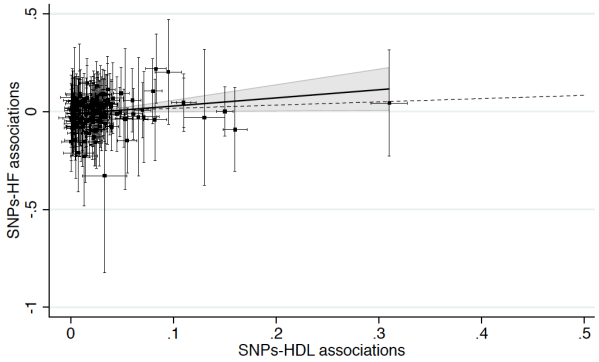
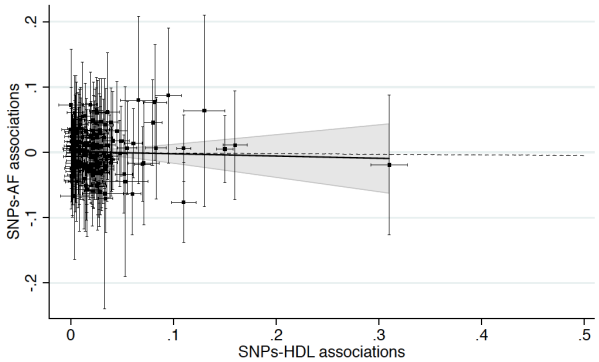


Figure S 5-18 Meta-analysis of trials on HDL-C raising drugs (13 trials) on the risk of MI

Heart failure (156 SNPs)



Atrial fibrillation (156 SNPs)



Myocardial infarction (156 SNPs)

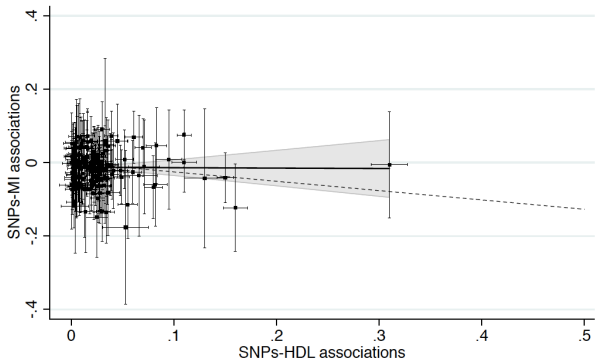


Figure S 5-19 Scatter plots of genotype-HDL-C associations versus genotype-outcome associations

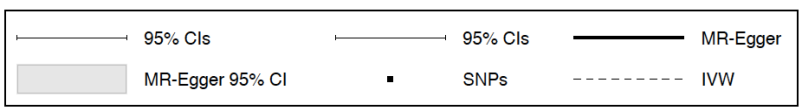


Table S 5-7 Testing for horizontal pleiotropy

Outcomes	Egger-intercept coefficient (p-values)	Q statistics, degree of freedom (p-values)
Heart failure		
UK Biobank (156 SNPs)	-1.31×10^{-2} (0.09)	124.77, 155 (0.96)
HERMES (167 SNPs)	-3.04×10^{-3} (0.14)	Not available
Atrial fibrillation (156 SNPs)	1.30×10^{-3} (0.69)	176.10, 155 (0.11)
Myocardial infarction (156 SNPs)	**-1.29×10^{-2} (0.010)**	**222.18, 155 (<0.001)**
Coronary artery disease (157 SNPs)	**-1.09×10^{-2} (0.008)**	**356.18, 156 (<0.001)**

Note: Significant coefficient and p-value (embolden figures) suggest potential horizontal pleiotropy. Significant Q statistics p-values might suggest either directional or balanced pleiotropy.

Table S 5-8 Power calculation of MR at two-sided alpha of 0.05

Parameter	HF (UKB)	AF (UKB)	MI (UKB)	CAD (CARDIoGRAMplusC4D)
Number of cases*	512	3,349	1,761	63,746
Number of controls*	110,884	108,047	109,635	130,681
Odds ratio to be detected	1.24	0.92	1.24	0.92
R^2 (SNPs HDL-C)	0.065	0.065	0.065	0.092
Calculated power	23.52%	23.95%	62.67%	>99%

Note: *Number of cases and controls derived from SNPs-outcome consortium (N of UKB = 111,396 and N of CARDIoGRAMplusC4D = 194,427). Power was calculated based on the method explained in Hermani *et al.*[223]

Table S 5-9 Correlation between instrumental variable effect and exposure effect

Outcomes	Correlation coefficients (p-values)
Heart failure (UK Biobank: 156 SNPs)	-2.15×10^{-2} (0.79)
Atrial fibrillation (156 SNPs)	9.50×10^{-3} (0.90)
Myocardial infarction (156 SNPs)	-2.36×10^{-2} (0.77)
Coronary artery disease (157 SNPs)	0.10 (0.18)

Note: Highly correlated coefficients and significant p-values might suggest the invalidity of InSIDE (Instrumental Strength Independent of Direct Effect) assumption.

CHAPTER 6 TRIGLYCERIDES AS A CAUSE OF HEART FAILURE AND ATRIAL FIBRILLATION: COHORT, TRIAL, AND GENETIC EVIDENCE

6.1 Key messages

What is already known?

- Observational evidence suggested that higher TG levels might be associated with increased risk of atherosclerotic cardiovascular diseases (ASCVDs), but their effects on heart failure (HF) and atrial fibrillation (AF) have not been well established due to ambivalent findings.
- Previous meta-analyses of randomised controlled trials have shown moderate cardiovascular benefits of fibrates in both primary and secondary prevention.
- Genetic evidence from Mendelian randomisation (MR) has shown that TG might be causally relevant to coronary artery disease (CAD), but the evidence on HF and AF is still lacking.

What does this study add?

- For HF and TG, we observed an inverse association from a cohort study; however, meta-regression did not support the observational findings. MR results from two sources (UK Biobank and HERMES) were conflicted.
- We found a strong inverse association between TG and AF from a cohort study, which was consistent with genetic findings; however, evidence from meta-regression was not concordant.
- Our study suggested that TG and AF might be a causally inverse association. Future work needs to confirm the protective role of an increased TG level on AF.
- We found that the observed positive (direct) association between TG and incident MI or CAD might be bias due to confounders, especially to other lipid fractions and chronic conditions.

6.2 Abstract

Objective: To evaluate the causal relevance of triglyceride (TG) levels for the risk of heart failure (HF) and atrial fibrillation (AF).

Design, setting, and participants: We compared the evidence from three study designs: 1) population-based cohort study of people free of CVDs at baseline using the linked electronic health records (CALIBER platform); 2) meta-regression of randomised controlled trials of lipid-lowering agents; and 3) Mendelian randomisation (MR) using summary-data from GLGC, UK Biobank (UKB), HERMES, and the CARDIoGRAMplusC4D consortium

Main exposure: TG

Main outcome measures: HF and AF

Results: During the median follow-up of 5.3 years (IQR 0.1-19.5 years) between 1st Jan 1997 and 30th Jun 2016, we identified new 30,007 HF and 53,746 AF cases amongst 1,262,280 participants from CALIBER who were free from CVDs at study entry. For HF and TG, we observed an inverse association from the cohort study, corresponding to HR per log mmol/L decrease in TG levels of 1.11 (95%CI 1.08 to 1.15). However, the results from the meta-regression of 24 trials (5,707 HF event) were not consistent with observational findings (RR per 1 mmol/L decrease in TG was 0.77 [95%CI 0.55 to 1.09]). Furthermore, genetic evidence showed conflicting results between UKB (OR per 1-SD [1 mmol/L or 88.57 mg/dL] genetically determined TG reduction was 0.94 [0.63 to 1.40]) and HERMES (adjusted OR: 0.94 [0.91, 0.98]). For AF outcomes, we found a positive association of TG from the cohort study (HR 1.30 [95%CI 1.28 to 1.33]), which was consistent with genetic findings of first event cases (OR 1.25 [95%CI 1.03 to 1.52]) whereas those of any event cases were borderline significant (OR 1.18 [1.00 to 1.38]). However, findings from the meta-regression of 18 trials (5,140 AF cases) did not support observed and genetic evidence (RR 0.96 [95%CI 0.64 to 1.46]).

Conclusion: While the association between TG and HF remains unclear, our study suggested that the decrease in TG levels might be causally associated with an increased risk of AF, and this should be further investigated.

6.3 Introduction

6.3.1 Clinical Importance

Triglyceride (TG) has been investigated for a causal association with myocardial infarction (MI) for a while. Although observational studies showed null association between TG and the risk of incident coronary heart disease (CHD),[95] most genetic evidence suggested the potential causal relevance (see Chapter 2). However, the European Society of Cardiology (ESC) recently recommended that the association observed from genetic studies might be confounded by the effect of other lipid particles that are closely related to TG, especially Apolipoprotein B (ApoB) particles.[36] In addition, to date, TG has no role in the risk prediction of cardiovascular disease (CVD) and MI (see Chapter 7). This raises concerns whether measuring TG is clinically valuable for the sake of cardiovascular protection.

In May 2019, the European Union (EU) approved a new drug - volanesorsen (Waylivra®) - which inhibits ApoCIII mRNA leading to a dramatic reduction of fasting TG levels by 70-75%, for the treatment of a rare metabolic disorder called Familial Chylomicronemia Syndrome (FCS).[40] This gives researchers an opportunity to explore new indications for targeting TG or to find out what to be monitored to prevent potential adverse effects from profoundly lowering TG levels.

Heart failure (HF) and atrial fibrillation (AF) have become increasingly prevalent globally. Importantly, there is no specific recommendation for primary prevention for both cardiac diseases, especially in healthy populations.[1,5] Due to the fact that MI is the major risk factor for both diseases,[42] and genetic evidence collectively suggested the potential causal relationship between TG (or lipid particles that closely related to TG) and the risk of MI.[36] Therefore, it is worth investigating whether TG have a direct role in the occurrence of HF and AF.

6.3.2 Uncertainty

It is uncertain whether TG plays a causal role in initially CVD-free populations in the incidence of HF and AF, having a separate role, if any, in causing MI. Admittedly, there have been no previous large-scale studies examining both cardiac conditions, which often co-exist and share common risk factors and pathophysiological mechanisms.[8,42]

Results from previous epidemiological studies of TG on HF and AF were inconsistent. Some observational studies suggested a positive association between TG and incident HF.[78,112,113] Others, on the other hand, showed no association.[108,227] In terms of incident AF, while most of the previous studies failed to demonstrate any associations with TG levels,[32,69,70,73,76] Alonso *et al*. had shown that increased TG levels might be linked to an increased risk of AF.[31] Moreover, a recent post hoc analysis of VA-HIT trials suggested that gemfibrozil, a medication in the fibrate drug class, cannot prevent AF in males who had existing coronary heart disease (CHD).[128] However, there is no such evidence on healthy individuals. Consequently, the role of TG in HF and AF still requires further investigation.

6.3.3 Objectives

In this study, we provided three new pieces of evidence from observational, trial, and genetic (Mendelian randomisation) studies to assess the association between TG and the risk of HF and AF. We used MI (and CAD) as a control to validate whether our cohorts and methodology used throughout this study can reproduce results from the existing evidence. There were three specific objectives in this study. First, we aimed to conduct a large observational study examining incident HF and AF in the same cohorts based on EHRs across a wide range of TG levels (i.e., TG 0.02 mmol/L [1.8 mg/dL] to 20 mmol/L [1,771 mg/dL]) and to further evaluate the role of intercurrent cardiovascular diseases (CVDs) on the association. Second, we aimed to examine whether changes in the risk of HF and AF are associated with the difference in TG levels between the active and the control groups over the follow-up period in randomised controlled trials of lipid-lowering agents. Third, we aimed to investigate whether the genetic evidence using the Mendelian randomisation (MR) approach supports findings from both cohort and trial studies.

6.4 Methods

6.4.1 Population-based cohort study

In the cohort part of this study, we used a longitudinal design from which participants were followed-up over the period between 1st January 1997 and 30th June 2016. Exposure and outcomes were ascertained through the linked EHRs amongst general practices (GPs), hospital admission records, and national death registry of England. Reports on the cohort section had followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[195,196] and the REporting of studies Conducted using Observational Routinely-collected health Data (RECORD)[197] recommendations (Table S 6-1).

Data sources and studied population

As described in Chapter 4, our studied populations were drawn from the CALIBER (ClinicAl research using LInked Bespoke studies and Electronic health Records) platform.[170] In this study, 3.6 million patients from 387 GPs across England from CPRD database were initially included. We identified all patients aged 18 years or older who registered between 1st January 1997 and 30th June 2016 and had been followed-up with their GPs for at least one year. Individuals who had a history of CVDs at the baseline TG measurement were excluded.

Exposure: Triglyceride (TG) as an EHR phenotype

We used ambulatory care triglyceride (TG) measurement sampled in clinics and hospital out-patients and electronically recorded in the primary care. The raw data included a total of 7,101,840 measurements (multiple records per patient), and plasma levels (3.4% of all records) were multiplied by a factor of 1.03 to convert to serum levels before analysis.[198] We excluded outlier values (i.e., TG $\geqslant$ 20 mmol/L [1,771 mg/dL]) from our analysis (0.04 % of all records). For patients who had more than one TG measurement within a year (27% of all patients), we used a yearly-averaged value, and this further refers to the term 'baseline TG'. The earliest date of TG measurement was used as the start of patient follow-up. For individuals with more than one measurement on

a given day (0.1 % of all TG records), the values were aggregated by taking the mean.

Covariates

Baseline covariates taken from the closest record to the baseline date (within a one-year interval) were selected based on their association with TG, HF, and AF from previous studies.[74,178,199,200] These included age, socioeconomic status (i.e., index of multiple deprivation), smoking, body mass index, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP: not specified assay method), diabetes, use of antihypertensive medications, and use of statins. Any missing value of covariates was imputed using multiple imputations by chained equations (MICE) (see the supplementary appendices). A complete list of codes used to identify all covariates in this study can be found at https://www.caliberresearch.org/portal.

Follow-ups for incident HF, AF, MI, and intercurrent CVDs

Endpoints including HF, AF, and MI were evaluated based on diagnostic codes (ICD-10 and Read codes), which included fatal- (ONS), hospitalised- (HES), and non-hospitalised (CPRD) cases. The definition of each endpoint and its validity was shown in Chapter 3.

Statistical analysis

We used the Cox proportional hazards model to calculate the hazard ratio (HR) from the time of blood sampling TG measurement to the time of the incident event, censoring (i.e., death or transfer out of practice), or end of the follow-up, whichever occurred first. All models were adjusted for baseline covariates and stratified by sex and primary care practice. Since TG levels were positively skewed with the skewness and kurtosis of 3.91 (ideally zero) and 34.57 (ideally 3), respectively, we logarithmically transformed all TG values before analysis. After logarithmic transformation, the histogram showed the normal distribution of the values (skewness and kurtosis of logarithmic TG values were 0.39 and 3.53, respectively).

We analysed baseline TG as both continuous and categorical variables to avoid presuming a particular shape of the association. For categorisation, we

used the cut-off for TG according to a clinical guideline[201] as follows: less than 0.85 mmol/L (75 mg/dL), 0.85-1.12 mmol/L (75-99 mg/dL), 1.13-1.40 mmol/L (100-124 mg/dL), 1.41-1.68 mmol/L (125-149 mg/dL), 1.69-2.25 mmol/L (150-199 mg/dL), 2.26-2.81 mmol/L (200-249 mg/dL), and 2.82 mmol/L (250 mg/dL) or higher. We chose the middle category (i.e., TG 1.41-1.68 mmol/L) as a reference group to avoid the potential impact of an outlier, if any, on the overall shape of the association. The associations of each endpoint with baseline TG were reported as per continuous (per natural-log one mmol/L [88.57 mg/dL] decrease in TG) or categorical baseline TG as described above. Sensitivity analyses had been carried out and were explained in the supplementary appendices.

All analyses in the cohort part have been done using STATA version 13 (MP version, StataCorp) with statistical significance defined by a two-tailed P-value of < 0.05. The Bonferroni method was used, as applicable, for multiple comparison adjustment.

6.4.2 Trial-level meta-analysis of randomised controlled trials

Study selection and outcome data

We included randomised controlled trials of lipid-modifying agents published up to July 2019. At first, since we considered our outcomes of interest as hard outcomes, we included only major trials with at least 1,000 participants who were followed up for at least one year to perform the meta-analysis. To further expand our search, we additionally included small trials (i.e., N<1,000) from trials of fibrates identified through previous Cochrane reviews.[231,232] Depending on the availability of reported data, HF and MI were defined as either fatal and non-fatal cases. AF was defined as either reported atrial fibrillation or cardiac arrhythmia. All included studied were assessed for their quality using the Cochrane risk of bias tool, and the results were shown in Chapter 2.

Statistical analysis

We calculated relative risk (RR) of outcome of interest per one mmol/L (88.57 mg/dL) decrease in TG levels between the active and the control groups at the end of follow-up period using the fixed-effect meta-regression method.

Alternatively, a random-effect was used if we found evidence of heterogeneity [i.e., I^2 > 75% or P-value for heterogeneity < 0.1][233]). For visualisation, we created bubble plots for the association between the relative risk of disease on the y-axis versus the absolute reduction (in mmol/L) of TG between the active and the control groups on the x-axis. Each bubble represents each trial, and its size depends on the variance of each trial. Then the association between changes in TG and the risk ratio of outcomes was the beta-coefficient of slope from the linear equation derived from the estimated straight line. For trials with no events with active or control arm, a nominal amount (0.5 cases) was added to the results for both trial groups.

The main results were derived from univariable meta-regression models. Potential publication bias was assessed by visualising a funnel plot of log RR (x-axis) and standard error (y-axis) of a model without a moderator (i.e., change in TG levels) and by Egger's test P-value. We also analysed multivariable meta-regression models and performed subgroup and sensitivity analyses (supplementary appendices). All analyses in this part were done by using 'metafor' and the 'CALIBERdatamanage' package in R version 3.3.2.

6.4.3 Mendelian randomisation (MR)

Data Sources

We used the same data sources as in Chapters 4 and 5. Full details were provided in the supplementary appendices.

Selection of SNPs and MR Analyses

We used 185 lipid-associated SNPs initially identified by Willer *et al.* to generate a series of genetic instruments for TG.[202] Since nearby SNPs might violate the independent assortment rules, all 185 SNPs were further pruned to a set of low linkage disequilibrium (pairwise R^2 < 0.05) within a window of 10,000 kb using the UKB10K LD reference. This process yielded a final set of 156 SNPs from UKB and 105 SNPs from CARDIoGRAMplusC4D. SNPs and effect allele frequency across the consortia (e.g. GLGC and UKB) were checked to ensure that the same strand was used throughout the analysis.

We employed the two-sample MR approach with various sensitivity methods, including i) inverse variance weighted (IVW) MR, ii) MR-Egger, iii) weighted

median MR, and iv) multivariate MR (MVMR) analyses to show the robustness of our findings and potential horizontal pleiotropy. We took the main results from the MVMR model since it took into account the genetic variability of other lipid traits (i.e., LDL-C and HDL-C). Details about testing the MR assumption and power calculation can be found in the supplementary section (see supplementary appendices). All analyses in the MR part were performed using the 'TwoSampleMR' package in R version 3.3.2, and the 'mrrobust' package in STATA version 13 (MP version, StataCorp).

6.5 Results

6.5.1 Results from an observational study (CALIBER)

After the median follow-up period of 5.3 years (interquartile range [IQR] 0.1 to 19.5 years) out of the total of 1,262,280 individuals (Figure S 6-1), we identified 30,007, 53,746, and 20,933 new cases of HF, AF, and MI, respectively (Figure S 6-2 and Figure S 6-3). At baseline, the study population had a mean age of 55.5 years with the median TG level of 1.3 mmol/L (115 mg/dL) (IQR: 0.9-1.9 mmol/L [80-168 mg/dL]). From Table 6-1 and Table S 6-3, we observed that higher baseline TG was associated with the male gender, a higher proportion of current or ex-smokers, higher BMI, higher blood pressure, and higher levels of LDL-C and CRP but lower HDL-C levels. Moreover, higher TG was also related to a higher prevalence of type 2 diabetes. Additionally, there was a positive trend towards the use of lipid-lowering agents, antihypertensive agents, and antiplatelet agents as TG levels increased.

From Table S 6-2, we noticed that the incidence rate of HF increased as TG levels increased and reached the plateau at around 4.4 per 1,000 person-years from 1.69-2.25 mmol/L group upwards. Furthermore, we observed a frown-shape pattern of the incidence rate of AF across TG strata with the highest incidence rate of 7.9 (7.74-8.06) per 1,000 person-years in the 1.13-1.40 mmol/L group.

Figure 6-1 showed the different patterns of the observed association between TG and incident HF and AF. In the age and sex-adjusted model (complete-case analysis), there was a direct association between TG and incident HF; however, the pattern of the association had changed to a mirrored J-shape (or

inverse association) in the fully adjusted model, corresponding to an HR per one log mmol/L decrease in TG of 1.11 (95%CI 1.08, 1.15). For the incident AF, we found a weakly inverse association across TG strata in the age- and sex-adjusted model, which became stronger after full adjustment, corresponding to an HR per one log mmol/L decrease in TG of 1.30 (95%CI 1.28 to 1.33). Surprisingly, the association of TG levels with the incidence of MI had paradoxically changed from a strong direct association in the age- and sex-adjusted model to a weakly inverse one in the fully adjusted model, corresponding to an HR of 1.07 (95%CI 1.04, 1.10) per one log mmol/L decrease in TG levels. Further serial adjustment (Table S5) revealed that the pattern of the association between TG and incident MI had changed after taking into account chronic conditions.

Subgroup analyses of HF, AF, and MI were given in Figure S 6-4. Overall, subgroup analyses showed a robust direction of the associations, compared with the main findings, except for MI outcomes, in which the analysis only amongst women yielded a borderline positive association (HR: 0.95 [95%CI 0.89, 1.00]). Also, the analysis only amongst patients with baseline comorbidities (i.e., COPD, cancer, and chronic kidney disease) showed a direct, but not significant, association between TG and the incidence of MI (HR per log mmol/L decrease in TG was 0.93 [95%CI 0.85 to 1.02]). Moreover, age group, gender, and intercurrent CVD might modify the strength of the association between TG and incident HF, whereas age group, intercurrent MI, intercurrent AF, intercurrent HF, and intercurrent CVDs might affect the relationship between TG and incident AF. Schoenfeld residual plots and proportional hazard plots did not show any apparent violation of the proportional hazards assumption (Figure S 6-5 and Figure S 6-6).

Further analysis on subtypes of HF and AF revealed two important points (Figure 6-2). First, we found an inverse association between TG levels and systolic HF (HR 1.40 [95%CI 1.16, 1.68]). Second, analysis on AF subtypes showed robust findings throughout all subtypes of AF (i.e., paroxysmal AF, persistent/ permanent AF, atrial flutter, and other AF). Additional examining on mediating effect of intercurrent MI suggested that the association between TG and HF and AF was unlikely to be mediated through MI. Also, MI might be an

effect modifier of the association between TG and AF since we observed stronger association among patients without intercurrent MI (HR 1.31 [1.28, 1.33]) than that among individuals with intercurrent MI (HR 1.09 [0.93, 1.27]).

6.5.2 Results from meta-regression of RCTs

According to Figure 6-3, from 49 RCTs that reported changes in TG between the active and the control groups, 32 and 22 trials had reported HF (8,230 events out of 259,538 individuals) and AF (6,016 events out of 189,478 individuals) as one of their endpoints, respectively. Meta-regression analysis had shown no significant association of a decrease in TG levels with either the risk of HF (RR 0.77 [95%CI 0.55 to 1.09]) or that of AF (RR 0.96 [95%CI 0.64 to 1.46]). Besides, I^2 and p-value for heterogeneity did not reveal a significant degree of statistical heterogeneity from the univariable model with I^2 of 0.0% (p-value 0.42) and 12.6% (p-value 0.13) for the HF and AF endpoints, respectively. Summaries of the included trials are provided in Chapter 2 (Table S 2-10).

According to Figure S 6-7 and Figure S 6-8, subgroup analyses did not reveal significant associations between subgroups. However, we noticed that most of the results from subgroup analyses were less precise due to a wide confidence interval. Interestingly, the multivariable meta-regression model showed a significant positive association of a decrease in TG with the risk of MI with RR of 0.50 (95%CI 0.34 to 0.73), and I^2 had reduced from 63.9% (p-value < 0.001) in the univariable model to 36.9% (p-value 0.007) in the multivariable model. In addition, reducing TG levels by statins and fibrates was significantly associated with a decrease in the risk of MI by 51% (8% to 73%) and 48% (5% to 71%), respectively. Moreover, funnel plots between the log risk ratio and the standard error did not show an apparent asymmetrical pattern with Egger's p-value > 0.05 (Figure S 6-9), suggesting that publication bias was unlikely to be a significant concern.

6.5.3 Results from the genetic study

We extracted 156 SNPs from 520 cases, 3,213 cases, and 1,741 cases of the first diagnostic HF, AF, and MI in UK Biobank, respectively, and 105 SNPs from 63,746 CAD cases from the CARDIoGRAMplusC4D, which explained

around 6% of the variability of TG levels. In the HERMES consortium, the results were derived from 110 SNPs, which were from 47,309 HF cases out of 977,323 individuals. Scatter plots for genotype-TG associations and genotype-outcome associations are given in Figure S 6-10.

We found conflicting genetic evidence of the association between TG and the risk of HF. According to Figure 6-4, we found no association between one SD genetically determined decrease in TG and the risk of heart failure identified from UKB regardless of how the cases were identified (e.g., first event cases or any event cases): the MVMR model showed the odds ratio (OR) of 0.94 (95%CI 0.63 to 1.40) while sensitivity analysis did not show the deviated results from the MVMR model. In contrast, the results from HERMES suggested a direct association, and the OR remained statistically significant (0.94 [95%CI 0.91 to 0.98]) even after the adjustment for CAD. However, no evidence of horizontal pleiotropy had been found in HF outcomes (Table S 6-5).

Interestingly, we found that TG levels might be causally relevant to the risk of AF. From Figure 6-4, in the MVMR model of first event cases, one SD genetically determined decrease in TG was inversely associated with the risk of AF (OR 1.25 [95%CI 1.03 to 1.52]) although most of the results from sensitivity analyses and any event cases had shown only a borderline significant association.

Regarding MI outcomes, we found the evidence of horizontal pleiotropy (p-value for Egger's intercept was 0.008, Table S 6-5) and MR-Egger showed the null association with TG (OR 0.87 [95%CI 0.63 to 1.21]). Additionally, although most of the genetic findings had shown a significant direct association with the risk of CAD, taking into account other genetically determined lipid traits (MVMR) regressed the association towards null (OR 0.88 [95%CI 0.77 to 1.01]), suggesting that the association between TG and the risk of CAD might be mediated through LDL-C or HDL-C. Sensitivity analysis by less stringent pruning criteria ($R^2 < 0.2$) did not change our conclusion (results not shown).

When putting together, a summary of findings from cohorts, trials (meta-regression), and genetic (MR) studies is given in Figure 6-5. An inverse association of TG with the risk of HF observed from the cohort study was not supported by trials or genetic evidence from UKB. In contrast, genetic evidence from the biggest consortium of HF (HERMES) even showed conflicting results. Meanwhile, an inverse association of TG with the risk of AF, which was not supported by trials, was partially consistent with genetic findings. However, the association we found between TG and the risk of MI or CAD was likely to be explained by other lipid traits, such as LDL-C and HDL-C or by horizontal pleiotropy (i.e., TG genetic instrument might be associated with MI through other pathways that were not related to TG levels).

6.6 Discussion

In this study, we reported the first evaluation of the causal relevance of TG in the two most common cardiac diseases globally: HF and AF. We compared higher resolution observational cohorts (more than ten times the number of participants of previous studies), new meta-regression of trials on lipid-lowering agents, and new MR with the largest GWAS on HF outcomes (HERMES). We found inverse associations extending into low TG levels with incident HF and AF. However, trials evidence did not support a causal role of TG in both outcomes, while genetic evidence (MR) showed conflicting results with HF. Interestingly, genetic evidence suggested a causal role of TG in incident AF. Taken together, these three forms of evidence did support a strategy of lowering TG for the primary prevention of AF, whereas its role in HF was still inconclusive.

6.6.1 Heart failure - what is new about our observation?

We conducted the first study showing a mirrored J-shape (inverse) association of TG levels with incident HF (30,007 cases). To date, only a few (much smaller) observational cohorts have reported the effect of TG on the risk of HF, and their findings were mixed. Three studies suggested a positive association[78,112,113], whereas two of them showed null results.[108,227] In our analysis, intercurrent MI and CVD did not significantly modify the association (p-value for heterogeneity of subgroup > 0.05). Nonetheless, we found that

gender (i.e., stronger effect amongst males, $p < 0.001$), age group (i.e., stronger effect amongst the elderly aged more than 75 years old, $p = 0.002$), and intercurrent AF (i.e., stronger effect amongst individuals with intercurrent AF, $p = 0.005$) significantly modified the magnitude, but not the direction, of the association between TG and HF (Figure S 6-4). Additionally, an inverse association between TG and risk of systolic HF might be worth investigating further.

6.6.2 TG and HF - How we extended knowledge from trials and genetic evidence?

Our meta-regression of trials did not reveal any association of TG with HF, which was independent upon lipid-lowering agents, settings (i.e., primary-, secondary-, and mixed prevention), and comparisons (i.e., head-to-head trials and placebo-controlled trial). To our best knowledge, there is no previous meta-analysis of TG-regulating agents, such as fibrates, on the risk of HF. However, genetic evidence using different sources showed contradictory findings: those from UK Biobank were null, whereas those from HERMES, even after being adjusted for coronary artery disease, showed a robust direct association between genetically determined TG and the risk of HF. No genetic study on the association between TG and HF has been previously reported.

6.6.3 TG and HF – Possible explanation

Confounders or reverse causation cannot fully explain the inverse association observed in our cohort study. We noticed that characteristics that increase the risk of HF, such as hypertension, diabetes, metabolic syndrome, and atherosclerotic CVDs (ASCVDs), were not prominent amongst individuals in the lowest TG levels (i.e., < 0.85 mmol/L). According to Table 6-1 and Table S 6-3, those in that group were likely to be younger, less male predominant, fewer smokers, and have lower BMI, SBP, DBP, and CRP levels. In addition, they also had the lowest prevalence of diabetes, chronic kidney disease, cancer, and COPD compared with individuals in higher TG level groups. In other words, people with higher TG levels tend to have a higher risk of HF. This argument could be further supported by the results from the age- and sex-adjusted model in which a direct association had been first observed, with its

shape being significantly transformed into the inverse one after a full adjustment for covariates.

In contrast to previous studies,[78,112,113] we did not find a mediation effect of preceded MI on the association between TG and incident HF. On the other hand, our results suggested that AF might mediate the association instead. Admittedly, reverse causation is less likely to explain our results as excluding HF cases identified within four years prior to the formal diagnosis did not profoundly change our conclusion.

6.6.4 Atrial fibrillation - what does the observation add?

We found a strong and monotonic inverse association extending from low (i.e., < 0.85 mmol/L or < 75.3 mg/dL) to high (i.e., > 2.81 mmol/L or > 248.9 mg/dL) TG levels and incident AF. The strength of the association became even stronger after full adjustment. Compared with previous studies, we presented the first findings of a negative (inverse) association between TG levels and incident AF. Almost all of the prior studies failed to show the association between TG and AF[32,69,70,73,75,76,107,110,111] while just one study had suggested that the risk of AF increased as TG levels increased.[31] Moreover, the consistent findings in all AF subtypes even in the subtype that had a very small number of events (n=67) might confirm the true association between TG and AF.

6.6.5 What does trials and genetic evidence add on TG and AF?

To date, there is no previous meta-analysis of TG levels *per se* on the risk of AF, and the evidence from trials did not reveal significant findings. Genetic evidence (MR), although not robust across all sensitivity MR models, was still directionally consistent with the observational results. Our genetic findings, however, were discordant with the prior work from which the odds ratio (OR) of AF per one gene score increase in TG was 0.99 (95%CI 0.97 to 1.02).[92] It is worth noticing that the previous work might have an issue of statistical underpowering and fail to detect the small effect size, since the calculated power using the formulae given by Brion *et al*.[244] yielded only 5%.

6.6.6 TG and AF – possible explanation

The biological mechanism underlying the association between TG and incident AF is less clear, and further studies are needed to provide an insight into this area. Alternative explanations for the paradoxical association, such as residual confounders and reverse causation, cannot fully explain our findings. This is because when we consider three main aetiological factors related to AF, namely i) male gender and older age; ii) thyroid dysfunction; and iii) inflammation, they are less likely to explain our findings. For instance, individuals in the lowest TG strata (i.e., TG levels < 0.85 mmol/L) were likely to be of younger age and be female. Moreover, their lipid profile patterns did not give a hint for potential hyperthyroidism (i.e., low LDL-C and TC but still high HDL-C).

Importantly, the strength of the association was even stronger after excluding intercurrent CVDs cases (p-value for heterogeneity of subgroup < 0.001), which further supported the direct link between TG levels and the risk of AF. Also, the robust results obtained after excluding AF cases identified within four years prior to the disease evaluation implied that the reverse causation could be less of a concern. Compared with previous works, other potential confounders that we had not adjusted for in our models included menopausal status, hormone replacement therapy, and alcohol consumption.[76]

6.6.7 Positive control: myocardial infarction and further insight

We found a significant transformation of the shape of the association between TG levels and incident MI from a strong direct association in the age- and sex-adjusted model to a weakly inverse one in the fully adjusted model, particularly after being collectively adjusted for a list of chronic conditions (i.e., blood pressure, LDL-C, HDL-C, CRP, use of statins, use of antihypertensive medications, and prevalence diabetes) (Table S4). Moreover, we still noticed the positive association amongst females and those with baseline comorbidity (i.e., CKD, COPD, and cancer). When analysing TG as a categorical variable, our results were similar to previous findings by the Emerging Risk Factors Collaboration (ERFC) in which multivariable adjustment attenuated the positive association between TG and incident CHD towards null (see Chapter 3, Figure 3-3).[95]

In meta-regression results, we found a significantly positive association only in the multivariable-adjusted model. However, our subgroup results, in which reducing TG levels by fibrates was related to a decreased risk of MI, were consistent with previous Cochrane reviews where the use of fibrates had shown to be associated with the decrease in fatal and non-fatal MI risks in both primary[231] and secondary preventions.[232] We did not find any high-quality evidence on the change in TG levels and the risk of MI that has previously been published.

Besides, our genetic findings suggested that the genetic instrument of TG might be associated with MI outcomes through other pathways not related to TG levels (i.e., horizontal pleiotropy, p-value for Egger's test = 0.008), while the adjustment for other lipid traits (MVMR) weakened the association towards null. Our findings were supported by the fact that genetic variants of high TG levels are always co-associated with reduced HDL-C levels[98], as well as in previous MR that showed an unbalanced pleiotropy between genetic variants of TG and the risk of CAD.[62] Therefore, the evidence here collectively indicated that the direct (or positive) association between TG and MI was most likely due to residual confounders.

6.6.8 Strengths

This was the first study that comprehensively investigated the association and causation of TG on the two most common, but less-well studied, CVDs using three different study designs (i.e., cohort, trial, and genetic studies). The strengths of our study were as follows: 1) Compared to prior cohort studies (see Chapter 2, Table S 2-8 and Table S 2-9), our results were derived from huge and representative cohorts (N=1,262,280), which were ten times larger than the previous most extensive cohort study.[113] This enabled us to subcategorise our cohorts into seven strata with very high statistical power (>90%) to capture even the very least effect size. Also, having a substantial sample allowed us to study HF, AF, and MI together, and improved the ability to evaluate intercurrent diseases. Furthermore, this was the first time that we could examine an association of the disease with low TG levels (i.e., TG < 0.85 mmol/L or 75.3 mg/dL); 2) This was the first meta-analysis focusing on the role of TG *per se,* regardless of lipid-lowering agents, on the risk of HF and AF; 3)

A numbers of MR sensitivity analyses were applied in at least three data sources (i.e., UK Biobank, CARDIoGRAMplus4CD, HERMES) to ensure the validity and robustness of genetic findings; and 4) MR analysis on a large consortium (HERMES) secured generalisability, especially in European populations.

6.6.9 Limitations

As discussed in Chapter 4, using EHR might be biased due to misclassification of outcomes. However, in Chapter 3, we showed that our definition of outcomes using ICD-10 and Read Codes was reasonably valid. Therefore, this issue was not a major problem in our study.

Another limitation of using EHR due to the incompleteness of data collection was unmeasured confounders. As discussed, we did not adjust our models for several factors that might be related to outcomes, such as menopausal status, use of hormone replacement therapy, alcohol consumption, and baseline thyroid function. However, collective results from other study designs, such as meta-analysis of RCTs (whose confounders were equally distributed between arms due to the randomisation process) or Mendelian randomisation (whose genetic instrument was not related to confounders and occurred before an outcome of interest), might introduce less insight bias than from the cohort design alone.

In addition, we could not distinguish the state of measuring TG from CPRD (i.e., fast VS non-fast) since 98.52% of all TG values in our cohorts were coded as either “serum triglycerides” or “plasma triglyceride level” without specific state of measuring (Chapter 3, Table 3-4). However, the most recent guideline for the management of dyslipidaemias from the European Society of Cardiology (ESC) has recommended that non-fasting samples of TG might not be required, as they often show non-clinically significantly higher TG levels of around 0.3 mmol/L (27 mg/dL), compared with the fasting state. Therefore, this limitation should not profoundly affect our findings.

Regarding the limitation of meta-regression, as previously discussed in Chapter 4, the numbers of people with HF and AF might be underestimated and not adjudicated. Also, we are aware of the fact that we could not exclude

prevalent cases of HF and AF, and our meta-regression results cannot be generalised to the incident cases and inform primary prevention.

Lastly, although our instrumental variable used in MR was valid with no apparent evidence of horizontal pleiotropy, except for MI outcomes, we still faced the issue of statistical underpowering (Table S 6-6). Such a limitation might explain the discrepancy of genetic findings between UKB and HERMES. For UKB, we were able to extract genetic data from first event cases separated from any event cases; however, it had low statistical power. For HERMES, while the statistical power was higher than in UKB, we could not separately analyse first event cases and any event cases. Also, we did not have MVMR results from HERMES. Therefore, we cannot ascertain whether the association of TG and HF we found would be confounded by the genetic effects on other lipid traits, such as LDL-C and HDL-C.

Potential bias from the observational study design are as follows:

Selection bias: patients who were recruited in my analysis are the individuals with lipid measurement. Therefore, only patients with an indication to measure blood lipids will be included. This would limit the generalisibility of my findings rather than artifact the results. Also, the selection bias can be arisen when there is a discrepancy in the quality of care at practice level. For example, the same patient might be eligible to have lipid measured in one GP but ineligible if he or she goes to another GP. To minimise the bias due to the variation of practice level, I have stratified all analysis by gender and practice level.

Misclassification and information bias:

- Extracted phenotypes of outcome are mainly based on signs and symptoms but not based on an objective confirmation, such as echocardiogram results. For instance, there were only 3% of HF cases who had codes for echocardiography that confirmed HF. Therefore, it is likely to include other health conditions that are mimic signs and symptoms of HF, such as chronic respiratory disease exacerbation as HF cases (i.e., false positive cases).

- Diagnosis taken from EHRs is based solely from one physician (not by adjudication committee), and this might vary according to level of expertise of physicians. Therefore, this prone to misclassification bias.

These two scenarios above would result in misclassification bias and increasing false positive cases. In other words, this would lead to decrease in specificity due to increasing false positive cases, which leads to type I error, and could inflating the observation. However, subgroup analysis of HF based solely on echocardiography codes (i.e., systolic and diastolic HF) had shown the consistent findings between TG and systolic HF (Figure 6-2). Therefore, misclassification bias is not a major concern.

- We are unable to identify whether TG were measured during the fasting state or not.

This scenario can cause measurement error and lead to information bias and would unpredictably bias results in either directions (inflating or attenuating findings). However, some methods to control for this bias, such as excluding outlier lipid levels, using one-year averaged lipid levels (and compared with the results from using single lipid levels), and stratifying results by practice levels, can be used to attenuate the impact of measurement error and misclassification bias. In addition, the most recent ESC clinical recommendations have suggested that using non-fasting levels of lipid do not clinically differ from fasting levels.[36]

Attrition bias (i.e., bias due to loss to follow-up or dropping out): In my study, there were 5% of studied populations who were censored due to death from other causes, and this might compete the outcome of interest. However, in age and sex-adjusted model, further adjusting for competing risk (Figure S) did not significantly deviate the findings.

6.6.10 Implications of findings

The current clinical guidelines have no specific recommendations for primary prevention of HF and AF, and our results suggested that increased TG levels might be causally relevant to the decreased risk of AF. Although increasing TG levels seems clinically irrational since it can promote other adverse events, such as acute pancreatitis, it might be worth monitoring patients who have very low TG levels for the occurrence of AF. Regarding HF outcomes, due to the

conflicting results and limitations of our study, the research was inconclusive and needs further investigation. Moreover, TG should not be a target for MI prevention since our evidence had collectively revealed that the direct (or positive) association found between TG and incident MI could be biased due to confounders.

Concerning research implications, our study is an example of the use of different study designs (e.g., EHR cohort, trial, and MR studies) to triangulate and tackle a particular research question. This should be encouraged since we can strengthen the evidence and make the best use of available data sources nowadays. Furthermore, a gap in CVD research still exists regarding temporal relationships amongst the three most common CVDs (i.e., MI, HF, and AF) and needs to be addressed.

6.7 Conclusion

We found a strong, inverse observational association between TG levels and incident AF, which was supported by Mendelian randomisation. Therefore, TG *per se* is likely to be causally relevant to the onset of AF, and TG might have a protective effect on AF. However, the evidence surrounding TG and HF was less clear and requires further investigation. Also, our study suggested that a positive association between TG and MI was likely to be biased and unreliable.

Table 6-1 Observational cohort: participant characteristics of the population-based EHR cohort (n= 1,262,280)

Baseline characteristics	Triglyceride level at baseline (mmol/L)				P-values
	< 0.85	1.41 - 1.68	> 2.81	Total	
N	250,849	128,772	117,656	1,262,280	
Female	64.0%	51.0%	34.8%	52.7%	P < 0.001
Age (year)	52.8 (13.3)	57.0 (13.3)	54.0 (12.0)	55.5 (13.3)	P < 0.001
White	83.7%	90.4%	91.7%	89.0%	P < 0.001
Missing*	47.6%	40.1%	37.2%	41.8%	
Health behaviours, physical and laboratory measurements at baseline					
Non-smokers	66.1%	57.1%	46.8%	58.3%	P < 0.001
Missing*	0.7%	0.8%	1.0%	0.8%	
BMI (kg/m^2)	25.4 (4.8)	28.5 (5.6)	30.0 (5.3)	27.8 (5.6)	P < 0.001
Missing	5.0%	4.6%	4.0%	4.6%	
SBP (mmHg)	130.8 (17.3)	138.1 (16.5)	139.9 (15.8)	136.2 (17.0)	P < 0.001
Missing	1.4%	1.0%	0.9%	1.0%	
DBP (mmHg)	78.7 (9.5)	82.2 (9.1)	84.1 (8.9)	81.4 (9.4)	P < 0.001
Missing	1.4%	1.0%	0.9%	1.0%	
eGFR (mL/min/1.73m^2)	78.4 (18.6)	84.7 (22.3)	87.6 (22.4)	83.2 (20.9)	P < 0.001
Missing	4.4%	3.0%	2.6%	3.4%	
CRP (mg/L), median (IQR)	3.0 (1.0-5.4)	4.9 (2.0-8.3)	5.0 (2.5-9.0)	4.0 (2.0-8.0)	P < 0.001
Missing	56.3%	56.3%	57.3%	56.5%	
LDL cholesterol (mmol/L)	2.9 (0.8)	3.5 (0.9)	3.3 (1.1)	3.3 (0.9)	P < 0.001
Missing	12.9%	15.4%	27.0%	15.6%	
HDL cholesterol (mmol/L)	1.7 (0.5)	1.4 (0.4)	1.1 (0.3)	1.5 (0.4)	P < 0.001
Missing	7.7%	8.3%	9.2%	8.2%	
Total cholesterol (mmol/L)	5.0 (0.9)	5.6 (1.0)	6.1 (1.1)	5.5 (1.0)	P < 0.001
Missing	6.1%	5.6%	5.1%	5.4%	
Triglyceride (mmol/L), median (IQR)	0.7 (0.6-0.8)	1.5 (1.5-1.6)	3.6 (3.1-4.4)	1.3 (0.9-1.9)	P < 0.001
Health conditions at baseline					
Diabetes type 2	2.0%	5.3%	11.1%	5.0%	P < 0.001
Chronic kidney disease	2.5%	3.6%	3.3%	3.3%	P < 0.001
Cancer	2.9%	3.6%	3.0%	3.3%	P < 0.001
COPD	1.3%	1.9%	1.8%	1.8%	P < 0.001
Medications					
Statins (at baseline)	6.2%	13.8%	23.6%	12.9%	P < 0.001
Statins (at follow-up)	15.5%	35.7%	56.6%	32.5%	P < 0.001
Other lipid-lowering	0.3%	0.6%	2.0%	0.7%	P < 0.001
Antihypertensive drugs	19.7%	33.3%	37.5%	29.8%	P < 0.001
Antiplatelet drugs	4.2%	6.9%	7.1%	6.1%	P < 0.001

Note: *Percentages of the missing category were separately calculated from complete cases. Values are presented as numbers (percentage) or mean (standard deviation) or median (interquartile range) as appropriate. Corresponding values for TG are: 0.85 mmol/L = 75.28 mg/dL; 1.12 mmol/L = 99.20 mg/dL, 1.40 mmol/L = 124.00 mg/dL, 1.68 mmol/L = 148.80 mg/dL, 2.25 mmol/L = 199.28 mg/dL, 2.81 mmol/L = 248.88 mg/dL. To convert mmol/L of cholesterol (i.e., total cholesterol, LDL-C, and HDL-C) and triglyceride to mg/dL, multiply by the factors of 38.67 and 88.57, respectively.

Abbreviations: CRP; C-Reactive Protein, eGFR; estimated glomerular filtration rate, COPD; chronic obstructive pulmonary disease, HDL; high density lipoprotein, LDL; low density lipoprotein.

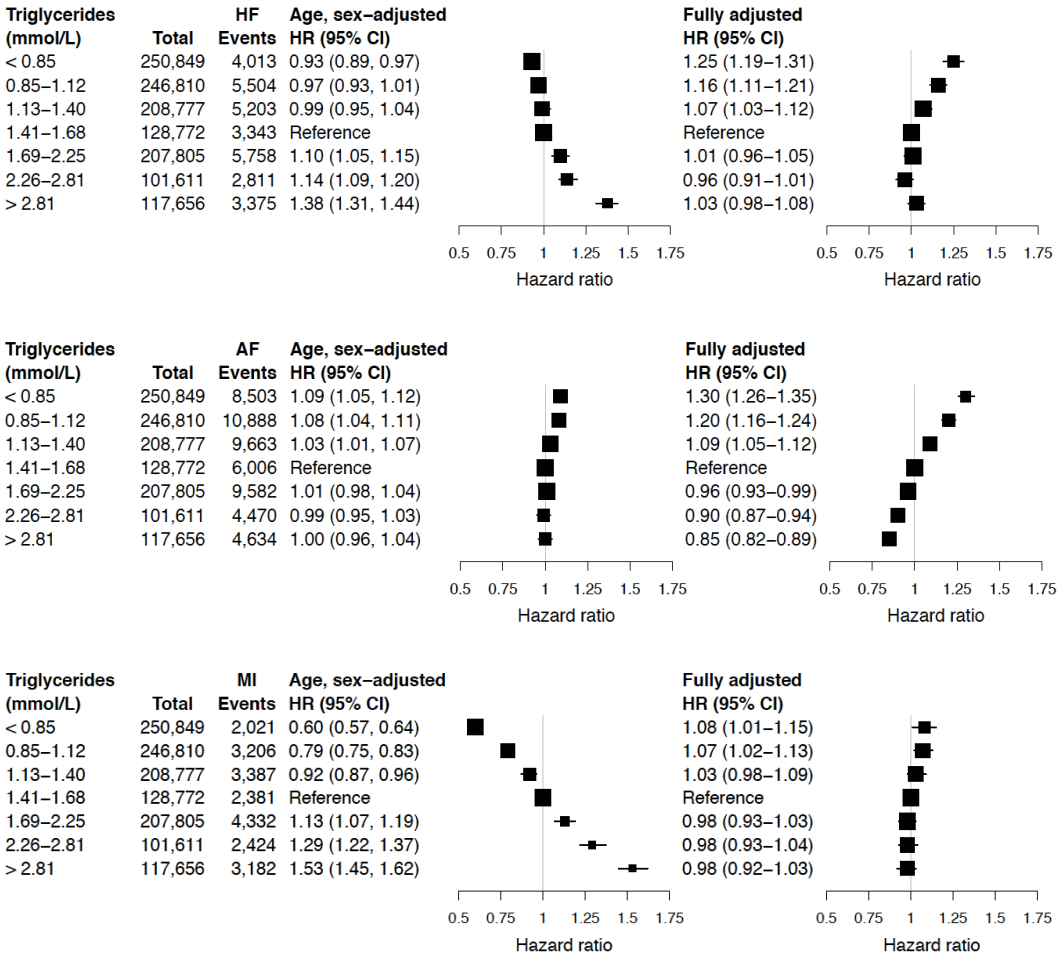


Figure 6-1 Observational cohort: The association of different levels of TG and incident HF (total 7,543,757 person-years), AF (total 7,449,706 person-years), and MI (total 7,547,422 person-years) among 1,262,280 individuals without diagnosed CVD at baseline over the median follow-up of 5 years (interquartile range: 2-9 years).

Note: Fully adjusted models were stratified for gender and primary care practice and adjusted for age, socioeconomic status, smoking, body mass index, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, diabetes, use of blood pressure-lowering drugs and use of statins. The size of the boxes varies based on the inverse variance of the data in each category.

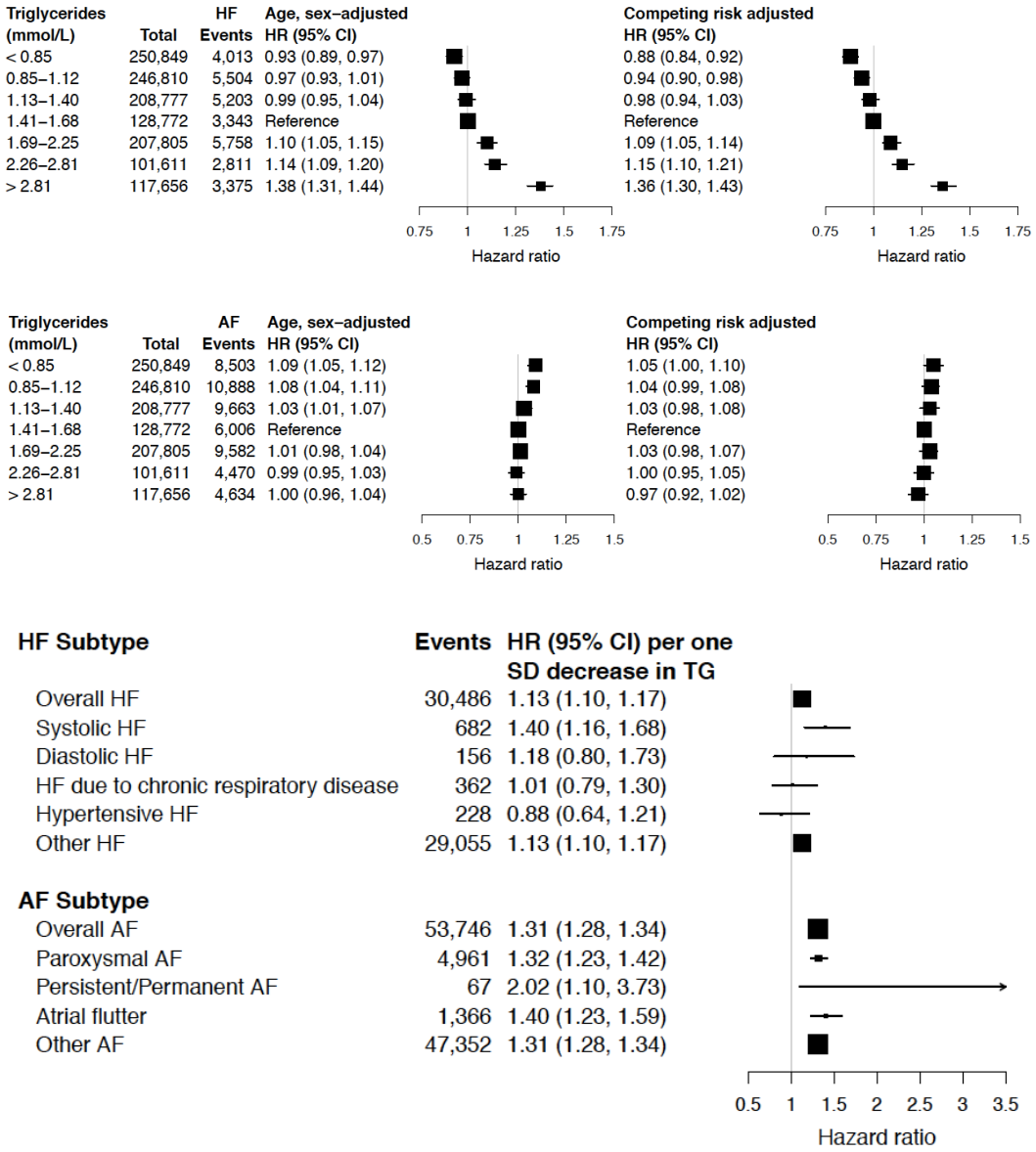


Figure 6-2 Competing risk adjustment and subtype analysis

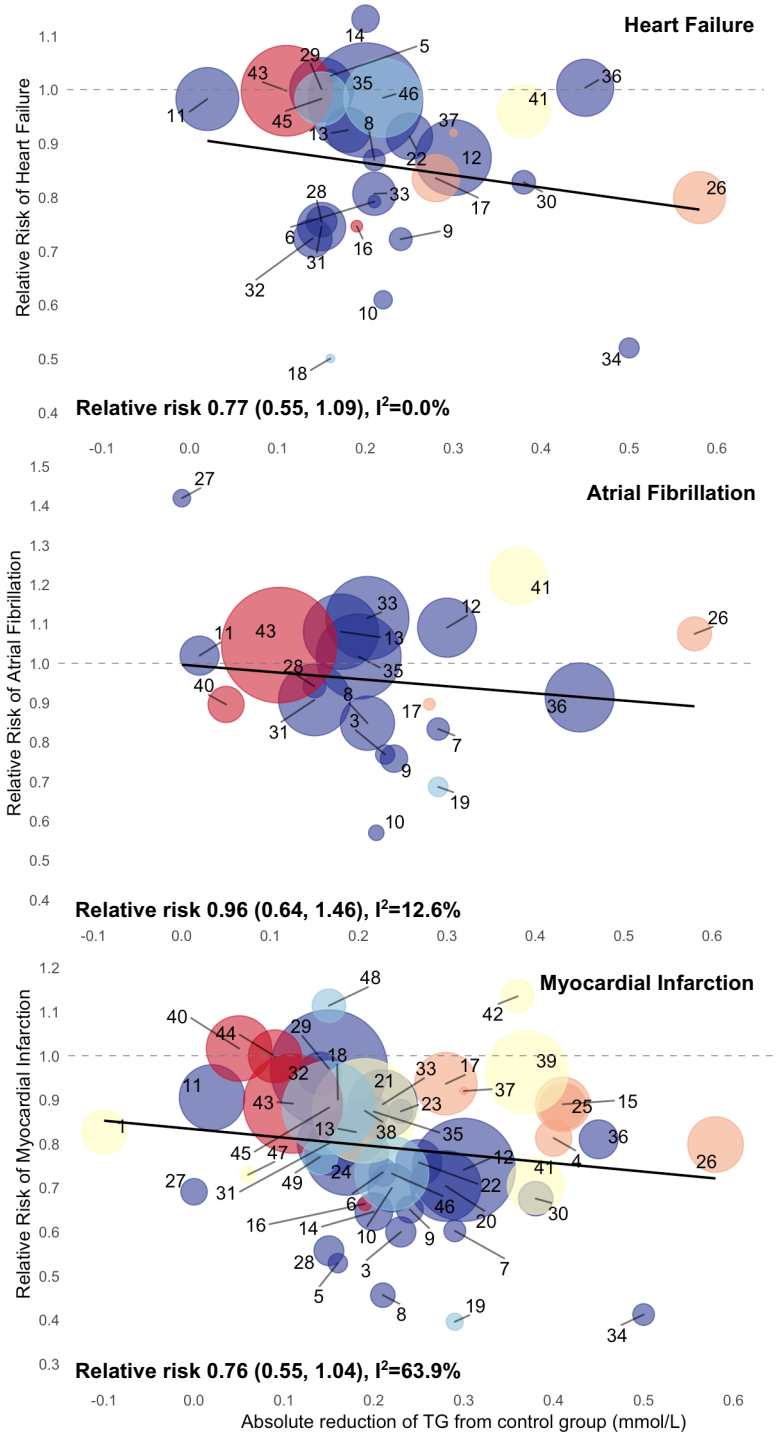


Figure 6-3 RCTs of lipid-lowering agents: Univariable meta-regression of the risk of HF (32 trials: 8,230 events), AF (22 trials: 6,016 events), and MI (49 trials: 26,276 events) per absolute reduction in TG (mmol/L) from control group.

CETP inhibitors Fibrates Others PCSK-9 Inhibitors Statins

Note: Numbers designated in plots represent study identification (Chapter 2, Table S 2-10 and Table S 2-11), and the size of bubbles was proportional (weighted) to inverse-variance.

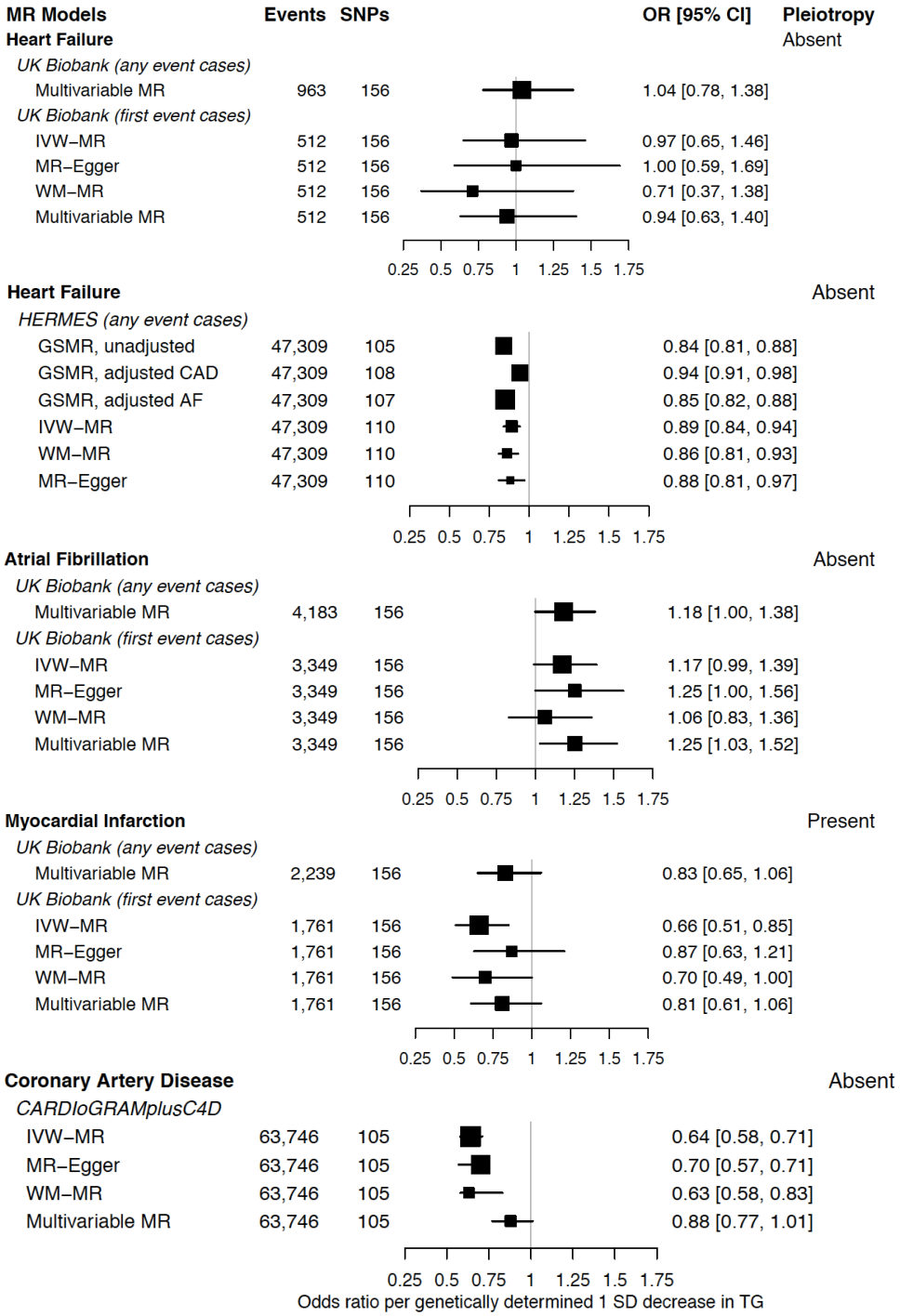


Figure 6-4 Genetic (MR) study of associations between genetically determined 1 SD (1 mmol/l) lower TG and the risk of HF, AF, MI (or CAD)

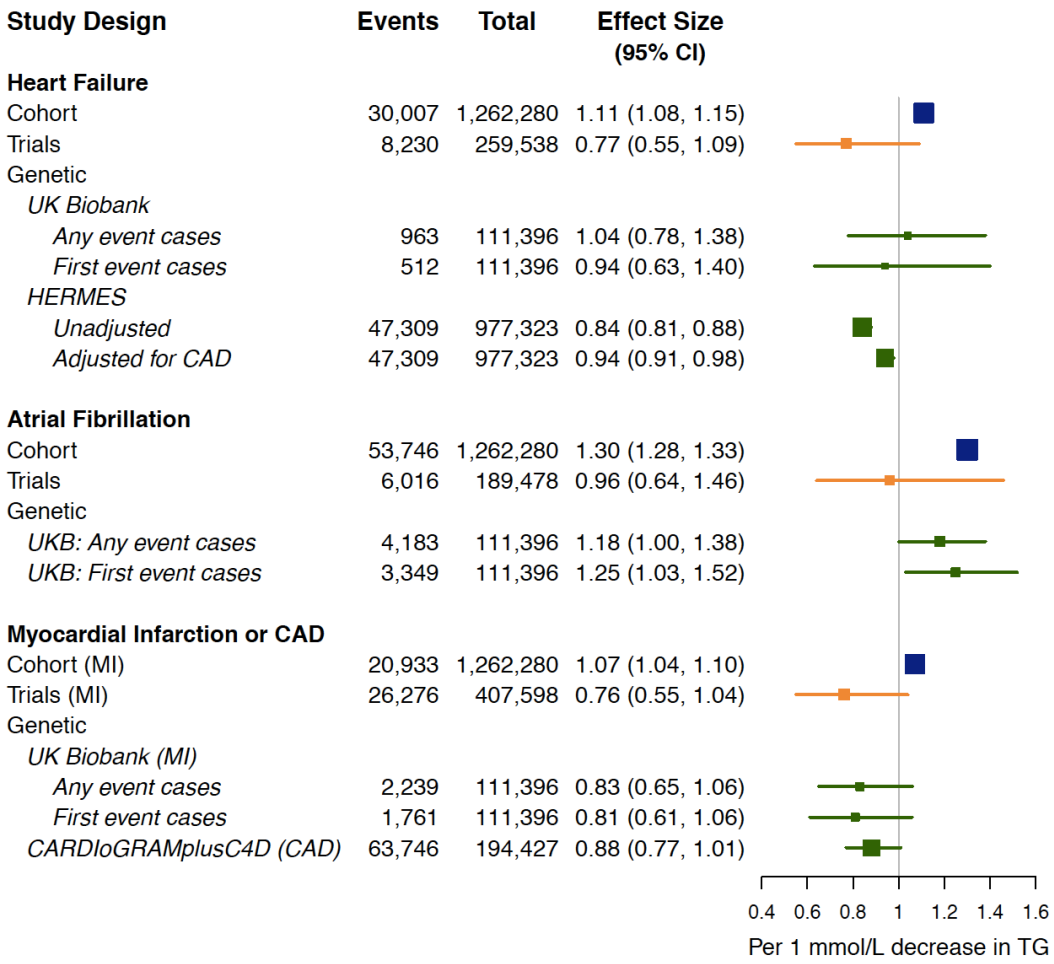


Figure 6-5 Comparison of the estimated associations between TG and the risk of HF, AF, and MI (or CAD) from the cohort (hazard ratio), RCTs (risk ratio), and MR study (odds ratio)

6.8 Chapter Supplementary

Table S 6-1 The STROBE and RECORD checklist

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 289	RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	1.1) – 1.3) Page 289
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 290-291		
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 292		
Methods					
Study Design	4	Present key elements of study design early in the paper	Page 292		
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 292		
Participants	6	*(a) Cohort study* - Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed	a) Page 292 Supplementary appendices b) Not applicable	RECORD 6.1: The methods of study population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided.	6.1) Page 292 Supplementary appendices 6.2) Page 293 (section of follow up for incident HF, AF, MI, and intercurrent CVDs)

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
		Case-control study - For matched studies, give matching criteria and the number of controls per case		RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	6.3) Figure S 6-2
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Page 293	RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Supplementary appendices Details can be found from CALIBER portal at: https://www.caliberresearch.org/portal
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 293, Details can be found from CALIBER portal at: https://www.caliberresearch.org/portal		
Bias	9	Describe any efforts to address potential sources of bias	Page 293-294		
Study size	10	Explain how the study size was arrived at	Supplementary appendices		
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 294		
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses	a) Page 293-294, b) Supplementary appendices c) Supplementary appendices d) Not applicable e) Supplementary appendices		
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population.	12.1) Page 292-293

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
				RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	12.2) Page 292-293
Linkage		..		RECORD 12.3: State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	12.3) Page 292, Refer to CALIBER portal, which includes extensive information.) Also, refer to a paper explaining the CALIBER platform (S Denaxas et al, Int J Epidemiol)
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	a) – c) Figure S 6-1	RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	Figure S 6-1
Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)	a) – b) Table 6-1 and Table S 6-3 c) Page 296-297 in a section of 'Results from an observational study (CALIBER)		
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or summary measures	Page 296-297 in a section of 'Results from an observational study (CALIBER), Figure S 6-3 and Table S 6-2		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized	a) - b) Figure 6-1		

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location in manuscript where items are reported
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	c) Not applicable		
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Table S 6-4 and Figure S 6-4		
Discussion					
Key results	18	Summarise key results with reference to study objectives	Page 300, 302		
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 305-307	RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	Page 305-307
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 307-308		
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 306		
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Not relevant		
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	Not relevant

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press..*Checklist is protected under Creative Commons Attribution (CC BY) license.

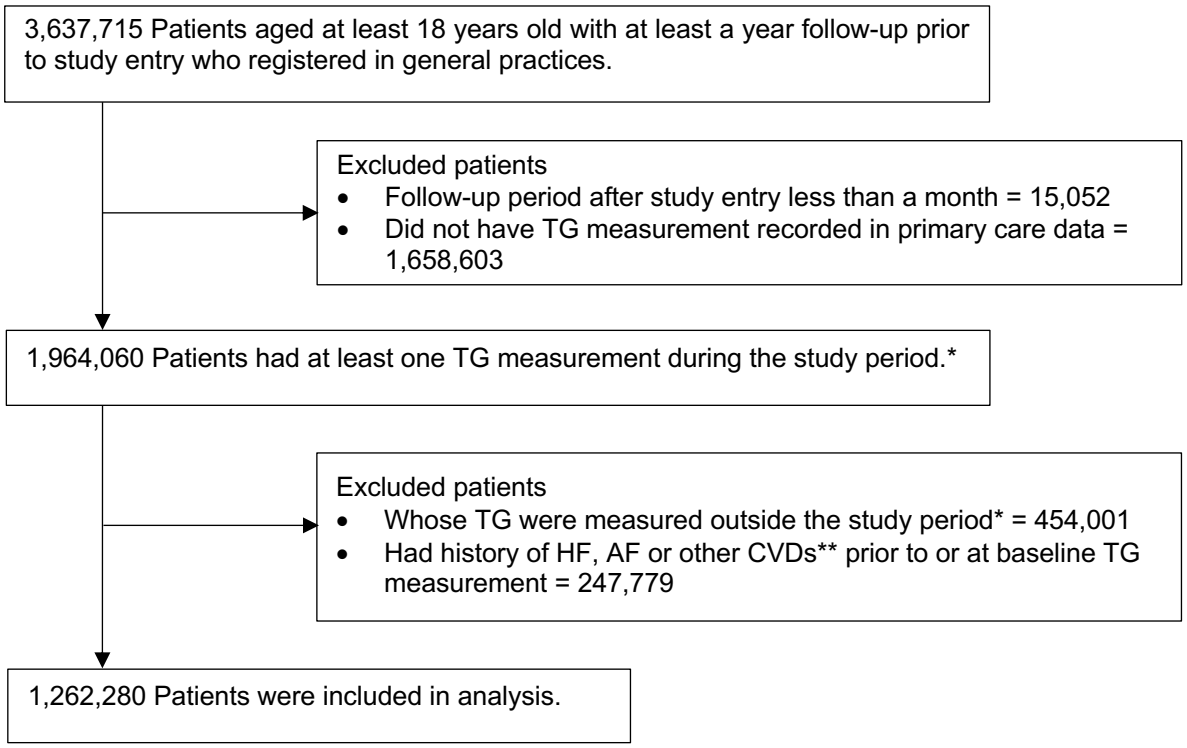


Figure S 6-1 Study flow diagram

*Study period is between 1st Jan 1997 and 30th Jun 2016.

**CVDs include 1) coronary artery disease (i.e., myocardial infarction, unstable angina, and stable angina), 2) stroke (i.e., haemorrhagic stroke, ischaemic stroke, and unclassified stroke), 3) transient ischaemic attack, 4) abdominal aortic aneurysm, 5) peripheral arterial disease, and 6) sudden cardiac arrest.

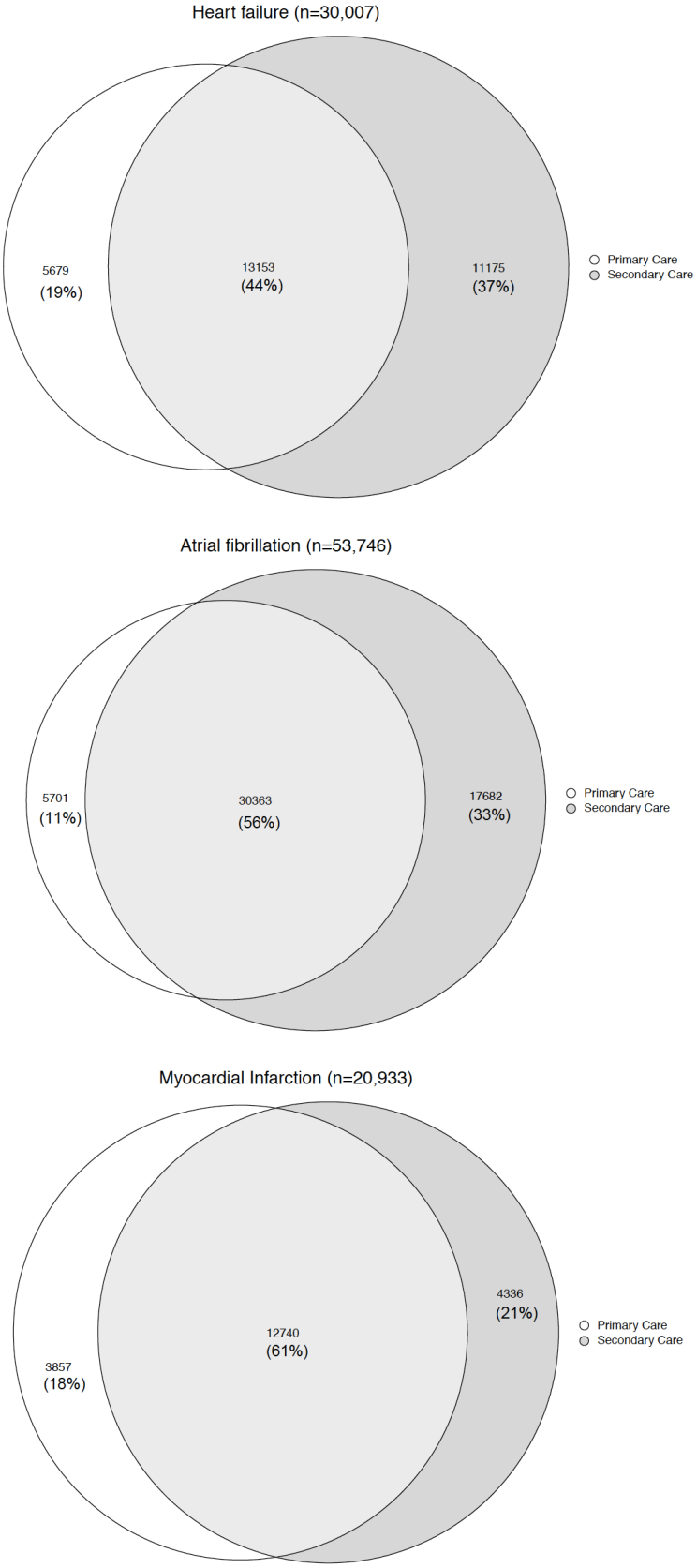


Figure S 6-2 Venn diagram to illustrate linkage process of HF (top) AF (middle) and MI (bottom)

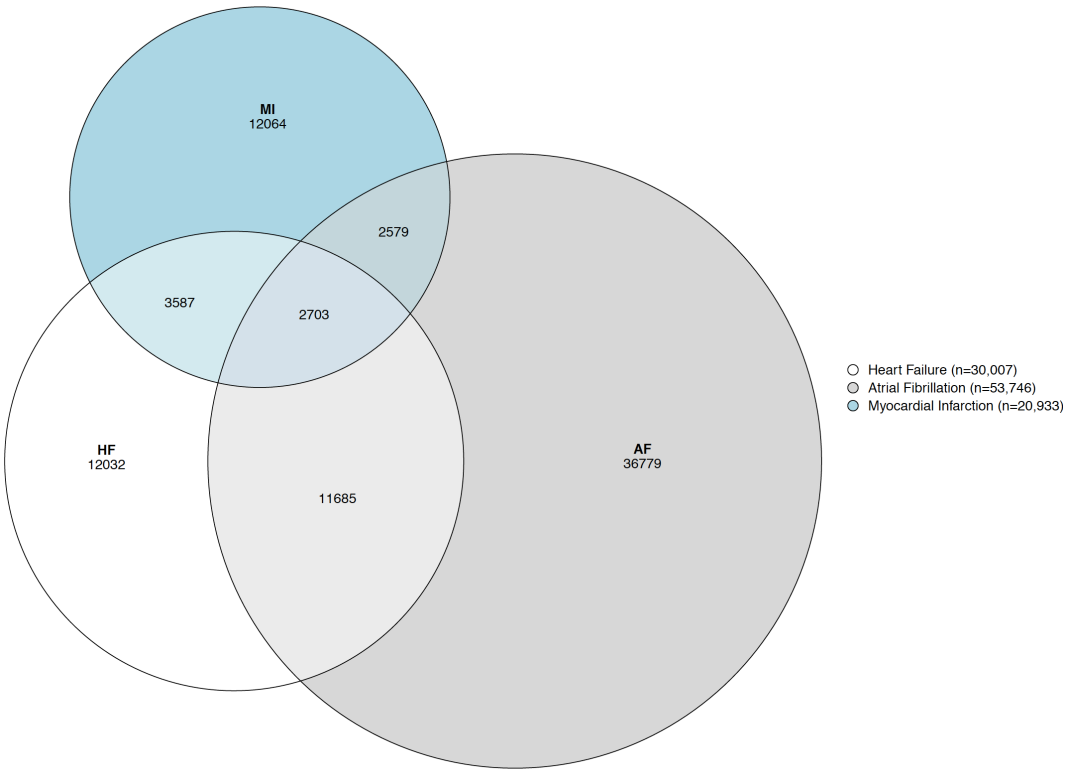


Figure S 6-3 Venn diagram for incident MI, HF, and AF in 1,262,280 CALIBER cohorts

Note: Median follow-up time was 5.3 years (IQR: 0.1 to 19.5 years)

Table S 6-2 Incidence rate of HF and AF by TG levels

TG levels (mmol/L)	< 0.85	0.85 – 1.12	1.13 – 1.40	1.41 – 1.68	1.69 – 2.25	2.26 – 2.81	> 2.81	Total
Heart failure								
Events	4,013	5,504	5,203	3,343	5,758	2,811	3,375	30,007
Person-years	1,399,747	1,426,284	1,240,284	787,077	1,286,232	643,300	760,825	7,543,757
Incidence rate (95%CI)	2.86 (2.77, 2.95)	3.85 (3.75, 3.96)	4.19 (4.08, 4.31)	4.24 (4.10, 4.39)	4.47 (4.36, 4.59)	4.36 (4.21, 4.53)	4.43 (4.28, 4.58)	3.97 (3.93, 4.02)
Atrial fibrillation								
Events	8,503	10,888	9.663	6,006	9,582	4,470	4,634	53,746
Person-years	1,382,954	1,405,571	1,222,416	776,338	1,270,559	636,530	755,338	7,449,706
Incidence rate (95%CI)	6.14 (6.01, 6.28)	7.74 (7.60, 7.89)	7.90 (7.74, 8.06)	7.73 (7.54, 7.93)	7.54 (7.39, 7.69)	7.02 (6.81, 7.23)	6.13 (5.96, 6.31)	7.21 (7.15, 7.27)

Note: Incidence rate per 1,000 person-years

Table S 6-3 Full participant characteristics of CALIBER population-based EHR cohort (n= 1,262,280) according to TG values at baseline

Baseline	Triglyceride levels at baseline (mmol/L)								P-values
	< 0.85	0.85 - 1.12	1.13 - 1.40	1.41 - 1.68	1.69 - 2.25	2.26 - 2.81	> 2.81	Total	
N	250,849	246,810	208,777	128,772	207,805	101,611	117,656	1,262,280	
Male	90,234 (36.0%)	103,812 (42.1%)	96,412 (46.2%)	63,141 (49.0%)	108,524 (52.2%)	57,642 (56.7%)	76,732 (65.2%)	596,497 (47.3%)	P < 0.001 P_{trend} < 0.001
Female	160,615 (64.0%)	142,998 (57.9%)	112,365 (53.8%)	65,631 (51.0%)	99,281 (47.8%)	43,969 (43.3%)	40,924 (34.8%)	665,783 (52.7%)	
Age (year)	52.8 (13.3)	56.0 (13.7)	56.8 (13.6)	57.0 (13.3)	56.7 (13.0)	55.9 (12.6)	54.0 (12.0)	55.5 (13.3)	P < 0.001 P_{trend} < 0.001
Ethnicity									
Caucasian	110,071 (83.7%)	122,852 (88.3%)	109,943 (89.7%)	69,729 (90.4%)	115,596 (91.0%)	57,628 (91.5%)	67,752 (91.7%)	653,571 (89.0%)	P < 0.001 P_{trend} < 0.001[$]
South Asian	6,759 (5.1%)	7,375 (5.3%)	6,767 (5.5%)	4,235 (5.5%)	7,043 (5.5%)	3,622 (5.7%)	4,179 (5.7%)	39,980 (5.4%)	
African	11,184 (8.5%)	5,856 (4.2%)	3,380 (2.8%)	1,653 (2.1%)	1,966 (1.5%)	686 (1.1%)	659 (0.9%)	25,384 (3.5%)	
Other	3,507 (2.7%)	3,025 (2.2%)	2,481 (2.0%)	1,517 (2.0%)	2,355 (1.9%)	1,077 (1.7%)	1,256 (1.7%)	15,218 (2.1%)	
Missing	119,328 (47.6%)	107,702 (43.6%)	86,206 (41.3%)	51,638 (40.1%)	80,845 (38.9%)	38,598 (38.0%)	43,810 (37.2%)	528,127 (41.8%)	
Health behaviors, physical and laboratory measurements at baseline									
Smoking									
Non-smokers	164,600 (66.1%)	150,676 (61.5%)	121,762 (58.8%)	72,909 (57.1%)	112,979 (54.8%)	52,306 (51.9%)	54,558 (46.8%)	729,790 (58.3%)	P < 0.001 P_{trend}< 0.001[$$]
Ex-smokers	54,009 (21.7%)	58,664 (23.9%)	52,255 (25.2%)	33,494 (26.2%)	56,199 (27.3%)	28,227 (28.0%)	33,830 (29.0%)	316,678 (25.3%)	
Current smokers	30,479 (12.2%)	35,633 (14.5%)	33,133 (16.0%)	21,340 (16.7%)	36,874 (17.9%)	20,168 (20.0%)	28,117 (24.1%)	205,744 (16.4%)	
Missing	1,761 (0.7%)	1,837 (0.7%)	1,627 (0.8%)	1,029 (0.8%)	1,753 (0.8%)	910 (0.9%)	1,151 (1.0%)	10,068 (0.8%)	
Body mass index (kg/m^2)	25.4 (4.8)	26.8 (5.3)	27.9 (5.5)	28.5 (5.6)	29.2 (5.6)	29.7 (5.5)	30.0 (5.3)	27.8 (5.6)	P < 0.001 P_{trend} < 0.001
Missing	12,645 (5.0%)	12,134 (4.9%)	9,663 (4.6%)	5,930 (4.6%)	9,027 (4.3%)	4,174 (4.1%)	4,692 (4.0%)	58,265 (4.6%)	
Systolic blood pressure (mmHg)	130.8 (17.3)	134.9 (17.1)	137.0 (16.8)	138.1 (16.5)	138.9 (16.3)	139.5 (16.0)	139.9 (15.8)	136.2 (17.0)	P < 0.001 P_{trend} < 0.001

Baseline	Triglyceride levels at baseline (mmol/L)								P-values
	< 0.85	0.85 - 1.12	1.13 - 1.40	1.41 - 1.68	1.69 - 2.25	2.26 - 2.81	> 2.81	Total	
Missing	3,388 (1.4%)	2,750 (1.1%)	2,082 (1.0%)	1,276 (1.0%)	1,795 (0.9%)	817 (0.8%)	1,026 (0.9%)	13,134 (1.0%)	
Diastolic blood pressure (mmHg)	78.7 (9.5)	80.5 (9.3)	81.5 (9.2)	82.2 (9.1)	82.8 (9.0)	83.4 (8.9)	84.1 (8.9)	81.4 (9.4)	P < 0.001 P_{trend} < 0.001
Missing	3,388 (1.4%)	2,750 (1.1%)	2,082 (1.0%)	1,276 (1.0%)	1,795 (0.9%)	817 (0.8%)	1,026 (0.9%)	13,134 (1.0%)	
eGFR (mL/min/1.73m^2)	78.4 (18.6)	81.5 (19.5)	83.4 (21.1)	84.7 (22.3)	85.5 (21.6)	86.5 (21.6)	87.6 (22.4)	83.2 (20.9)	P < 0.001 P_{trend} < 0.001
Missing	11,134 (4.4%)	8,858 (3.6%)	6,602 (3.2%)	3,859 (3.0%)	5,981 (2.9%)	2,758 (2.7%)	3,096 (2.6%)	42,288 (3.4%)	
CRP (mg/L)									
Mean (SD)	7.7 (19.5)	9.2 (22.0)	9.9 (23.3)	10.4 (22.9)	10.3 (22.5)	10.5 (23.1)	10.6 (23.0)	9.5 (22.1)	
Median (IQR)	3.0 (1.0-5.4)	3.8 (1.9-7.0)	4.0 (2.0-8.0)	4.9 (2.0-8.3)	5.0 (2.0-8.8)	5.0 (2.3-9.0)	5.0 (2.5-9.0)	4.0 (2.0-8.0)	P < 0.001 P_{trend} < 0.001
Missing	141,309 (56.3%)	138,645 (56.2%)	117,793 (56.4%)	72,511 (56.3%)	117,756 (56.7%)	57,664 (56.7%)	67,377 (57.3%)	713,055 (56.5%)	
LDL cholesterol (mmol/L)	2.9 (0.8)	3.3 (0.9)	3.4 (0.9)	3.5 (0.9)	3.6 (1.0)	3.6 (1.0)	3.3 (1.1)	3.3 (0.9)	P < 0.001 P_{trend} < 0.001
Missing	32,259 (12.9%)	34,035 (13.8%)	30,373 (14.5%)	19,491 (15.4%)	32,424 (15.6%)	16,909 (16.6%)	31,720 (27.0%)	197,211 (15.6%)	
HDL cholesterol (mmol/L)	1.7 (0.5)	1.6 (0.4)	1.5 (0.4)	1.4 (0.4)	1.3 (0.3)	1.2 (0.3)	1.1 (0.3)	1.5 (0.4)	P < 0.001 P_{trend} < 0.001
Missing	19,222 (7.7%)	19,532 (7.9%)	17,029 (8.2%)	10,739 (8.3%)	17,534 (8.4%)	8,943 (8.8%)	10,787 (9.2%)	103,786 (8.2%)	
Total cholesterol (mmol/L)	5.0 (0.9)	5.3 (1.0)	5.5 (1.0)	5.6 (1.0)	5.7 (1.0)	5.9 (1.0)	6.1 (1.1)	5.5 (1.0)	P < 0.001 P_{trend} < 0.001
Missing	15,352 (6.1%)	13,158 (5.3%)	11,345 (5.4%)	7,266 (5.6%)	10,343 (5.0%)	5,239 (5.2%)	5,987 (5.1%)	68,690 (5.4%)	
Triglyceride (mmol/L)									
Mean (SD)	0.7 (0.1)	1.0 (0.1)	1.3 (0.1)	1.5 (0.1)	1.9 (0.2)	2.5 (0.2)	4.2 (1.9)	1.6 (1.1)	
Median (IQR)	0.7 (0.6-0.8)	1.0 (0.9-1.1)	1.3 (1.2-1.4)	1.5 (1.5-1.6)	1.9 (1.8-2.1)	2.5 (2.4-2.6)	3.6 (3.1-4.4)	1.3 (0.9-1.9)	P < 0.001 P_{trend} < 0.001
Health conditions at baseline									
Diabetes type 1	714 (0.3%)	647 (0.3%)	437 (0.2%)	254 (0.2%)	457 (0.2%)	203 (0.2%)	437 (0.4%)	3,149 (0.2%)	P < 0.001 P_{trend} = 0.011$^{\#}$
Diabetes type 2	5,010 (2.0%)	7,768 (3.1%)	9,064 (4.3%)	6,804 (5.3%)	13,450 (6.5%)	8,162 (8.0%)	13,039(11.1%)	63,297 (5.0%)	P < 0.001 P_{trend}< 0.001$^{\#\#}$
Chronic kidney disease	6,165 (2.5%)	7,885 (3.2%)	7,374 (3.5%)	4,694 (3.6%)	7,750 (3.7%)	3,558 (3.5%)	3,825 (3.3%)	41,251 (3.3%)	P < 0.001 P_{trend} < 0.001
Cancer	7,219 (2.9%)	8,589 (3.5%)	7,585 (3.6%)	4,635 (3.6%)	7,294 (3.5%)	3,325 (3.3%)	3,588 (3.0%)	42,235 (3.3%)	P < 0.001 P_{trend} < 0.001

Baseline	Triglyceride levels at baseline (mmol/L)								P-values
	< 0.85	0.85 - 1.12	1.13 - 1.40	1.41 - 1.68	1.69 - 2.25	2.26 - 2.81	> 2.81	Total	
COPD	3,336 (1.3%)	4,479 (1.8%)	4,011 (1.9%)	2,453 (1.9%)	3,942 (1.9%)	1,943 (1.9%)	2,092 (1.8%)	22,256 (1.8%)	P < 0.001 P_{trend} < 0.001
Medications									
Statins (at baseline)	15,585 (6.2%)	23,699 (9.6%)	25,206 (12.1%)	17,807 (13.8%)	33,401 (16.1%)	19,311 (19.0%)	27,772 (23.6%)	162,781 (12.9%)	P < 0.001 P_{trend} < 0.001
Statins (during follow-up)	38,826 (15.5%)	59,692 (24.2%)	64,418 (30.9%)	46,016 (35.7%)	85,868 (41.3%)	48,675 (47.9%)	66,635 (56.6%)	410,130 (32.5%)	P < 0.001 P_{trend} < 0.001
Other lipid-lowering drugs	855 (0.3%)	1,108 (0.4%)	1,064 (0.5%)	751 (0.6%)	1,504 (0.7%)	1,025 (1.0%)	2,370 (2.0%)	8,677 (0.7%)	P < 0.001 P_{trend} < 0.001
Antihypertensive drugs	49,398 (19.7%)	65,759 (26.6%)	63,939 (30.6%)	42,936 (33.3%)	73,078 (35.2%)	36,989 (36.4%)	44,113 (37.5%)	376,212 (29.8%)	P < 0.001 P_{trend} < 0.001
Antiplatelet drugs	10,435 (4.2%)	13,915 (5.6%)	13,575 (6.5%)	8,844 (6.9%)	14,604 (7.0%)	7,302 (7.2%)	8,365 (7.1%)	77,040 (6.1%)	P < 0.001 P_{trend} < 0.001

Note: Values are presented as numbers (percentage) or mean (standard deviation) or median (interquartile range) as appropriate. Corresponding values for TG are: 0.85 mmol/L = 75.28 mg/dL; 1.12 mmol/L = 99.20 mg/dL, 1.40 mmol/L = 124.00 mg/dL, 1.68 mmol/L = 148.80 mg/dL, 2.25 mmol/L = 199.28 mg/dL, 2.81 mmol/L = 248.88 mg/dL.To convert mmol/L of cholesterol (i.e., total cholesterol, LDL-C, and HDL-C) and triglyceride to mg/dL, multiply by the factor of 38.67 and 88.57, respectively. $^{\$}$P trend for Caucasian vs other ethnicity $^{\$\$}$P trend for non-smokers vs others (ex-smokers and current smokers). $^{\#}$P trend for type 1 diabetes vs no diabetes $^{\#\#}$P trend for type 2 diabetes vs no diabetes

Abbreviations: CRP; C-Reactive Protein, eGFR; estimated glomerular filtration rate, COPD; chronic obstructive pulmonary disease, HDL; high density lipoprotein, LDL; low density lipoprotein.

Table S 6-4 Serial adjusted hazard ratio (HR) of the association between TG and incident MI

Model	Adjustment	HR (95% CI) per one log TG (mmol/L) decrease	Approach to analysis (Total = 1,262,280, Event = 20,933)
1	Unadjustment, unstratified	0.59 (0.58, 0.61)	Complete-case
2	Unadjustment, stratified by sex and practice	0.66 (0.64, 0.67)	Complete-case
3	Model 2 + additional adjustment for demographic data	0.60 (0.59, 0.62)	Complete-case
4	Model 3 + additional adjustment for health risks	0.65 (0.63, 0.67)	Multiple imputations
5	Model 4 + additional adjustment for chronic conditions	1.07 (1.04, 1.10)	Multiple imputations
5.1	Model 4 + additional adjustment for type 2 DM	0.67 (0.66, 0.69)	Multiple imputations
5.2	Model 4 + additional adjustment for use of statins	0.91 (0.88, 0.94)	Multiple imputations
5.3	Model 4 + additional adjustment for use of antihypertensive agents	0.66 (0.64, 0.67)	Multiple imputations
5.4	Model 4 + additional adjustment for HDL-C	0.74 (0.72, 0.76)	Multiple imputations
5.5	Model 4 + additional adjustment for LDL-C	0.67 (0.65, 0.69)	Multiple imputations
5.6	Model 4 + additional adjustment for CRP	0.65 (0.64, 0.67)	Multiple imputations

Note: Demographic data included age, age^2, and quintile of index of multiple deprivation; Health risks included BMI, smoking, and systolic and diastolic blood pressure; Chronic conditions included type 2 diabetes, treatment with antihypertensive agents, treatment with statins, HDL-C, LDL-C, and CRP levels at baseline

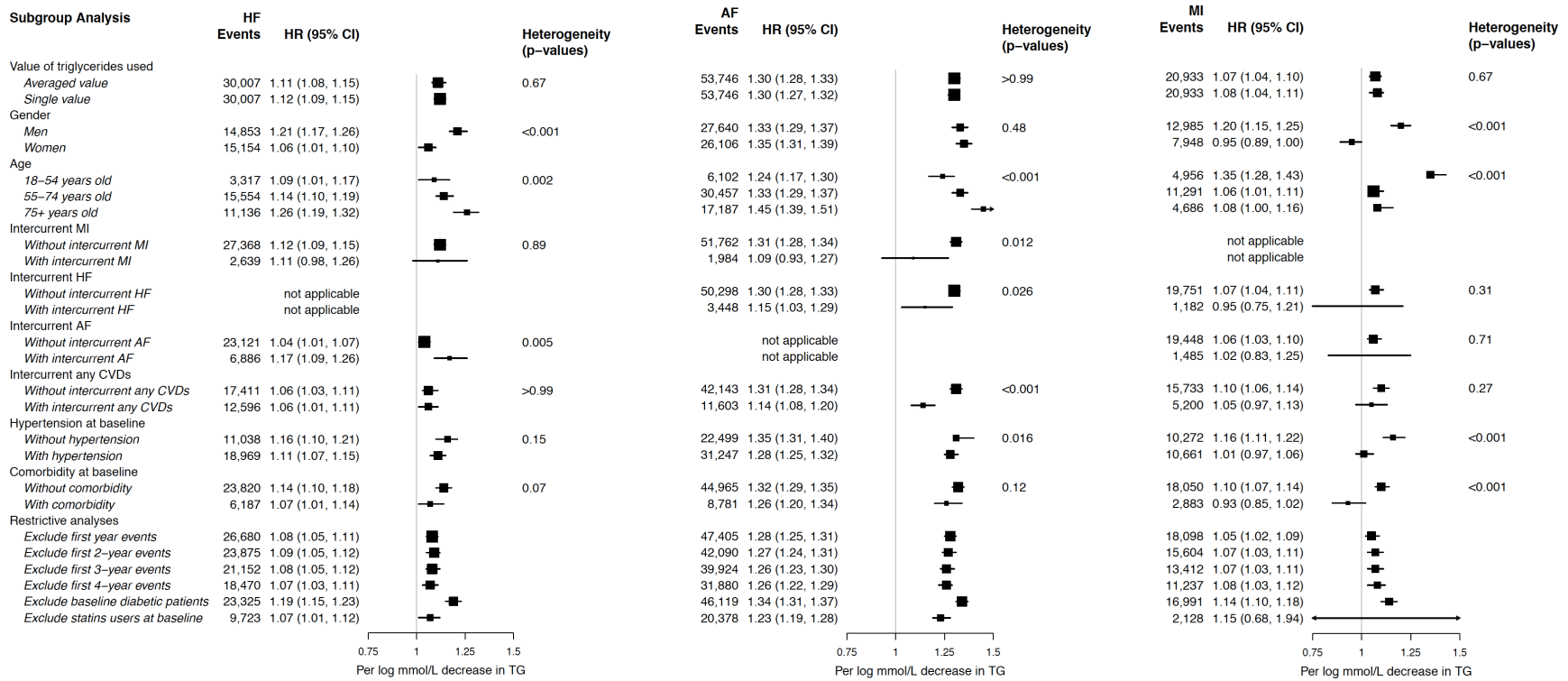


Figure S 6-4 Subgroup analyses of associations between TG and incident HF, AF, and MI

All models are stratified for gender and primary care practice and adjusted for age, body mass index, smoking, systolic blood pressure, diabetes, socioeconomic status, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, use of blood pressure lowering drugs and use of statins, and TG was logarithmic transformation. Baseline comorbidities include having diagnosed with cancer, kidney disease, and COPD at baseline LDL-C measurement. Any CVDs included acute MI, coronary revascularisation, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, peripheral arterial disease, ventricular fibrillation, heart failure (excluded in HF outcome), and atrial fibrillation (excluded in AF outcome).

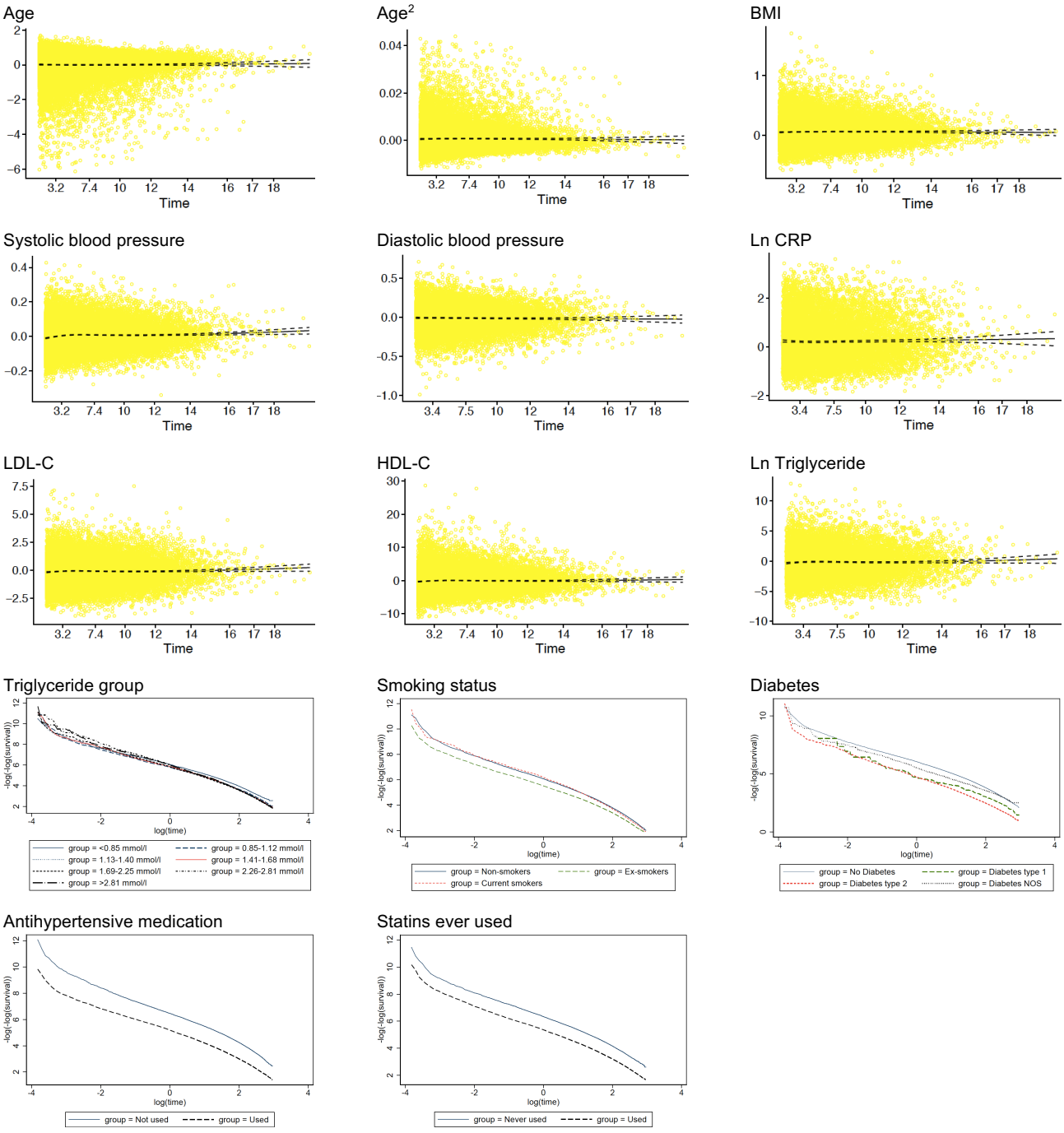


Figure S 6-5 Schoenfeld residual and proportional hazard plots on HF outcome

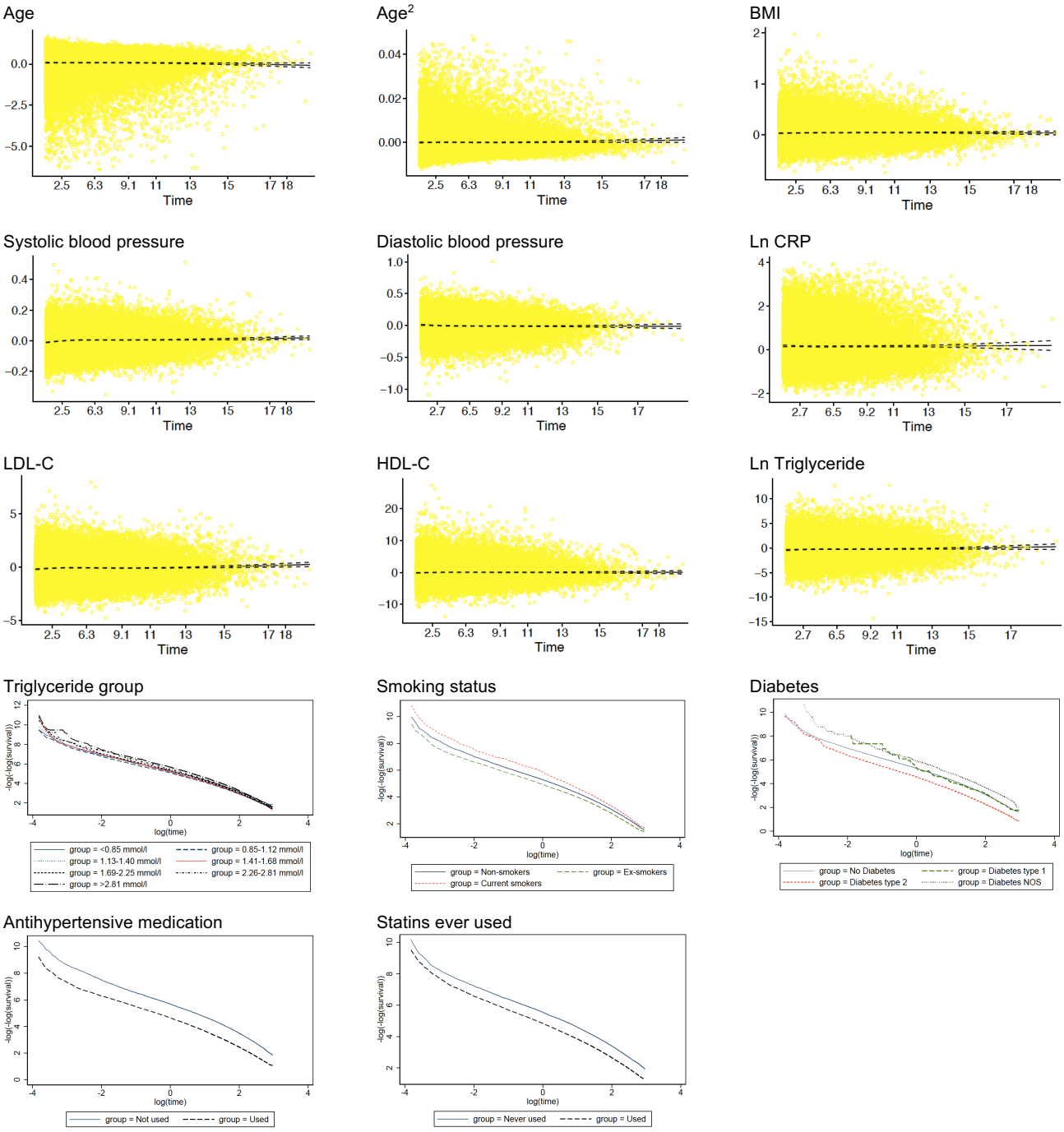
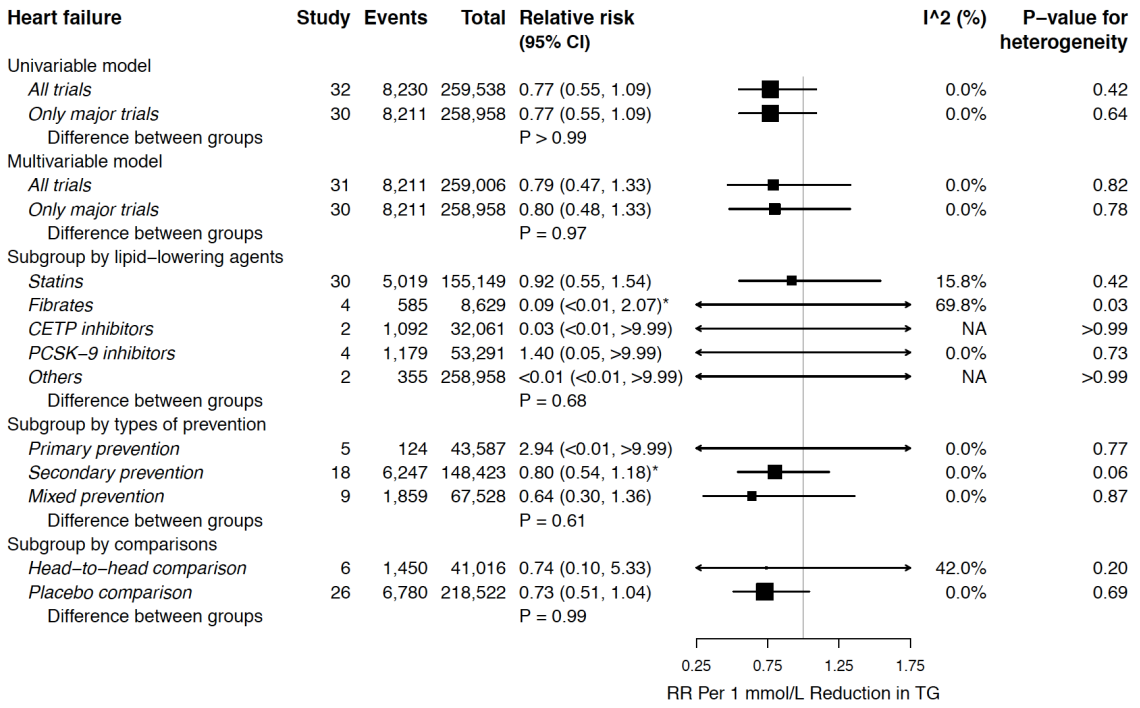


Figure S 6-6 Schoenfeld residual and proportional hazard plots on AF outcome



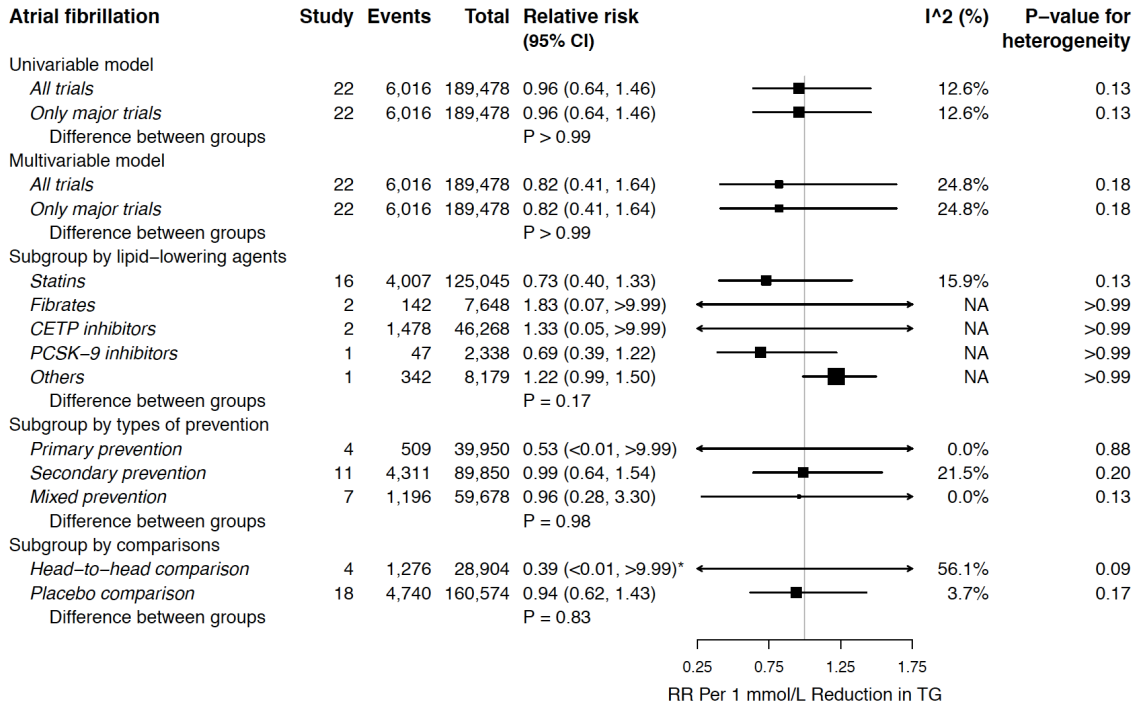


Figure S 6-7 Sensitivity and subgroup analysis of meta-regression of the change in TG levels and the risk of HF (top) and AF (bottom)

*Random effect model

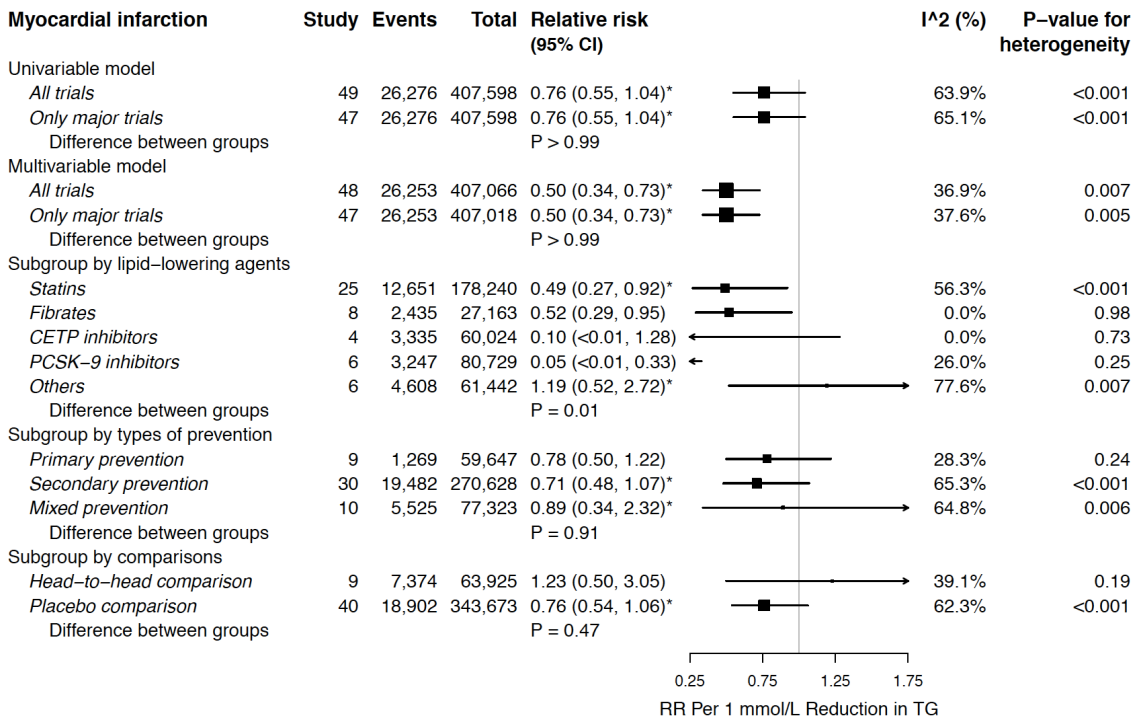


Figure S 6-8 Sensitivity and subgroup analysis of meta-regression of the change in TG levels and the risk of MI

*Random effect model

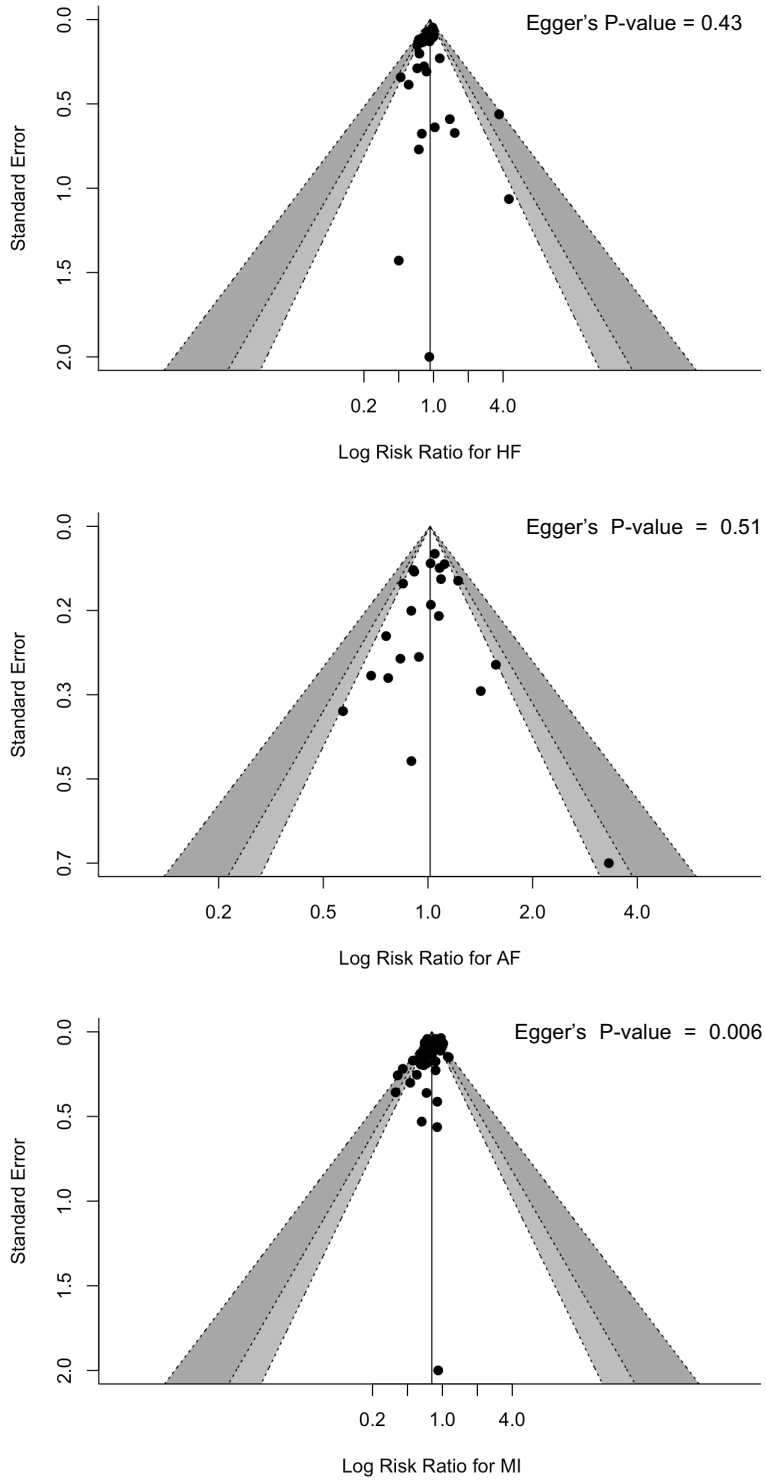
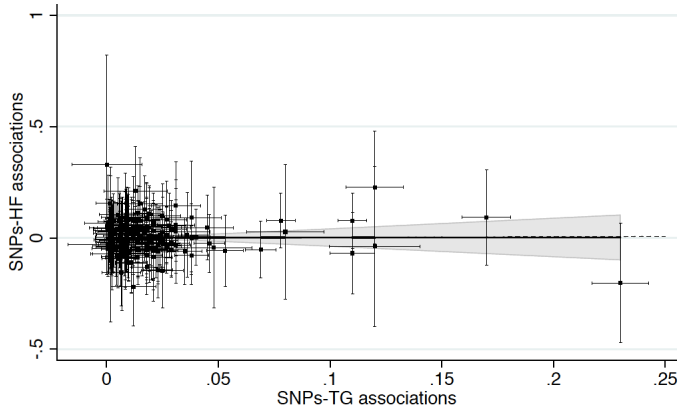


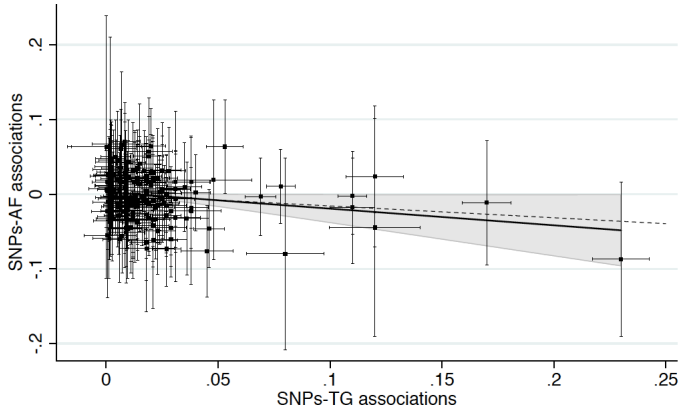
Figure S 6-9 Funnel plot of RCTs of HF (top), AF (middle), and MI (bottom) outcomes

Note: grey shade and dark grey shade represent areas of 95 and 99 % confidence interval, respectively.

Heart failure (156 SNPs)



Atrial fibrillation (156 SNPs)



Myocardial infarction (156 SNPs)

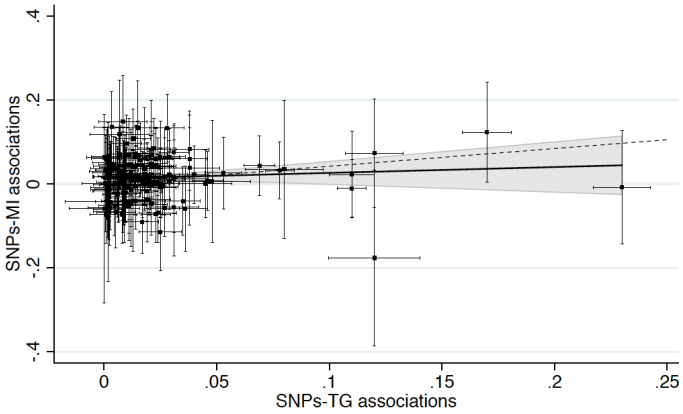


Figure S 6-10 Scatter plots of genotype-TG associations versus genotype-outcome associations

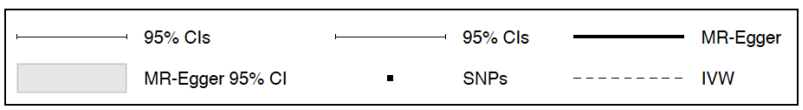


Table S 6-5 Testing for horizontal pleiotropy

Outcomes	Egger-intercept coefficient (p-values)	Q statistics, degree of freedom (p-values)
Heart failure (UK Biobank: 156 SNPs)	9.4×10^{-4} (0.90)	125.91, 155 (0.95)
Heart failure (HERMES: 110 SNPs)	-2.11×10^{-4} (0.92)	Not available
Atrial fibrillation (156 SNPs)	2.86×10^{-3} (0.37)	176.09, 155 (0.11)
Myocardial infarction (156 SNPs)	**1.24×10^{-2} (0.008)**	**214.06, 155 (0.001)**
Coronary artery disease (105 SNPs)	5.18×10^{-3} (0.21)	**215.63, 104 (<0.001)**

Note: Significant coefficient and p-value (embolden figure) suggest potential horizontal pleiotropy. Significant Q statistics p-values might suggest either directional or balanced pleiotropy.

Table S 6-6 Power calculation of MR at two-sided alpha of 0.05

Parameter	HF (UKB)		AF (UKB)		MI (UKB)		CAD (CARDIoGRAM plusC4D)
	Any event	First event	Any event	First event	Any event	First event	
Number of cases*	963	512	4,183	3,349	2,239	1,761	63,746
Number of controls*	110,433	110,884	107,213	108,047	109,157	109,635	130,681
Odds ratio to be detected	1.04	0.94	1.18	1.25	0.83	0.81	0.88
R^2 (SNPs TG)	0.056	0.056	0.056	0.056	0.056	0.056	0.067
Calculated power	4.71%	5.16%	70.03%	85.31%	54.20%	54.62%	>99%

Note: *Number of cases and controls derived from SNPs-outcome consortium. Power was at two-sided alpha of 0.05 and the calculation was based on the method explained in Hermani et al.[223]

Table S 6-7 Correlation between instrumental variable effect and exposure effect

Outcomes	Correlation coefficients (p-values)
Heart failure (UK Biobank: 156 SNPs)	-6.10×10^{-3} (0.93)
Atrial fibrillation (156 SNPs)	-5.80×10^{-3} (0.94)
Myocardial infarction (156 SNPs)	-3.3×10^{-3} (0.96)
Coronary artery disease (105 SNPs)	-5.1×10^{-2} (0.60)

Note: Highly correlated coefficients and significant p-values might suggest the invalidity of InSIDE (Instrumental Strength Independent of Direct Effect) assumption

CHAPTER 7 RISK PREDICTION OF INCIDENT HEART FAILURE AND ATRIAL FIBRILLATION: THE ROLE OF BLOOD LIPIDS

7.1 Key messages

What is already known?

- The role of blood lipids in the risk prediction of coronary artery disease (CAD) has been widely investigated, and most current risk prediction tools for CAD or MI have incorporated HDL-C into their models.
- However, it has not been established whether blood lipids may play any role in the risk prediction of two of the most common cardiac diseases: HF and AF, is not established. To date, there are only two risk prediction scores that have included blood lipids in their models: one for the prediction of HF in which HDL-C was incorporated into a risk prediction model as TC/HDL-C ratio, and another one for that of AF in which HDL-C levels were dichotomised to low and high HDL-C groups.
- In Chapters 4-6, we reported strong observational associations (mostly inverse) between blood lipids and the new-onset of HF and AF, but the causality is less likely in most cases.

What does this study add?

- This is the first time electronic health record (HER) data has been used to develop risk prediction for HF and AF in healthy English populations who were free of CVD at baseline.
- LDL-C did not have a significant incremental role in the risk prediction for HF and AF.
- Adding HDL-C did not improve the performance of risk prediction for HF and AF. However, it significantly improved the risk prediction for MI.
- Incorporating TG into the model can modestly improve the risk prediction for AF in both genders.

- Our risk prediction model showed a good discrimination metrics based on internally validated cohorts (C-statistic > 0.75), but slightly overfitted the data.
- Patients who were at low risk of MI might still face an increased risk of HF and AF. Therefore, clinicians should be aware of the different cardiovascular risk profiles of patients since these might inform distinct primary preventive strategies.

7.2 Abstract

Introduction: Previous chapters have shown strong associations between blood lipids and incident HF and AF. Although genetic and trial evidence does not support a causal role in most cases, whether lipids may add incrementally to risk prediction of HF and AF is uncertain.

Design, setting, and participants: We tapped the linked electronic health records (EHRs) of around three million participants aged at least 18 years old, registered in 387 general practices across England, without a previous medical history of cardiovascular diseases at baseline lipids measurements. Cohorts were followed-up between 1st January 1997 and 30th June 2016.

Main exposure: Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) recorded in primary care.

Main outcome of interest: 10-year risk of incident HF, AF, and MI

Results: Based on the complete case analysis of around 450,000 individuals with a median follow-up time for 6 years (interquartile range 3-9 years), we identified up to 14,114 HF cases, 25,060 AF cases, and 8,967 MI cases. We used a gender-specific Cox-model, which incorporated age, age^2, socioeconomic status, smoking status, body mass index, systolic blood pressure, diastolic blood pressure, HDL-C, LDL-C, TG, C-reactive protein, diabetes, use of antihypertensive agents, and use of statins. The model was internally validated using a geographically split approach, and the results showed a good discrimination (C-statistic > 0.75) but slightly overfitted the data (calibration slope < 1). We found that LDL-C and HDL-C did not improve the risk prediction of HF and AF. Adding TG, however, showed modest, but statistically significant, improvement on the risk prediction of AF. Likewise,

HDL-C was shown to significantly contribute to the risk prediction of MI. Moreover, we also noticed that almost 15% of patients who were at low risk of MI (i.e., 10-year risk for MI < 5%) still had an increased risk of HF (i.e., 10-year risk for HF > 5%), and more than one-third of individuals at low risk of MI still carried an increased risk of AF.

Conclusion: Our findings suggested that blood lipids have no role in the risk prediction of incident HF and AF, with the exceptions of TG in the risk prediction of incident AF, and the role HDL-C in that of incident MI. Furthermore, patients with a low MI risk may still have a high risk of HF and AF, and this would help inform different primary prevention strategies adopted by clinicians.

7.3 Introduction

In previous chapters, observational findings robustly indicated strong associations between blood lipids and the incidence of heart failure (HF) and atrial fibrillation (AF). Despite the findings that a causal role is not always corroborated, it is still worth investigating further whether blood lipids may add incremental information in the risk prediction of incident HF and AF. It is recognised that general biomarkers may still help in predicting the risk of developing disease, or to stratify patients into subgroups that are suitable for different therapies, even though there is no causal reletionship between those biomarkers and the disease. A good example is the utilisation of HDL-C for the risk prediction of CVD. Although previous trials and genetic studies suggested that HDL-C is not causally relevant to CVD,[36] most of the risk scores for CVD still incorporated HDL-C into their models and showed good model discrimination (C-statistic 0.70-0.88, see Table S 7-1).

Risk prediction tool for HF and AF may help devise and evaluate strategies for primary prevention, such as tailoring personalised medicine, and monitoring for early diagnosis. However, no such tool is currently used in clinical practice. Moreover, to date, only a few risk predictive models for HF and AF that take into account for lipid fractions (Table S 7-1). One predictive model for the 10-year risk of incident HF in diabetic patients incorporated HDL-C (as a total cholesterol per HDL-C ratio) into the model since it was significantly associated

with the incidence of HF in both genders.[245] The other study showed that adding HDL-C as a binary variable (i.e., low levels [< 40 mg/dL for male and < 50 mg/dL for female] vs. high levels of HDL-C) can improve model discrimination for the risk of incident AF: C-statistic increased from 0.697 (0.612–0.782) to 0.728 (0.645–0.811).[107] Therefore, here a research gap has been identified, indicating that the role of blood lipids in the risk prediction for both HF and AF still requires further investigation.

It is not known whether patients may be concurrently at low risk of MI while at high risk of HF and AF; such discordance might inform different treatment decisions (e.g., the choice of blood pressure lowering therapy).

In this chapter, we primarily aim to examine the role of blood lipids in the risk prediction of incident HF and AF. The specific objectives are: i) to develop and validate (geographical split) a clinically implementable model (used for analyses in Chapter 4-6) for the prediction of the risk of new-onset HF and AF at 10 years. This would imply whether EHRs can be used to predict HF and AF, ii) to examine whether adding blood lipids (i.e., LDL-C, HDL-C, and TG) improves model performance on the risk prediction of HF and AF, and iii) to compare the extent to which people at low risk of MI are at high risk of HF and AF or vice versa.

7.4 Methodology

7.4.1 Linked EHRs

The studied populations were taken from the linkage EHRs platform called CALIBER (ClinicAl research using LInked Bespoke studies and Electronic health Records) platform.[170] The link had been performed across primary care data (general practices [GPs] from Clinical Practice Research Datalink [CPRD][171]), secondary care data (hospital admission), and death registry (Office for National Statistic: ONS). CPRD is a database containing the EHRs of 10.5 million people from 548 GPs across the UK from which 411 GPs consented to the linkage.[171] It has been shown that the population drawn from CPRD is unselected and representative of the general English population in terms of age, sex, and overall mortality.[171,188] Details of the CALIBER platform are described in Chapter 3 and in the supplementary appendices. Approval of

this study was granted by the Independent Scientific Advisory Committee (ISAC) of the Medicines and Healthcare products Regulatory Agency (protocol number 12_153RARMnAR).

7.4.2 Population

In this study, we initially included 3.6 million patients from 387 GPs across England from the CPRD database. We identified all patients aged 18 years or older who registered between 1st January 1997 and 30th June 2016 and had followed-up with their GPs for at least one year. We excluded individuals with a history of CVDs at the baseline lipid measurement, including heart failure, atrial fibrillation, ventricular fibrillation, myocardial infarction, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, peripheral arterial disease, or had previously undergone coronary revascularisation.

Cases with baseline CVD were excluded because of three reasons. First, I mainly aim to study the association between blood lipids and incidence of HF and AF amongst relatively healthy populations, which are scarcely studied. Second, excluding CVD at baseline can minimise the potential confounder due to reverse causation (i.e., having HF or AF might endogenously affects lipid levels). Since HF and AF may occurred at the subclinical stage and having other CVDs can increase the risk of subclinical HF and AF. Therefore, patients with CVD at baseline should be excluded from the analysis. Third, one objective of this chapter is to validate the model used in chapter 4-6. Therefore, studied population should be consistent across chapters. Moreover, I did not aim to develop a new risk predictive tool for HF and AF. So, the interval validity seems to be more important than the external validity (i.e., generalisability) in this case.

7.4.3 Potential predictive factors

As described in Chapters 4-6, baseline covariates taken from the closest record to the baseline lipid measurement date were selected based on previous studies, regardless of their statistical significance shown in our regression model.[50,52,246–250] Included variables were as follows: age, age

squared, gender, socioeconomic status (in terms of the quintiles of the index of multiple deprivation), smoking status, body mass index, systolic blood pressure, diastolic blood pressure, LDL-C, HDL-C, TG (logarithmic transformation), C-reactive protein (logarithmic transformation), diabetes, use of antihypertensive medications, and use of statins. A complete list of codes used to identify all covariates in this study can be found at https://www.caliberresearch.org/ portal.

7.4.4 Blood lipids and endpoints

As described in Chapters 4-6, we used ambulatory care low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) measurement sampled in clinics and hospital out-patients and electronically recorded in the primary care. All values were converted to serum values and removed for outliers. Since more than one fourth of participants had more than one lipid measurement throughout the follow-up period, we used a yearly averaged value, and the earliest date of measurement was used as the start of follow-up.

Primary endpoints were the first occurrence of HF, AF, and MI (considered each outcome separately), which were evaluated based on diagnostic codes that were linked between hospitalised (HES: ICD-10 codes), and non-hospitalised (CPRD: Read codes) cases. Due to the fact that each individual might independently have measurement of LDL-C, HDL-C, and TG on different dates, we created separated cohorts for each lipid fraction. Patients were observed since the first time of measurement at GP (i.e., baseline lipids measurement) until the first presentation of endpoints of interest, death, or transferring out of practices, whichever occurred first. Endpoint definition and validation are described in Chapter 3.

7.4.5 Statistical analysis

<u>Development and internal validation of risk prediction model for HF and AF</u>

Model development

We evaluated associations between endpoints and predictive factors using Cox-proportional hazards models. Hazard ratio (HR) was calculated from the time of blood lipids measurement to the time of the incident event, censored

(i.e., death or transfer out of practice), or end of the follow-up, whichever occurred first. All models were stratified by sex. Logarithmic transformation was used to transform skewed variables, including TG and CRP, before analysis. All continuous variables were standardised to the mean of zero with the change per one standard deviation. In addition, model validity was visually checked for proportional hazard assumptions by using Schoenfeld residual and log(-log) plots as shown previously in Chapters 4-6.

Model validation

The geographical-split method is used to create a validation cohort. The geographical-split method was chosen instead of a random-split method. This is because the former (as parts of non-random split methods) makes validation dataset more likely to be different from the development data in some key characteristics, whereas the latter makes the validation and development dataset more comparable (i.e., no differences other than by chance) and thus the validation dataset is expected to show the same performance.[251] In other words, if a random-split method is used to derive the development and validation dataset, and the internal validation results show good performance of the model (i.e., high C-statistic, a calibration slope closes to one), we cannot distinguish whether the good performance is because of the performance of the model (which is desired) or because of similarity between development and validation dataset (which is artifact findings).

We grouped individuals from North East, North West, Yorkshire and the Humber, and East Midlands as a validation cohort. This is because people in the northern region are known to have a higher CHD risk and poorer health, compared with individuals in south.[252] Linear predictors of the validation cohort were calculated from beta-coefficient and baseline survival at 10 years directly taken from the development cohort. The linear predictor of the model was calculated as follows:

$$\begin{aligned}\text{Linear predictor} = {} & (\beta_1 * \text{LDL-C}) + (\beta_2 * \text{HDL-C}) + (\beta_3 * \log \text{TG}) + (\beta_4 * \text{age}) \\ & + (\beta_5 * \text{age}^2) + (\beta_6 * \text{IMD quintile } 2^{nd}) + (\beta_7 * \text{IMD quintile } 3^{rd}) \\ & + (\beta_8 * \text{IMD quintile } 4^{th}) + (\beta_9 * \text{IMD quintile } 5^{th}) \\ & + (\beta_{10} * \text{current smoker}) + (\beta_{11} * \text{ex-smoker}) \\ & + (\beta_{12} * \text{Systolic blood pressure}) + (\beta_{13} * \text{Diastolic blood}\end{aligned}$$

pressure) + (β_{14} * Body mass index) + (β_{15} * log C-reactive protein) + (β_{16} * Type 1 Diabetes) + (β_{17} * Type 2 Diabetes) + (β_{18} * Other types of diabetes)
+ (β_{19} * Use of antihypertensive agents)
+ (β_{20} * Use of statins)

Then the 10-year risk of event can be calculated as follows:

10-year risk of event = 1 - Baseline survival at 10 year $^{\wedge \exp(\text{Linear predictor})}$

To visually inspect model discrimination, we grouped patients into four risk groups (i.e., highest, high, low, and lowest risk) using cut-off threshold risk at the 16th, 50th and 84th percentiles and plotted each group as a cumulative risk (Y-axis) per change in the time of follow-up (X-axis). The justification for unequally sized groups is based on previous work suggesting that this can minimise information loss better than equal groupings.[253,254] We also calculated C-statistic for model discrimination. As to model calibration, we created calibration plots of observed (derived from KM estimation) versus expected (predicted) risk and calculated the calibration slope.

Examining the risk predictive role of blood lipids in HF and AF

To assess the predictive value of adding blood lipids to models, we compared various statistical metrics between the model, which were used throughout Chapters 4-6, with and without blood lipids. The compared statistical measures included the chi-squared test from the likelihood ratio statistic, Royston and Sauerbrei's adjusted R^2_D for the measurement of overall performance, calibration slope for the measurement of model agreement, and Harrell's C- and D-statistic for the measurement of model discrimination.

To visualise a model discrimination, we plotted the overlaid histograms of predicted risk between cases and non-cases, and the more separated the risk groups then the better the model discriminates. The model's calibration was further visualised by plotting bar graphs on an agreement between predicted and observed 10-year risk of event in deciles of predicted risk and by plotting the predicted and observed 10-year risk compared with a reference (diagonal) line. Degree of miscalibration can be observed from the agreement between predicted and observed risk in deciles of predicted risk and the alignment of

the plots along the reference line. We did not report p-value from extended Hosmer-Lemeshow Chi-square since the test is very sensitive to the sample size and choice of grouping and has poor interpretability due to the absence of direction or magnitude of miscalibration.[255,256]

Additionally, we calculated the net reclassification improvement (NRI) including both category based- and category free-NRIs (cfNRI). For the category-based NRI, we divided predictive risks into four groups as suggested by the recent guideline.[257] To avoid underestimation due to crossing thresholds, we also calculated the cfNRI since no threshold is required. Due to the longitudinal nature of our data, we need to take into account censoring. Therefore, we adjusted calculated NRI by using weighted Kaplan-Meier estimation at 10 years.[258] In addition, to improve statistical power, we used combined (i.e., development + validation) cohorts to assess the incremental value of adding blood lipids into the risk prediction model.

Comparing MI risk with HF and AF risk

We estimated 10-year risk for MI, HF, and AF using the model that we developed and internally validated in the previous section and grouped patients into four groups of risk levels, including <5%, 5 - < 7.5%, 7.5 - < 20%, and ≥ 20%, according to the cut-off used by the most recent clinical guideline.[257] We then constructed a 4-by-4-table comparing four risk groups between MI, HF, and AF. Because we developed 3 cohorts for each lipid fraction, the justification for the choice of cohorts should be used to compare MI, HF, and AF risk based on model discrimination and calibration from the internal validation results.

All analyses in this chapter were done based on a complete-case approach to avoid the potential missing-not-at-random (MNAR) assumption of missingness. All combined cohorts were used to compare risks. To acquire a 95% confidence interval for C-statistic, Sauerbrei's adjusted R^2_D, and NRI, we performed 500 bootstraps and use a percentile method. Details of each statistical term used in this chapter can be found in the chapter supplementary. All plots and calculations of C-statistic, D-statistics, adjusted R^2_D, calibration slope were done using STATA version 15 (StataCorp, College Station, Texas).

NRI were calculated using packages 'survNRI'[259] and 'nricens'[260] in R version 3.5.1. The two-sided p-value of < 0.05 was considered statistical significance.

7.5 Results

7.5.1 Model for the risk prediction of incident HF and AF

Baseline characteristic and development model

We observed that the development and validation cohorts had different incidence rates of HF, MI, and AF. According to Table S 7-4, we found that our development cohort had a lower incidence rate of HF and MI than the validation cohort, whereas the development cohort had a higher incidence rate of AF than the validation group. LDL-C cohort, for instance, the incidence rate of HF in the development cohort versus validation cohort was 4.61 (95%CI 4.53, 4.70) vs 5.09 (95%CI 4.92, 5.26) per 1,000 person-years. The incidence rate of MI was 2.87 (2.80, 2.94) in the development cohort and 3.41 (3.28, 3.56) in the validation cohort. For the incidence of AF, on the other hand, the figure in the development cohort was 8.64 (8.52, 8.76), which was slightly higher than that in the validation group (8.08 [7.87, 8.29] per 1,000 person-years). Moreover, this pattern is similar across different cohorts for each lipid fraction (i.e., LDL-C, HDL-C, and TG).

Baseline characteristics of participants in development and validation cohorts are shown in Table 7-1 (for LDL-C cohort) and in Table S 7-2 and Table S 7-3 (for HDL-C and TG cohort, respectively). The overall trend and pattern were similar across cohorts of three lipid fractions. In summary, approximately 60% of development cohorts was female with an average age of 57 ± 13 years. The prevalence of most of the risk factors was not clinically different between development and validation cohorts although P-value suggested was statistically significant in most cases. However, the proportion of non-smokers in development cohorts (59%) was slightly higher than that in validation cohorts (54%), whereas the proportion of patients who received statins at follow-up in the development cohorts (34-35%) was slightly lower, compared to the validation cohorts (38-39%).

During the median follow-up of 6 years (interquartile range 3 to 9 years), 6,607 incident MI cases, 10,605 incident HF cases, and 19,553 incident AF cases were identified in the development LDL-C cohort. In addition, we reported variables and their beta-coefficients in Table 7-2.

Validation of risk predictive model

Discrimination of the validation cohorts is shown in Figure S 7-1 to Figure S 7-3. In LDL-cohorts, the C-statistics (95% CI) for HF, AF, and MI outcomes were 0.798 (0.791, 0.804), 0.803 (0.796, 0.811), and 0.748 (0.741, 0.755), respectively. In HDL-C cohorts, the C-statistic (95% CI) was 0.794 (0.786, 0.802) for HF, 0.785 (0.778, 0.791) for AF, and 0.800 (0.793, 0.807) for the MI outcome. In TG cohorts, we observed a C-statistic (95%CI) of 0.805 (0.797, 0.812), 0.786 (0.779, 0.792), and 0.803 (0.796, 0.810) for HF, AF, and MI outcomes, respectively (Table 7-3). Although C-statistics suggested a decent degree of separation (i.e., >0.7), discrimination plots showed that our model can discriminate well only between individuals at high risk (i.e., predicted risk was in between 50^{th} and 84^{th} percentiles) and highest risk (i.e., $>84^{th}$ percentiles) group. Those in low risk (16^{th} - 50^{th} percentiles) and lowest risk groups ($<16^{th}$ percentiles) did not separate well, or the plots even overlapped in the MI outcome.

Regarding the calibration metrics of a 10-year risk, which was shown as calibration plots in Figure S 7-1 to Figure S 7-3. In LDL-C cohorts, the calibration slopes for HF, AF, and MI outcomes were 0.930, 0.597, 0.955, respectively. The calibration slope in HDL-C cohorts was 0.956 for HF, 0.948 for AF, and 0.958 for the MI outcome, whereas the figures for TG cohorts were 0.921, 0.960, and 0.963, for HF, AF, and MI outcomes, respectively. Overall, our models were overfitted since calibration slopes were less than 1, especially between LDL-C and AF from which the calibration slope was very low (0.597). This indicated that our models overestimated the high-risk group (i.e., individuals in the top deciles), and need further optimism adjustment.

7.5.3 Predictive value of adding blood lipids to the model

Heart failure (HF)

From Table 7-4, although adding LDL-C to the model caused a significant change in the likelihood ratio statistic (P<0.001) and modestly increased C-statistic (+0.001) and category-free net reclassification index (cfNRI) in both genders, it did not improve the net reclassification of four categories due to the confidence interval (CI) straddling zero. Furthermore, HDL-C and TG did not significantly improve model reclassification and discrimination. Figure S 7-4 to Figure S 7–12 showed the calibration plots by deciles of observed (Kaplan Meier) and predicted risk, suggesting that adding lipid fractions did not improve the model calibration.

Atrial fibrillation (AF)

According to Table 7-4, while LDL-C significantly improved in cfNRI but not in four-category NRI, HDL-C did not significantly affect either metrics. Interestingly, adding TG consistently improved model discrimination (in terms of C-statistic), four-category NRI, and cfNRI in both males and females. Regarding the four-category NRI, TG increased the metric by 0.009 (95%CI 0.001, 0.016) and 0.007 (95%CI 0.001, 0.015) in males and females, respectively. Incorporating TG also increased cfNRI by 0.090 (95%CI 0.066, 0.116) and 0.066 (95%CI 0.043, 0.088) in males and females, respectively.

Myocardial infarction (MI)

According to Table 7-4, LDL-C and TG did not contribute to significant model improvement for the 10-year risk prediction of incident MI. Importantly, we noticed that adding HDL-C significantly increased four-category NRI by 0.020 (95%CI 0.009, 0.038) in males but not in females. Additionally, HDL-C improved cfNRI in both genders, corresponding to the increased cfNRI of 0.157 (95%CI 0.123, 0.192) in males and 0.056 (95%CI 0.015, 0.101) in females.

Table S 7-5, S 7-7, and S 7-9 illustrate the comparison of HR between models with and without LDL-C, HDL-C, and TG, respectively, and details of performance statistic between models with and without each lipid fraction stratified by gender are illustrated in Table S 7-6, S 7-8, and S 7-10.

7.5.4 Comparing MI risk with HF and AF risk

We chose HDL-C cohort to calculate and compare the predicted 10-year risk of HF, AF, and MI because it had shown the best model discrimination and calibration, compared with LDL-C and TG cohorts (Table 7-3 and Figure S 7-2). Importantly, according to Table 7-5, we observed that even although people were at low risk of MI, they still carried residual risk for HF and AF. For example, when MI and HF risks were compared, individuals in the low risk group (i.e., <5%) were concordant (86% of all patients in the low risk of MI). However, among people who were at low risk of MI (i.e., 10-year risk <5%), 20,094 (5.5%), 23,613 (6.5%), and 6,475 (1.8%) of them still had borderline risk (i.e., 10-year risk 5-7.5%), intermediate risk (i.e., 10-year risk 7.5-20%), and high risk (i.e., 10-year risk >20%), respectively, for HF. Likewise, among 365,692 individuals who were at low risk of MI, approximately one-third also had borderline to high risk of AF.

7.6 Discussion

In this chapter, we examine the role of blood lipids in the risk prediction for HF and AF using MI as a positive control. We found that, in most cases, adding blood lipids did not significantly improve the risk prediction, with the exception of TG and the prediction of AF. With regard to the MI outcome, HDL-C had a significant predictive value, especially in males. In addition, we had shown the use of EHRs to develop a risk predictive model for HF and AF, which yielded good discrimination (i.e., C-statistics from internal validation > 0.75) and relatively good calibration performance (i.e., calibration slopes were closed to 1), and this can be formed the basis for further development. Lastly, we also found that approximately 10-20% of patients who were at low risk of MI still had an increased risk of HF and AF, suggesting that risk of MI is quite different from that of the other two cardiac diseases.

7.6.1 Risk prediction of HF and AF: Room for improvement

To improve the apparent performance of the models, there are some variables to be incorporated in the risk prediction model according to previous studies.

For HF, factors that had been previously reported to have a significant association are physical activity, haemoglobin, white blood cells, serum creatinine, N-terminal pro-B-type natriuretic peptide (NT-proBNP), myocardial infarction, and chronic kidney disease.[52] In specific subgroups of HF, troponin T, stroke, left bundle-branch block and left ventricular ejection fraction are among significant predictors of HF with reduced ejection fraction (HFrEF). Urinary albumin excretion (UAE), cystatin C, blood urea nitrogen, chronic obstructive pulmonary disease, atrial fibrillation, and anemia had been reported to be good predictors for HF with preserved ejection fraction (HFpEF: LVEF ≥ 50%).[50,246,250]

As to the AF outcome, ethnicity, height, waist circumference, alcohol drinking status, PR interval, left atrial enlargement, left ventricular hypertrophy, cardiac murmur, heart failure, and coronary heart disease are significantly associated with the risk of new-onset AF.[247–249]

Although EHR is a rich source of clinical records with wide range of disease and biomarkers, there is limitation about the availability of some variables. It should be noted that many factors are readily recorded in the EHR, such as diagnosis of disease, basic physical examination [e.g, weight, height], and routinely measured laboratory parameters [e.g., white blood cells, red blood cells, renal function test], whereas some factors are not EHR available since they are for research purpose (e.g., cystatin C). Moreover, some measurements, such as ECG, despite the EHR availability, are difficult to extract from the EHR.

However, it is worth mentioning that although the availability of data recorded in the EHR can limit the improvement of new risk prediction tool, it reflects the real-world data. Therefore, if we can develop a model from the EHR that can excellently perform, this model is likely to be easily adopted and widely used in clinical practice.

7.6.2 Strengths

To the best of our knowledge, there are a few previous studies showing the role of blood lipids in the prediction of incident HF and AF, and ours is among

the first that comprehensively examines the role of all three lipid fractions in both HF and AF in the same cohorts who were free of CVD at baseline.

Importantly, this is the first time EHR data is used to develop a risk prediction model for HF and AF in healthy populations across England. On the risk prediction of AF, previous EHR studies had been done in Taiwan,[261] Israel,[107] and China[262] but around 3-10% of the populations from those studies had CVD at baseline. In addition, those studies aimed to apply the existing risk score to predict thromboembolism (CHADS2 and CHA2DS2-VAS) rather than to develop a new one. Regarding the prediction of HF, a previous EHR study used CPRD data, but it focused on patients with diabetes rather than healthy individuals.[245] Regarding the QRISK score, which was also developed using EHR (CPRD) data, it is used to predict 10-year risk of cardiovascular event (i.e., composite outcome of coronary heart disease, ischaemic stroke, or transient ischaemic attack), but not to predict the new-onset HF or AF.[263,264]

The strength of our study lies in the use of a larger sample size, compared with most of previous reports (Table S 7-1). Also, we implemented various statistics, such as discrimination metrics, calibration metrics, and net reclassification improvement index to strengthen our findings. Moreover, unlike some previous risk prediction models for HF and AF that required information from ECG[261,265–270] or echocardiogram,[262] the risk prediction model that we developed used only a simple physical examination (i.e., blood pressure, BMI) and laboratory parameters that were routinely measured in clinical practice (i.e., blood lipids, CRP).

In addition, the use of the complete-case approach analysis, which yielded the robust results consistent with those from Chapters 4-6 (which used the multiple-imputations approach) ensures the robustness and reliability of our observational results throughout the whole thesis.

7.6.3 Limitations

Our Cox model used for the risk prediction might sufficiently explain the outcomes of interest since it can account for only 54% and 47% of the variability of incident HF and AF, respectively. Alternative models, such as parametric survival (i.e., restricted cubic spline or polynomial) can be used in

future research. Also, according to the findings in Chapter 5, we did not take into account the potential U-shaped association of HDL-C with the incidence of HF and AF, the issues of which could be explored in future studies. Also, drawing on EHRs alone has its own limitations, since the diagnosis of HF and AF was ascertained using ICD10 and Read codes from the medical records, which are likely to underestimate the true incidence. However, this would simply tend to tilt the results towards null because of the smaller number of cases, but it would not cause the findings to be falsely positive.[246] Therefore, the true associations observed in TG and AF and HDL-C and MI were likely to strengthen.

Certain metrics implemented in this study have weakness too. For instance, C-statistic (or AUC) is generally insensitive even after a very strong predictor has been incorporated, especially when the baseline model has performed well. It had been previously shown that adding a new predictor with a weak effect size (e.g., Cohen's d < 0.2, which approximately equals to odds ratio [OR] < 1.22) is sufficient to increase AUC from 0.50 to 0.50. To increase the AUC from 0.80 to 0.85, however, a new predictor with a very strong effect size (e.g., Cohen's d > 0.8, which approximately equals to OR > 2.23) is needed.[271]

Category based NRI relies on the threshold; if the new biomarker added to the model does not contribute to a significantly improvement and cross the threshold, it will not affect NRI. Despite the great dependence of category based NRI on the chosen threshold, there is no universally accepted threshold for the risk of both diseases. We applied a threshold from a 10-year risk of cardiovascular diseases suggested in the most recent guideline.[257] Nevertheless, the calculation of the category free NRI (cfNRI) considers only the direction, but ignores the magnitude of change since it will equally assign the value to any changes in the same direction (i.e., assign +1 to any improvement and -1 to any worsening reclassification). Therefore, it is likely to underestimate the prognostic value of the biomarker.

7.6.4 Different risk profile between MI, and HF and AF: Clinical implications

Evaluating disease specific risk might inform appropriate preventive decisions for specific CVDs. In the management of hypertension for the primary

prevention of HF, for instance, clinical guidelines have suggested that certain blood pressure lowering agents, such as angiotensin-converting enzyme inhibitors (ACEIs), angiotensin-receptor blockers (ARBs), diuretics, and beta-blockers are more effective than others, such as calcium channel blockers (CCBs), and alpha-blockers.[3,26] On the other hand, for hypertensive patients who did not have a high risk of HF, the reduction of blood pressure, regardless of antihypertensive class, produces similar effects on the primary prevention of coronary heart disease (CHD).[272] Therefore, predicting HF risk in hypertensive individuals in a primary prevention setting may assist clinicians in selecting the most appropriate blood pressure lowering drugs.

The residual cardiovascular risk observed in patients with low MI risk but high HF or AF risk indicates the different pathophysiological process between atherosclerotic cardiovascular disease (ASCVD) and HF and AF. Previous study has shown that the risk score for CHD cannot accurately predict the risk of AF, which is due partly to differences in the pathophysiological process between AF and CHD.[249] Therefore, further research is required to provide an insight into the residual cardiovascular risk among people who are at low risk of MI and seek for an appropriate measure to prevent HF and AF.

7.7 Conclusion

Our findings suggested that blood lipids have no role in the risk prediction of incident HF and AF, with the exceptions of TG on incident AF, and HDL-C on incident MI. Therefore, the role of TG on the risk prediction of AF needs further investigation. We also showed that EHRs can be used to develop a risk prediction model for HF and AF using simple measures, which provided a good basis for further study. Also, we found that patients who were at low risk of MI may still have an increased risk of HF and AF. Clinicians should be aware of the different CV risk profiles of patients, and further research within this group of population is required to be able to improve patients' outcomes.

Table 7-1 Characteristics of patients at baseline LDL-C measurement

Baseline characterisitics	Development cohort	Validation cohort	Total	P-values
N	375,239	103,464	478,703	
Female	59.4%	60.0%	59.5%	0.001
Age (year)	57.0 (13.2)	55.8 (13.0)	56.7 (13.2)	< 0.001
Health behaviors, physical and laboratory measurements at baseline				
Non-smokers	58.5%	54.3%	57.6%	< 0.001
Body mass index (kg/m^2)	27.8 (5.6)	28.0 (5.7)	27.8 (5.7)	< 0.001
Systolic blood pressure (mmHg)	135.3 (16.3)	135.3 (16.7)	135.3 (16.4)	0.58
Diastolic blood pressure (mmHg)	80.8 (9.0)	80.2 (9.0)	80.7 (9.0)	< 0.001
eGFR (mL/min/1.73m^2)	81.5 (21.1)	82.5 (20.0)	81.7 (20.9)	< 0.001
CRP (mg/L), median (IQR)	4.0 (2.0, 7.8)	4.0 (2.0, 7.4)	4.0 (2.0, 7.7)	0.51
LDL cholesterol (mmol/L)	3.4 (1.0)	3.4 (1.0)	3.4 (1.0)	< 0.001
HDL cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	< 0.001
Total cholesterol (mmol/L)	5.5 (1.0)	5.5 (1.0)	5.5 (1.0)	< 0.001
Triglyceride (mmol/L), median (IQR)	1.3 (0.9, 1.8)	1.4 (1.0, 2.0)	1.3 (0.9, 1.9)	< 0.001
Health conditions				
Diabetes type 2	5.5%	5.4%	5.5%	< 0.001
Chronic kidney disease	4.7%	4.0%	4.6%	< 0.001
Cancer	4.1%	3.4%	3.9%	< 0.001
COPD	2.0%	2.9%	2.2%	< 0.001
Medication				
Statins (at baseline)	15.4%	14.7%	15.2%	< 0.001
Statins (during follow-up)	35.2%	38.8%	36.0%	< 0.001
Other lipid-lowering drugs	1.0%	0.9%	1.0%	< 0.001
Antihypertensive drugs	31.4%	31.7%	31.5%	0.049
Antiplatelet drugs	6.9%	7.6%	7.1%	< 0.001

Table 7-2 Beta-coefficients and baseline survival at 10 year for the calculation of 10-year risk of HF, AF, and MI from the development cohort of each lipid fraction

Variables	LDL-C (n=375,239)			HDL-C (n=350,693)			TG (n=346,589)		
	HF	AF	MI	HF	AF	MI	HF	AF	MI
LDL-C (per SD)	-0.0895	-0.0965	0.0951	-0.1019	-0.1022	0.0642	-0.0981	-0.1004	0.0786
HDL-C (per SD)	-0.0049	0.0016	-0.1708	-0.0098	0.0028	-0.1762	0.0013	0.0043	-0.1787
Ln TG (per SD)	-0.0759	-0.1360	-0.0353	-0.0819	-0.1350	-0.0436	-0.0816	-0.1322	-0.0465
Age (per SD)	-0.0335	1.1438	-1.4040	0.0113	1.1774	-1.3466	-0.0169	1.1437	-1.3668
Age^2 (per SD)	1.0592	-0.0647	1.8219	1.0247	-0.0910	1.7659	1.0497	-0.0648	1.7829
2nd IMD quintile	0.0486	-0.0424	-0.0073	0.0617	-0.0362	-0.0033	0.0862	-0.0369	0.0055
3rd IMD quintile	0.1375	0.0209	-0.0120	0.1420	0.0124	-0.0147	0.1567	0.0199	-0.0089
4th IMD quintile	0.1883	-0.0332	0.0728	0.1797	-0.0426	0.0694	0.1929	-0.0441	0.0819
5th IMD quintile	0.1117	-0.0404	0.0632	0.1011	-0.0372	0.0653	0.1239	-0.0297	0.0878
Ex-smoker	0.1666	0.1049	0.1450	0.1765	0.1171	0.1685	0.1899	0.1156	0.1703
Current smoker	0.5248	0.1668	0.5646	0.5145	0.1678	0.5618	0.5291	0.1615	0.5602
SBP (per SD)	0.1167	0.0575	0.1027	0.1123	0.0542	0.0930	0.1064	0.0528	0.1016
DBP (per SD)	-0.1038	-0.0349	-0.0341	-0.1024	-0.0365	-0.0324	-0.0941	-0.0315	-0.0379
BMI (per SD)	0.3048	0.2027	-0.0587	0.3044	0.2049	-0.0548	0.3056	0.2037	-0.0557
Ln CRP (per SD)	0.2387	0.1625	0.1091	0.2334	0.1599	0.1103	0.2329	0.1602	0.1058
Type 1 DM	0.3872	-0.2338	0.0621	0.4737	-0.1503	0.1017	0.4465	-0.1802	-0.0006
Type 2 DM	0.3370	-0.0309	0.1906	0.3250	-0.0395	0.1404	0.3363	-0.0311	0.1812
DM other types	-0.1465	-0.7594	-0.3411	-0.0892	-0.7405	-0.3703	-0.1586	-0.7526	-0.3760
Use of antiHTN	0.3246	0.2479	-0.0458	0.3191	0.2493	-0.0583	0.3426	0.2570	-0.0336
Use of statins	0.4498	0.3240	2.4569	0.4727	0.3351	2.5051	0.4714	0.3312	2.4813
Baseline survival									
Male	0.9861	0.9572	0.9958	0.9868	0.9590	0.9959	0.9873	0.9590	0.9959
Female	0.9897	0.9699	0.9975	0.9902	0.9713	0.9975	0.9905	0.9713	0.9976

Note: Beta-coefficients of all continuous variables are per SD increase.

Abbreviations: AF = Atrial fibrillation, antiHTN = Antihypertensive drugs, BMI = Body mass index, CRP = C-reactive protein, DBP = Diastolic blood pressure, DM = Diabetes mellitus, HDL-C = High-density lipoprotein cholesterol, HF = Heart failure, IMD = Index of multiple deprivation, LDL-C = Low-density lipoprotein cholesterol, MI = Myocardial infarction, SBP = Systolic blood pressure, SD = Standard deviation, TG = Triglyceride.

Table 7-3 Numbers of events, discrimination, and calibration statistics of 10-year risk of HF, AF, and MI in the development and validation cohorts

	HF		AF		MI	
	Development samples	Validation samples	Development samples	Validation samples	Development samples	Validation samples
LDL-C						
Total	375,239	103,464	375,239	103,464	375,239	103,464
Events	10,605	3,509	19,553	5,507	6,607	2,360
C-statistic (95% CI)	0.831 (0.827,0.835)	0.803 (0.796,0.811)	0.795 (0.792,0.799)	0.748 (0.741,0.755)	0.822 (0.818,0.825)	0.798 (0.791,0.804)
Calibration slope	1.000	0.930	1.000	0.597	1.000	0.955
HDL-C						
Total	350,693	95,920	350,693	95,920	350,693	95,920
Events	10,016	3,212	18,408	5,046	6,265	2,215
C-statistic (95% CI)	0.832 (0.828,0.837)	0.794 (0.786,0.802)	0.797 (0.794,0.801)	0.785 (0.778,0.791)	0.824 (0.820,0.828)	0.800 (0.793,0.807)
Calibration slope	1.000	0.956	1.000	0.948	1.000	0.958
TG						
Total	346,589	95,941	346,589	95,941	346,589	95,941
Events	9,694	3,216	17,957	4,993	6,118	2,191
C-statistic (95% CI)	0.833 (0.829,0.837)	0.805 (0.797,0.812)	0.797 (0.794,0.800)	0.786 (0.779,0.792)	0.825 (0.822,0.829)	0.803 (0.796,0.810)
Calibration slope	1.000	0.921	1.000	0.960	1.000	0.963

Table 7-4 Summary of predictive values of adding blood lipids in the risk prediction of HF, AF, and MI

Blood lipids	Parameters	Heart failure		Atrial fibrillation		Myocardial infarction	
		Male	Female	Male	Female	Male	Female
LDL-C	Events (Total)	6,664 (193,898)	7,450 (284,805)	5,192 (193,898)	3,775 (284,805)	12,105 (193,898)	12,889 (284,805)
	LR-chi^2 (p-value)	49.52 (< 0.001)	75.63 (< 0.001)	43.16 (< 0.001)	20.63 (< 0.001)	113.39 (< 0.001)	111.69 (< 0.001)
	C-statistics Baseline value ΔC-statistics	0.803 +0.001	0.848 +0.001	0.773 +0.001	0.816 +0.002	0.793 +0.001	0.842 0.000
	Four-category NRI	0.006 (-0.003,0.013)	0.007 (-0.001,0.015)	0.001 (-0.010,0.010)	-0.006 (-0.010,0.009)	0.006 (-0.001,0.011)	-0.001 (-0.005,0.005)
	cfNRI	0.065 (0.032, 0.095)	0.038 (0.003, 0.067)	0.101 (0.070, 0.134)	0.105 (0.066, 0.142)	0.059 (0.034, 0.086)	0.050 (0.027, 0.075)
HDL-C	Events (Total)	6,251 (179,925)	6,977 (266,688)	4,911 (179,925)	3,569 (266,688)	11,394 (179,925)	12,060 (266,688)
	LR-chi^2 (p-value)	0.99 (0.319)	3.04 (0.081)	124.70 (<0.001)	27.94 (<0.001)	13.25 (0.0003)	6.83 (0.009)
	C-statistics Baseline value ΔC-statistics	0.776 0.000	0.819 0.000	0.791 +0.005	0.843 +0.001	0.806 0.000	0.850 0.000
	Four-category NRI	0.000 (-0.004,0.005)	-0.002 (-0.004,0.005)	0.020 (0.009,0.038)	0.001 (-0.006,0.014)	-0.001 (-0.005,0.004)	-0.002 (-0.004,0.002)
	cfNRI	0.002 (-0.019,0.035)	-0.003 (-0.032,0.031)	0.157 (0.123, 0.192)	0.056 (0.015, 0.101)	0.020 (-0.004,0.038)	0.020 (-0.002,0.042)
TG	Events (Total)	6,096 (178,098)	6,814 (264,432)	4,793 (178,098)	3,516 (264,432)	11,146 (178,098)	11,804 (264,432)
	LR-chi^2 (p-value)	69.18 (<0.001)	6.18 (0.013)	31.34 (<0.001)	2.21 (0.137)	137.81 (<0.001)	153.16 (<0.001)
	C-statistics Baseline value ΔC-statistics	0.806 +0.001	0.850 0.000	0.774 +0.001	0.818 +0.001	0.798 +0.001	0.844 0.000
	Four-category NRI	-0.000 (-0.006,0.013)	0.000 (-0.005,0.006)	0.006 (-0.005,0.015)	0.000 (-0.004,0.008)	0.009 (0.001, 0.016)	0.007 (0.001, 0.015)
	cfNRI	0.088 (0.055, 0.123)	-0.032 (-0.061,0.005)	0.053 (0.017, 0.085)	0.059 (-0.038,0.101)	0.090 (0.066, 0.116)	0.066 (0.043, 0.088)

Abbreviations: AF = atrial fibrillation, cfNRI= category-free net reclassification improvement, HDL-C = high-density lipoprotein cholesterol, HF = heart failure, LDL-C = low-density lipoprotein cholesterol, LR= Likelihood ratio, MI = myocardial infarction, TG = triglyceride.

Table 7-5 Comparison of 10-year risk of HF, AF, and MI (n = 446,613)

10-year risk of MI (events = 8,480)	Predicted 10-year risk of HF (events = 13,228)			
	<5%	**5% to <7.5%**	**7.5% to <20%**	**20% or higher**
<5%	315,510 (86.3%)	20,094 (5.5%)	23,613 (6.5%)	6,475 (1.8%)
5% to <7.5%	19,661 (48.3%)	8,568 (21.1%)	11,156 (27.4%)	1,297 (3.2%)
7.5% to <20%	4,721 (12.6%)	4,923 (13.1%)	18,914 (50.4%)	8,963 (23.9%)
20% or higher	0	0	165 (6.1%)	2,553 (93.9%)
10-year risk of MI (events = 8,480)	**Predicted 10-year risk of AF (events = 23,454)**			
	<5%	**5% to <7.5%**	**7.5% to <20%**	**20% or higher**
<5%	245,059 (67.0%)	41,693 (11.4%)	60,186 (16.5%)	18,754 (5.1%)
5% to <7.5%	7,734 (19.0%)	5,964 (14.7%)	21,932 (53.9%)	5,052 (12.4%)
7.5% to <20%	2,016 (5.4%)	1,560 (4.2%)	14,222 (37.9%)	19,723 (52.6%)
20% or higher	0	0	28 (1.0%)	2,690 (99.0%)

Note: Calculation was based on complete-case approach using data from HDL-C cohort

Abbreviations: AF; Atrial fibrillation, HF; Heart failure, MI; Myocardial infarction

7.8 Chapter Supplementary

Explanatory of terms used in this chapter

Overall measures of model fit

Model fit statistics measures the overall performance of the model. Genearalisations of R^2 for time-to-event outcomes have been proposed, such as Cox-Snell R^2, Nagelkerke's R^2, O'Quigley's R^2, Royston's R^2, and Royston and Sauerbrei's R^2_D. As a typical R^2 for continuous outcomes, these R^2 measures provides the proportion of variance of outcome values that is explained by the model, with values closer to one preferred. Often they are multiplied by one hundred, to give the percentage of variation explained.[273]

Calibration statistics and plots

Calibration examines the agreement between predicted and observed outcome risks, which should be examined across the whole spectrum of predicted values, and at each relevant time point. Good calibration means that model-based predicted event rates closely match those observed in practice.[274] It can be summarised by measures, such as the calibration slope (ideal value of 1), calibration-in-the-large (ideal value of 0), and the observed/expected ratio (ideal value of 1). The calibration slope is one measure of agreement between observed and predicted risk of the outcome across the whole range of predicted values. A slope <1 indicates that the model is overfitted mostly due to some predictions that are too extreme (e.g. prediction close to 1 are too high, and prediction close to 0 are too low) and a slope >1 indicates that predictions are too narrow (i.e., predictions are not varied enough therefore model is likely to be underfitted). To estimate the calibration slope, a calibration model can be fitted in the validation dataset. For example, for time-to-event outcome, a survival model could be fitted. Then beta-coefficient is estimated calibration slope. The calibration slope derived using individual predicted values (for the linear prediction) and does not require grouping. Often, a calibration slope can be estimated in the developed model itself and then is shrunk for optimism by a uniform shrinkage factor, such as Heuristic shrinkage factor.[275]

Discrimination statistic

Discrimination refers to how well predictions discriminate (separate) between those participants who do (cases) and do not (non-cases) develop the outcome of interest. Discrimination is formally measured by the Concordance (C) statistic (index) from which the value of 1 indicates the model has perfect discrimination whilst a value of 0.5 indicates the model discriminates no better than chance. Generalisations of the C statistic have been proposed for time-to-event models, most notably Harrell's C statistic.[276,277] This is the proportion (ranging from 0.5 to 1) of all possible pairs of study participants in which the individual with the hither predicted survival probability indeed survived longer than the other individual. Pairs in which both individuals are censored before the outcome occurrence, or both have the outcome at the same survival time, or where one individual is censored at an earlier time than the other individual's survival time, cannot be ordered and therefore are not included in the calculation of Harrell's C statistic. Another discrimination measure for time-to-event outcomes is Royston's D statistic, which can be interpreted as the log hazard ratio comparing low and high-risk groups, where these two equally sized groups are defined by dichotomising at the median value of the linear predictor from the developed model. Higher values for the D statistic indicate greater discrimination.[273]

Net reclassification improvement.

In terms of understanding the prognostic value of the new biomarker, net reclassification improvement (NRI) can be very useful, and there are two types of NRI: i) category-based NRI and ii) category-free NRI (cfNRI or NRI(>0)). Category-based NRI was firstly introduced in 2008[278] then it was extended in 2011 to the category-free version.[258] To calculate category-based NRI, each individual is assigned to a risk category based on the event probability calculated by the reference risk predictive model. A second model is constructed by adding the biomarker of interest (i.e., LDL-C in our study) to the reference model then each individual is reassigned to a risk category. The net proportion of patients with events reassigned to a higher risk category (NRI_{events}) and of patient without events reassigned to a lower risk category ($NRI_{nonevents}$) is calculated.[278] The NRI is the sum of NRI_{events} and $NRI_{nonevents}$.

It is interpreted as the proportion of patients reclassified to a more appropriate risk category. Amongst those with event, if the addition of the biomarker of interest to the model results in more individuals being reclassified to higher risk categories than to lower ones, then NRI_{events} is positive. Conversely, amongst those without events, if more are assigned to lower than higher risk categories, then the $NRI_{nonevents}$ is positive.[279]

On the other hand, the cfNRI (also known as continuous NRI) counts the direction of change for every individual regardless of whether they crossed of a threshold of risk.[258] Each patient is counted as either +1 or -1 depending on whether the change in calculated risk was in the correct direction (higher for those with events, lower for those without events). The cfNRI is the sum of the $cfNRI_{events}$ and $cfNRI_{nonevents}$, where the $cfNRI_{events}$ is the proportion of patients with events who have an increase in calculated risk minus the proportion with a decrease and the $cfNRI_{nonevents}$ is the proportion of patients without events who have a decrease in calculated minus the proportion with an increase.[279] Table S 7-0 illustrates example of how metrics compared

Table S 7-0 Illustrated changes in NRIevents NRInonevents cfNRIevents and cfNRInonevents for individual patients.

Patients	Have an event	Calculated risk		Threshold	Contribution			
		Reference model	New model		NRI_{events}	$NRI_{nonevents}$	$cfNRI_{events}$	$cfNRI_{nonevents}$
1	Yes	0.19	0.22	0.20	+1	-	+1	-
2	Yes	0.21	0.18	0.20	-1	-	-1	-
3	Yes	0.17	0.19	0.20	0	-	+1	-
4	Yes	0.19	0.16	0.20	0	-	-1	-
5	No	0.19	0.22	0.20	-	-1	-	-1
6	No	0.21	0.18	0.20	-	+1	-	+1
7	No	0.17	0.19	0.20	-	0	-	-1
8	No	0.19	0.16	0.20	-	0	-	+1

Abbreviations: NRI; net reclassification improvement, cfNRI; category-free net reclassification improvement.

Table S 7-1 Summary of evidence on the risk prediction score for incident HF, AF, and MI

Score, Published year	Outcome to predict	EHR study	Variables included in the risk score				Population			AUC or C-statistic* (Method of validation)
			LDL-C	HDL-C	TG	Others	Total	Cases	Characteristics	
Atrial fibrillation										
FHS-AF, 2009[247]	AF (10-year risk)	○	○	○	○	Age, gender, BMI, SBP, HTN medication, PR interval, cardiac murmur, HF	4,764	457	55% Female, Age 45-95 (mean 61 ± 10 years), 4% MI, 1% HF, FU 10 years	0.76 (Bootstrap)
ARIC-AF, 2011[249]	AF (10-year risk)	○	○	○	○	Age, race, height, SBP, HTN medication, smoking status, precordial murmur, LVH, Left atrial enlargement, DM, HF, CHD	14,546	515	77% Whites, 55% Female, Age 45-64 years, 5% CHD, 5% HF, FU 10 years	0.77 (Bootstrap)
CHARGE-AF, 2013[280]	AF (5-year risk)	○	○	○	○	Age, race, height, weight, SBP, DBP, current smoking, HTN medication, DM, MI, and HF	18,556	1,186	81% Whites, 55%-66% Female, Mean age ranged 60-73 years, 4-9% MI, 0.5-5.8% stroke, 0.6-7.2% HF	0.665-0.716 (Externally)
$CHADS_2$, 2013[261]	AF	●	○	○	○	$CHADS_2$: CHF (C), HTN (H), age ⩾ 75 years (A), and DM (D), previous stroke or TIA (S2). CHA_2DS_2-VASc: age ⩾ 75 years (A2), history of stroke, TIA, or thromboembolism (S2), CHF (C), HTN (H), DM (D), age 65 to 75 years (A), vascular disease (VA) (defined as previous MI, complex aortic plaque, carotid stenosis, and PAD), and female sex category (Sc).	702,502	9,187	Chinese individuals from Taiwan, Age 41 ± 16 years, 49% Female, 2% CHD, 2% stroke/TIA, 0.4% HF, mean FU 9 ± 2.2 years	0.713 (not validated)
$CHADS_2$ and CHA_2DS_2-VASc, 2016[281]	AF	●	○	○	○		1 M	23,223	Individuals from Israel, Age > 50 (mean 66 ± 12 years), 55% Female, 11% vascular disease, 7% stroke, 3% HF, FU 2 years	0.728 ($CHADS_2$) 0.744 (CHA_2DS_2-VASc) (not validated)
$CHADS_2$-HDL and CHA_2DS_2-VASc-HDL, 2017[107]	AF	○	○	●[a]	○		1,223	34	Dyslipidaemic individuals, Age 48-71 years, 53% Female, 15-24% CVD, Mean FU 6 years	0.690 ($CHADS_2$-HDL) 0.707 (CHA_2DS_2-VASc-HDL) (not validated)
C2HEST, 2019[262]	AF	●	○	○	○	CAD/COPD (1 point each); H: HTN (1 point); E: elderly (age ≥ 75 years, 2points); S: systolic HF (2points); and T: thyroid disease (hyperthyroidism, 1 point).	471,446	921	Chinese populations, Mean age ranged from 47 ± 16 years (subjects without AF) to 62 ± 12 years (subjects with AF), 3% CAD, FU 11 years	0.75 (Bootstrap)

Score, Published year	Outcome to predict	EHR study	Variables included in the risk score				Population			AUC or C-statistic* (Method of validation)
			LDL-C	HDL-C	TG	Others	Total	Cases	Characteristics	
Hamada, 2019[248]	AF (7-year risk)	○	○	○	○	Age, waist circumference, DBP, alcohol consumption, HR, cardiac murmur	65,984	349	Japanese populations, age 40-79 years, 35% Female, FU 5.5 years	0.77 (Bootstrap)
My PhD	AF (10-year risk)	●	●	●	●	Age, age^2, gender (stratified), smoking, BMI, SBP, DBP, HTN drugs, statins	442,538 - 478,703	22,950-25,060	Free of CVDs, 89% White, 60% Female, Age 56 ± 13 years, median FU 6 (IQR 3-9) years.	0.748-0.786 (Geographical split)
Heart failure										
FHS-HF, 1999[270]	HF (4-year risk)	○	○	○	○	Age, gender, FVC, SBP, HR, LVH on ECG, CHD, Valvular disease, DM, cardiomegaly	15,267	486	Existing CHD, HTN, or valvular heart disease, age 45-94 years (42% Female). FU 38 years	Not reported
Health ABC-HF, 2008[282]	HF (5-year risk)	○	○	○	○	Age, smoking status, SBP, HR, serum glucose, SCr, and albumin levels, LVH, and CHD,	2,935	258	Elderly individuals, age 70-79 (mean 74 ± 3) years, 52% Females, 59% Whites, 16.5% CHD, FU 6.5 years	0.72 (Bootstrap)
ARIC-HF, 2012[265]	HF (10-year risk)	○	○	○	○	Age, gender, race and NT-proBNP	13,555	1,487	55% Female, 74% Whites, age 45-64 years. No CHD at baseline, Mean FU 15.5 years.	0.794 (Bootstrap)
Schnabel, 2013[283]	HF (10-year risk)	○	○	○	○	Age, BMI, LVH on ECG, DM, significant murmur, and MI	725	161	AF patients mean age 73 (range 39-96 years), 45% Female, 48% Prevalent CVD, FU 10 years	0.71 (not validated)
Hippisley-Cox, 2015[245]	HF (10-year risk)	●	○	●	○	Age, gender, BMI, SBP, TC/HDL ratio, HbA1c, material deprivation, ethnicity, smoking, duration and type of DM, AF, CVD, and chronic renal disease	437,806	25,408	Diabetic patients (94% T2DM, 6% T1DM), 44% Female, age 25-84 (mean 60 ± 14 years), 17% CVD	0.77-0.78 (female) 0.76-0.77 (male), (Externally)
MESA-HF, 2015[267]	HF (5-year risk)	○	○	○	○	Age, gender, BMI, smoking status, SPB, HR, DM, and NT proBNP natriuretic peptide	6,814	176	Free of CVD at baseline, mean age 62 ± 10 years, 53% Female, 38.5% Caucasian, 11.8% Chinese, 27.8% African-American and 22% Hispanic, Median FU 4.7 years	0.91 (Cox) 0.87 (PBS) (Bootstrap)
Ho, 2016[284]	HF (10-year risk)	○	○	○	○	Age, gender, SBP, BMI, HTN treatment, MI (HFpEF) with additional smoking status, LVH, left bundle branch block, and DM for HFrEF	28,820	1,891	Mean age ranged 49-73 years, 50-57% Female, 7% CHD, and 982 (HFpEF) 909 (HFrEF) occurred. Median FU 12 years	0.80 (HFpEF) 0.82 (HFrEF) (Random split)
HOMAGE, 2017[285]	HF (5-year risk)	○	○	○	○	Age, gender, BMI, smoking status, DM, CAD, HTN treatment, SBP, HR, SCr	10,326	470	Elderly individuals (mean age 74.5 years, range 65-86 years), 51% Female, FU 3.5 years	0.706 (Bootstrap)

Score, Published year	Outcome to predict	EHR study	Variables included in the risk score				Population			AUC or C-statistic* (Method of validation)
			LDL-C	HDL-C	TG	Others	Total	Cases	Characteristics	
PCP-HF, 2019[286]	HF (10-year risk)	○	○	○	○	Age, gender, race, blood pressure (treated or untreated), fasting glucose (treated or untreated), body mass index, cholesterol, smoking status, and QRS duration	11,771	1,339	Free of CVD at baseline, 58% Female, 78% Whites, mean age of 52 ± 12 years, FU 10 years	0.71-0.87 (Random split)
My PhD	HF (10-year risk)	●	●	●	●	Age, age^2, gender (stratified), smoking, BMI, SBP, DBP, HTN drugs, statins	442,538 - 478,703	12,910-14,114	Free of CVD at baseline, 89% White, 60% Female, Age 56 ± 13 years, median FU 6 (IQR 3-9) years.	0.794-0.805 (Geographical split)
Myocardial infarction										
PROCAM, 2002[266]	Major coronary event (10-year risk)	○	●	●	●	Age, smoking, SBP, Family history MI, DM	5,389	352	Male aged 35-65 (mean 47 ± 7.5 years), and measure ACS events. FU 10 years	0.824 (PBS) 0.829 (Cox) (not validated)
SCORE, 2003[268]	CVD mortality (10-year risk)	○	○	●	○	Age, gender, TC or TC/HDL-C ratio, SBP, smoking status	205,178	7,934	Individuals from 12 European countries, Age 45-64 years, 43% Female,	0.71-0.84 (Country specific) (not validated)
CUORE, 2005[269]	CVD event (10-year risk)	○	○	●	○	Age, TC, SBP, HTN medication, smoking status, Family history CHD, DM	6,865	312	Male aged 35-69 years, conducted in Italy	0.75 (random split)
ASSIGN, 2007[287]	CVD event (10-year risk)	○	○	●	○	Age, gender, TC, SBP, smoking (number of cigarettes), DM, Area-leveled index of deprivation, family history of CVD event	13,297	1,165	Individuals aged 30-74 (mean 49 ± 0.1 years), 51% Female	Not report
Reynolds (female), 2007[288]	CV event (10-year risk)	○	○	●	○	Age, SBP, smoking status, TC, family history of premature MI (<60 years), HbA1c (if DM)	24,558	766	Female aged > 45 (median 52 [48-59] years),	0.808 – 0.809 (Random split)
Reynolds (male), 2008[183]	CV event (10-year risk)	○	○	●	○	Age, SBP, smoking status, TC, C-reactive protein, family history of premature MI (<60 years), HbA1c (if DM)	10,724	1,294	Male aged 50-80 (median 63 [57-70] years), and 1,294 CV events occurred.	0.700 (CHD) 0.708 (CVD) (not validated)
FHS, 2008[182]	CHD event (10-year risk)	○	○	●	○	Age, gender, TC, SBP, smoking status, HTN mediation	8,491	1,174	Individuals aged 30-74 (mean 49 ± 11 years), 53% Female,	0.763 (male) 0.793 (female) (Bootstrap)
QRISK2, 2008[264]	CVD event (10-year risk)	●	○	●	○	Age, gender (stratified), TC to HDL-C ratio, SBP, smoking status, DM, area-leveled index of deprivation, family history of CHD, BMI, HTN medication, ethnicity, RA, CKD stage 4-5, AF	1.5 M	96,709	Individuals aged 35-74, 39% Whites, 50% Female	0.792 (male) 0.817 (female) (Random split)
PCE, 2014[184]	CVD event (10-year risk)	○	○	●	○	Age, gender, TC, DM, smoking status, SBP, HTN mediation, race	24,626	2,689	Free of CVD at baseline, 83% Whites, 55% female aged 40-79 years	0.704-0.814 (10-fold cross validation)

Score, Published year	Outcome to predict	EHR study	Variables included in the risk score				Population			AUC or C-statistic* (Method of validation)
			LDL-C	HDL-C	TG	Others	Total	Cases	Characteristics	
Globorisk, 2015[289]	CVD mortality (10-year)	○	○	○	○	Age, gender, TC, smoking status, SBP, DM	50,129	2,265	Age > 40 years, 33% Female	0.760 (cross validation)
QRISK3, 2017[263]	CVD event (10-year risk)	●	○	●	○	Age, gender, TC to HDL-C ratio, SBP, SBP variability, smoking status, DM, are-leveled index of deprivation, family history, BMI, HTN medication, ethnicity, RA, CKD stage 3-5, AF, Corticosteroid use, SLE, Second generation "atypical" antipsychotic use, Severe mental illness, HIV or AIDS, Erectile dysfunction	7.89 M	363,565	Age 25-84 (mean 43 ± 15 years), 51% Female,	0.860 (male) 0.880 (female) (Random split)
My PhD	MI (10-year risk)	●	●	●	●	Age, age^2, gender (stratified), smoking, BMI, SBP, DBP, HTN drugs, statins	442,538 - 478,703	8,309 - 8,967	Free of CVD at baseline, 89% White, 60% Female, Age 56 ± 13 years, median FU 6 (IQR 3-9) years.	0.798-0.803 (Geographical split)

Note: ● indicates 'yes' or that variables were included in the risk prediction model, ○ indicates 'no' or that variables were NOT included in the risk prediction model

[a] Low levels of HDL-C **(i.e., <** 40 and **<**50 mg/dL for male and female subjects, respectively**),** ***AUC or C-statistic from internal validation (if available)

Abbreviations: AUC; Area under the receiver operating characteristic curve, AF; Atrial fibrillation, BMI; Body mass index, CAD; Coronary artery disease, CHD; Coronary heart disease, CHF; Congestive heart failure, COPD; Chronic obstructive pulmonary disease, CV; Cardiovascular, CVD; Cardiovascular disease, DBP; Diastolic blood pressure, DM; Diabetes mellitus, ECG; Electrocardiogram, EHR; Electronic health record, FVC; Forced vital capacity, FU; Follow-up, HbA1c; Glycosylated haemoglobin, HDL-C; High-density lipoprotein cholesterol, HF; Heart failure, HR; Heart rate, HTN; Hypertension, LBBB; Left bundle branch block, LVH; Left-ventricular hypertrophy, MI; Myocardial infarction, PAD; Peripheral arterial disease, PBS; Point-based score, RA; Rheumatoid arthritis, SBP; Systolic blood pressure, SCr; Serum creatinine, SLE; Systemic lupus erythematosus, TC; Total-cholesterol

Table S 7-2 Characteristics of patients at baseline HDL-C measurement

Baseline characterisitics	Development cohort	Validation cohort	Total	P-values
N	350,693	95,920	446,613	
Female	59.6%	60.2%	59.7%	0.001
Age (year)	56.6 (13.2)	55.4 (13.0)	56.3 (13.2)	< 0.001
Health behaviors, physical and laboratory measurements at baseline				
Non-smokers	58.6%	54.4%	57.7%	< 0.001
Body mass index (kg/m^2)	27.7 (5.6)	27.9 (5.7)	27.8 (5.6)	< 0.001
Systolic blood pressure (mmHg)	135.2 (16.5)	135.2 (16.9)	135.2 (16.6)	0.15
Diastolic blood pressure (mmHg)	80.8 (9.0)	80.3 (9.1)	80.7 (9.1)	< 0.001
eGFR (mL/min/1.73m^2)	81.4 (20.8)	82.4 (19.8)	81.6 (20.6)	< 0.001
CRP (mg/L), median (IQR)	4.0 (2.0, 7.6)	4.0 (2.0, 7.4)	4.0 (2.0, 7.5)	< 0.001
LDL cholesterol (mmol/L)	3.3 (0.9)	3.3 (0.9)	3.3 (0.9)	< 0.001
HDL cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	< 0.001
Total cholesterol (mmol/L)	5.5 (1.0)	5.5 (1.0)	5.5 (1.0)	< 0.001
Triglyceride (mmol/L), median (IQR)	1.3 (0.9, 1.8)	1.4 (1.0, 2.0)	1.3 (0.9, 1.9)	< 0.001
Health conditions				
Diabetes type 2	5.1%	5.1%	5.1%	< 0.001
Chronic kidney disease	4.4%	3.7%	4.2%	< 0.001
Cancer	4.0%	3.3%	3.8%	< 0.001
COPD	2.0%	2.8%	2.2%	< 0.001
Medication				
Statins (at baseline)	13.8%	13.1%	13.6%	< 0.001
Statins (during follow-up)	34.7%	38.1%	35.4%	< 0.001
Other lipid-lowering drugs	0.8%	0.7%	0.8%	< 0.001
Antihypertensive drugs	29.8%	30.2%	29.9%	0.042
Antiplatelet drugs	6.4%	7.1%	6.6%	< 0.001

Table S 7-3 Characteristics of patients at baseline TG measurement

Baseline characterisitics	Development cohort	Validation cohort	Total	P-values
N	346,589	95,941	442,538	
Female	59.6%	60.2%	59.8%	0.001
Age (year)	56.5 (13.2)	55.4 (13.0)	56.3 (13.2)	< 0.001
Health behaviors, physical and laboratory measurements at baseline				
Non-smokers	58.5%	54.3%	57.6%	< 0.001
Body mass index (kg/m^2)	27.7 (5.6)	27.9 (5.7)	27.7 (5.6)	< 0.001
Systolic blood pressure (mmHg)	135.1 (16.5)	135.2 (16.8)	135.2 (16.6)	0.33
Diastolic blood pressure (mmHg)	80.8 (9.1)	80.2 (9.1)	80.7 (9.1)	< 0.001
eGFR (mL/min/1.73m^2)	81.3 (20.7)	82.3 (19.9)	81.5 (20.5)	< 0.001
CRP (mg/L), median (IQR)	4.0 (2.0, 7.5)	4.0 (2.0, 7.4)	4.0 (2.0, 7.5)	0.002
LDL cholesterol (mmol/L)	3.3 (0.9)	3.3 (0.9)	3.3 (0.9)	0.007
HDL cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	< 0.001
Total cholesterol (mmol/L)	5.5 (1.0)	5.5 (1.0)	5.5 (1.0)	< 0.001
Triglyceride (mmol/L), median (IQR)	1.3 (0.9, 1.8)	1.4 (1.0, 2.0)	1.3 (0.9, 1.9)	< 0.001
Health conditions				
Diabetes type 2	5.0%	4.9%	5.0%	< 0.001
Chronic kidney disease	4.4%	3.8%	4.3%	< 0.001
Cancer	3.9%	3.3%	3.8%	< 0.001
COPD	2.0%	2.9%	2.2%	< 0.001
Medication				
Statins (at baseline)	13.7%	13.2%	13.6%	< 0.001
Statins (during follow-up)	34.2%	37.8%	35.0%	< 0.001
Other lipid-lowering drugs	0.8%	0.7%	0.8%	< 0.001
Antihypertensive drugs	29.6%	30.0%	29.7%	0.010
Antiplatelet drugs	6.3%	7.0%	6.4%	< 0.001

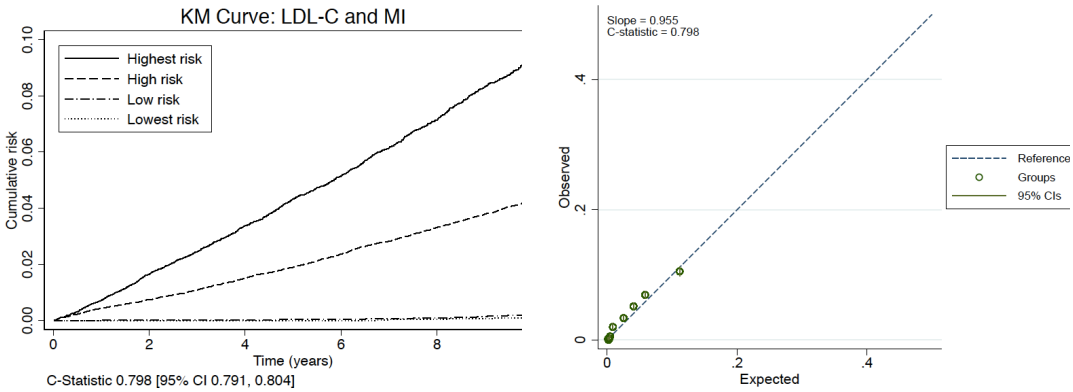
Table S 7-4 Incidence rate per 1,000 person-years of HF, AF, and MI according to geographical split cohort

Outcomes	Development cohort		Validation cohort[$]		Total	
	Person-year	Incidence rate (95%CI)	Person-year	Incidence rate (95%CI)	Person-year	Incidence rate (95%CI)
LDL-C cohort (n=478,703)						
HF (14,114 cases)	2,298,683	4.61 (4.53, 4.70)	689,950	5.09 (4.92, 5.26)	2,988,633	4.72 (4.65, 4.80)
AF (25,060 cases)	2,263,002	8.64 (8.52, 8.76)	681,914	8.08 (7.87, 8.29)	2,944,916	8.51 (8.40, 8.62)
MI (8,967 cases)	2,302,741	2.87 (2.80, 2.94)	691,127	3.41 (3.28, 3.56)	2,993,868	3.00 (2.93, 3.06)
HDL-C cohort (n=446,613)						
HF (13,228 cases)	2,193,683	4.57 (4.48, 4.66)	644,669	4.98 (4.81, 5.16)	2,838,352	4.66 (4.58, 4.74)
AF (23,454 cases)	2,159,450	8.52 (8.40, 8.65)	637,369	7.92 (7.70, 8.14)	2,796,819	8.39 (8.28, 8.49)
MI (8,480 cases)	2,197,239	2.85 (2.78, 2.92)	645,560	3.43 (3.29, 3.58)	2,842,799	2.98 (2.92, 3.05)
TG cohort (n=442,530)						
HF (12,910 cases)	2,149,938	4.51 (4.42, 4.60)	639,962	5.03 (4.85, 5.20)	2,789,899	4.63 (4.55, 4.71)
AF (22,950 cases)	2,116,437	8.48 (8.36, 8.61)	632,877	7.89 (7.67, 8.11)	2,749,314	8.35 (8.24, 8.46)
MI (8,309 cases)	2,153,149	2.84 (2.77, 2.91)	640,972	3.42 (3.28, 3.56)	2,794,121	2.97 (2.91, 3.04)

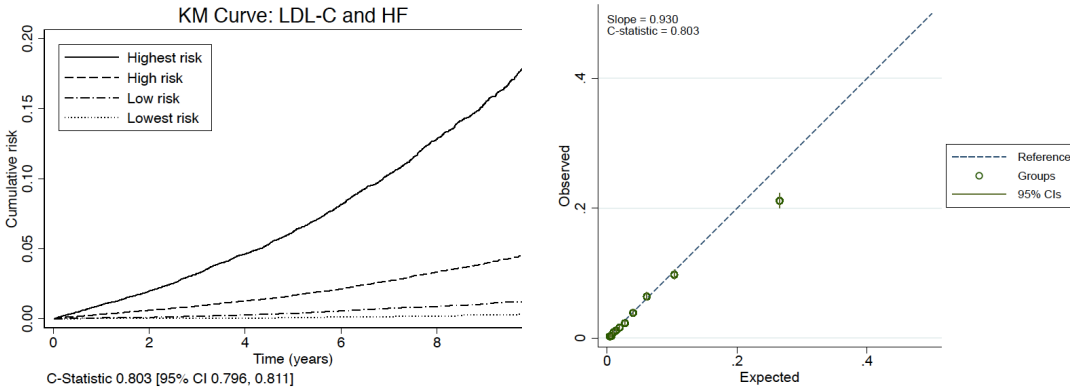
Note: [$]Populations in validation cohort were from North East, North West, Yorkshire and the Humber, and East Midlands.

Abbreviations: AF; Atrial fibrillation, CI; Confidence interval, HDL-C; High-density lipoprotein cholesterol, HF; Heart failure, LDL-C; Low-density lipoprotein cholesterol, MI; Myocardial infarction, TG; Triglyceride

Myocardial infarction



Heart failure



Atrial fibrillation

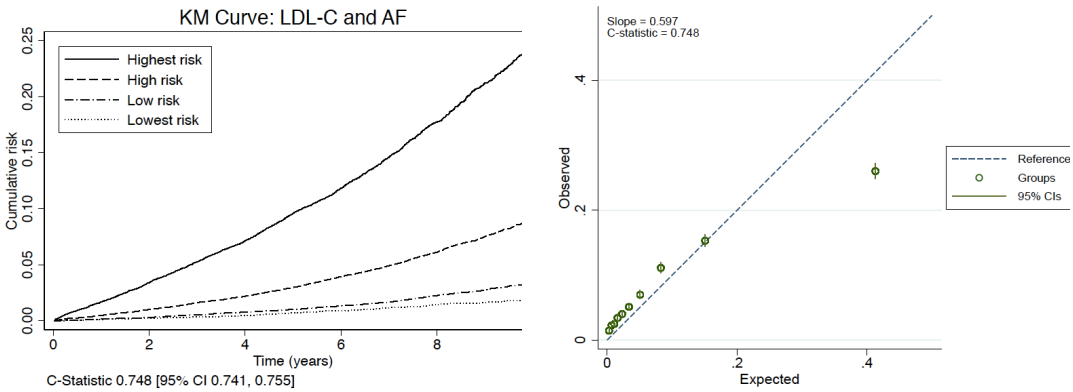
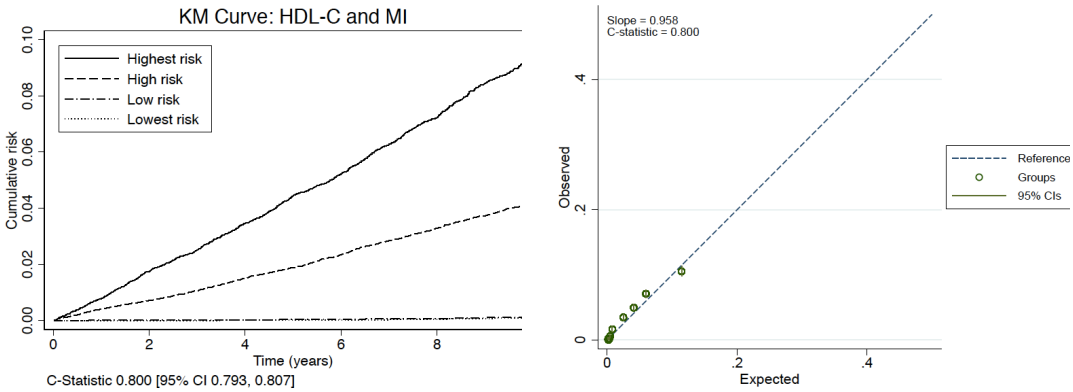


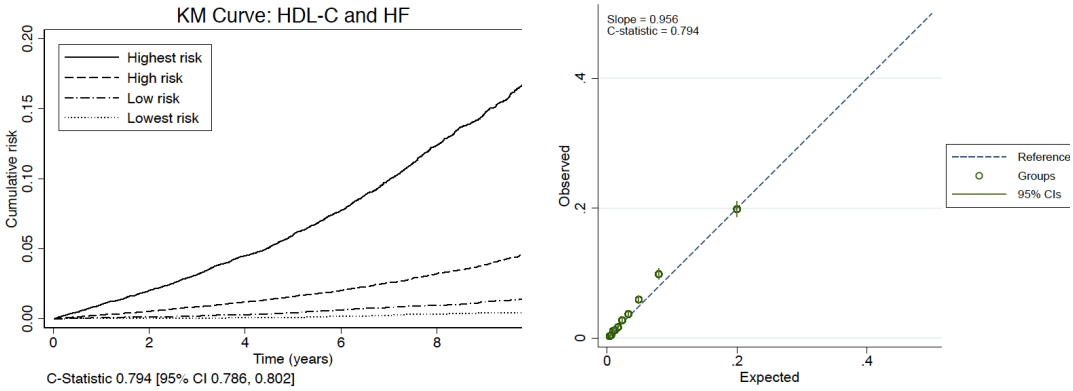
Figure S 7-1 Geographical validation: model discrimination by risk groups and calibration plots (validation LDL-C cohort, n = 103,464)

Note: Participants were grouped into deciles according to their expected (predicted) risk at 10 years and the average expected risk was compared to an average observed risk derived from Kaplan-Meier (KM) estimation

Myocardial infarction



Heart failure



Atrial fibrillation

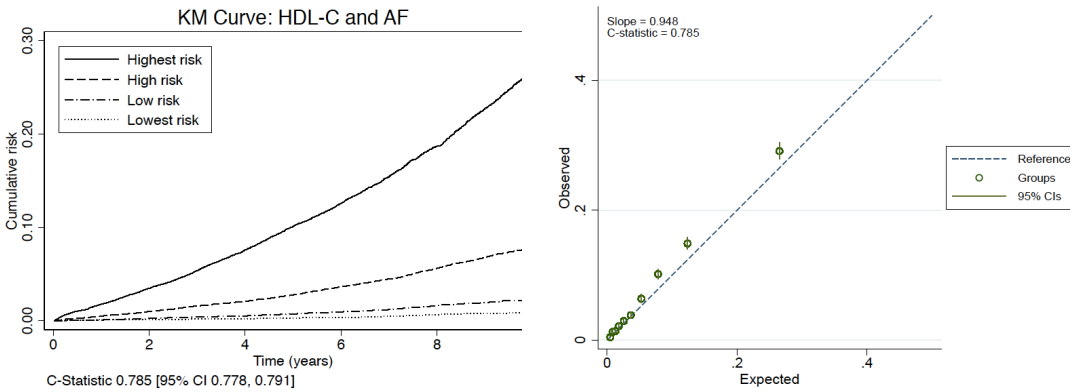
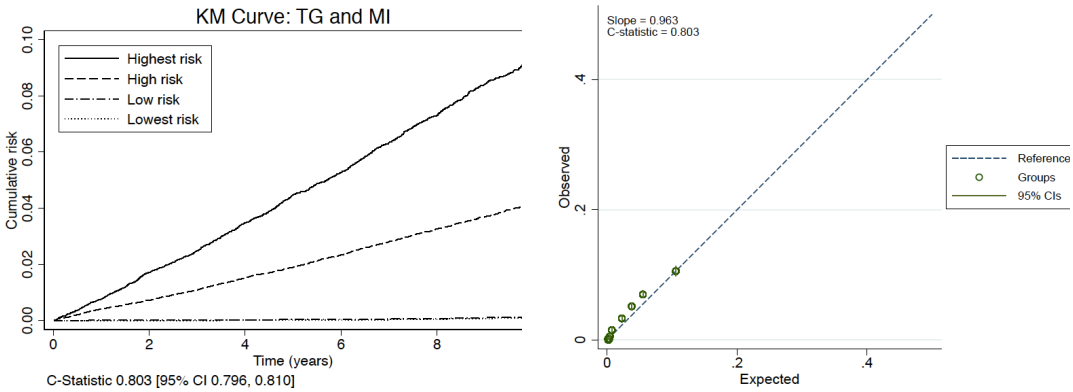


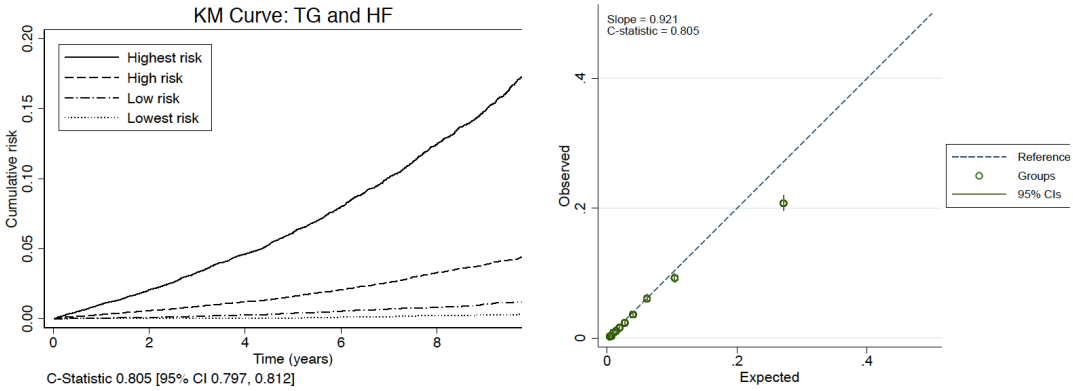
Figure S 7-2 Geographical validation: model discrimination by risk groups and calibration plots (validation HDL-C cohort, n = 95,920)

Note: Participants were grouped into deciles according to their expected (predicted) risk at 10 years and the average expected risk was compared to an average observed risk derived from Kaplan-Meier (KM) estimation

Myocardial infarction



Heart failure



Atrial fibrillation

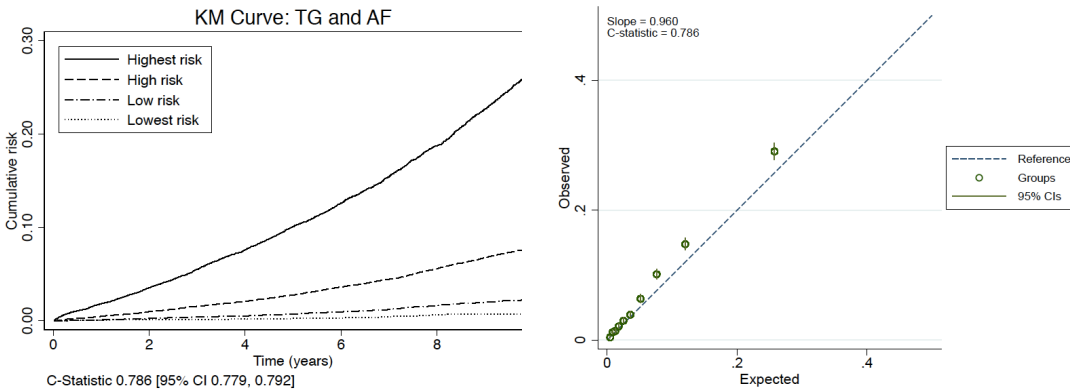


Figure S 7-3 Geographical validation: model discrimination by risk groups and calibration plots (validation TG cohort, n = 95,941)

Note: Participants were grouped into deciles according to their expected (predicted) risk at 10 years and the average expected risk was compared to an average observed risk derived from Kaplan-Meier (KM) estimation

Table S 7-5 Comparisons of point estimation (hazard ratio) and performance of risk predictive models for MI, HF, and AF with and without LDL-C variable (n=478,703)

Covariates in the model	Myocardial infarction (event = 8,967)		Heart failure (event = 14,114)		Atrial fibrillation (event = 25,060)	
	With LDL-C	Without LDL-C	With LDL-C	Without LDL-C	With LDL-C	Without LDL-C
LDL-C (per SD decrease)	0.9156 (0.0114)***	-	1.0975 (0.0080)***	-	1.0997 (0.0079)***	-
Age	0.2471 (0.0244)***	0.2487 (0.0246)***	0.9583 (0.0876)	0.9426 (0.0863)	2.9868 (0.2175)***	2.9090 (0.2121)***
Age^2	6.0720 (0.5533)***	5.9908 (0.5457)***	2.8556 (0.2257)***	2.9030 (0.2296)***	0.9771 (0.0618)	1.0016 (0.0634)
IMD 2^{nd} quintile vs. 1^{st} quintile	0.9992 (0.0343)	1.0011 (0.0344)	1.0058 (0.0278)	1.0031 (0.2296)	0.9473 (0.0187)**	0.9447 (0.0186)**
IMD 3^{rd} quintile vs. 1^{st} quintile	0.9919 (0.0352)	0.9945 (0.0353)	1.1099 (0.0311)***	1.1081 (0.0310)***	1.0250 (0.0207)	1.0228 (0.0206)
IMD 4^{th} quintile vs. 1^{st} quintile	1.0958 (0.0371)**	1.1000 (0.0373)**	1.1645 (0.0314)***	1.1598 (0.0313)***	0.9457 (0.0190)**	0.9418 (0.0189)**
IMD 5^{th} quintile vs. 1^{st} quintile	1.1659 (0.0389)***	1.1660 (0.0389)***	1.1870 (0.0322)***	1.1873 (0.0322)***	0.9918 (0.0200)	0.9921 (0.0200)
Ex-smokers vs. non-smokers	1.1559 (0.0283)***	1.1521 (0.0282)***	1.1939 (0.0227)***	1.1978 (0.0228)***	1.0944 (0.0155)***	1.0983 (0.0155)***
Current smokers vs. non-smokers	0.9497 (0.0142)**	1.7188 (0.0505)***	1.7076 (0.0450)***	1.7182 (0.0453)***	1.1814 (0.0253)***	1.1906 (0.0255)***
Systolic blood pressure (SBP)	1.0996 (0.0164)***	1.1048 (0.0165)***	1.1132 (0.0128)***	1.1072 (0.0127)***	1.0564 (0.0093)***	1.0517 (0.0092)***
Diastolic blood pressure (DBP)	0.9949 (0.0017)**	0.9565 (0.0143)**	0.9034 (0.0106)***	0.8959 (0.0105)***	0.9658 (0.0086)***	0.9583 (0.0085)***
BMI	0.9386 (0.0126)***	0.9329 (0.0124)***	1.3457 (0.0121)***	1.3539 (0.0121)***	1.2278 (0.0089)***	1.2351 (0.0090)***
Ln Triglyceride (TG)	0.9787 (0.0127)	0.9878 (0.0127)	0.9313 (0.0099)***	0.9198 (0.0098)***	0.8768 (0.0070)***	0.8644 (0.0069)***
HDL-C	0.8485 (0.0122)***	0.8489 (0.0122)***	0.9952 (0.0104)	0.9972 (0.0105)	1.0075 (0.0077)	1.0098 (0.0078)
Ln C-Reactive Protein (CRP)	1.1296 (0.0115)***	1.1309 (0.0115)***	1.2735 (0.0099)***	1.2725 (0.0099)***	1.1765 (0.0070)***	1.1758 (0.0070)***
Diabetes type I vs. no diabetes	1.1355 (0.1787)	1.0613 (0.1668)	1.5856 (0.1996)***	1.7026 (0.2140)***	0.7688 (0.1004)*	0.8259 (0.1078)
Diabetes type II vs. no diabetes	1.2306 (0.0393)***	1.1528 (0.0357)***	1.4073 (0.0341)***	1.5034 (0.0351)***	0.9989 (0.0209)	1.0703 (0.0217)**
Other diabetes vs. no diabetes	0.6591 (0.0422)***	0.6136 (0.0389)***	0.8070 (0.0412)***	0.8640 (0.0437)**	0.4570 (0.0225)***	0.4912 (0.0241)***
Blood pressure medication use	0.9702 (0.0224)	0.9423 (0.0215)**	1.3758 (0.0264)***	1.4108 (0.0268)***	1.2839 (0.0181)***	1.3165 (0.0184)***
Statins use	11.535 (0.4893)***	11.884 (0.5029)***	1.5871 (0.0318)***	1.5647 (0.0313)***	1.3687 (0.0197)***	1.3469 (0.0193)***
Model performance						
Likelihood ratio chi^2 (P-value)	69.83 (<0.001)		21.14 (<0.001)		190.59 (<0.001)	

[#]1 SD of LDL-C ~ 0.94 mmol/L (36.35 mg/dL), All continuous variables were reported HR as per SD increase as follows: 1 SD of age = 13.25 years, 1 SD of age^2 = 1,541.28 years^2, 1 SD of SBP = 16.69 mmHg, 1 SD of DBP = 9.26 mmHg, 1 SD of BMI = 5.59 kg/m^2, 1 SD of TG = 0.84 mmol/L, 1 SD of HDL-C = 0.43 mmol/L, and 1 SD of CRP = 21.81 mg/L. *, **, *** represent P-values of < 0.05, < 0.01, and < 0.001, respectively. Values are hazard ratio (standard errors). All models are stratified by gender. **Abbreviations:** LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, BMI; body mass index.

Table S 7-6 Performance statistic between the model with and without LDL-C (reference model) stratified by genders

Endpoints	Myocardial infarction		Heart failure		Atrial fibrillation	
	Male	Female	Male	Female	Male	Female
Events (n)	5,192	3,775	6,664	7,450	12,105	12,889
Nonevents (n)	188,706	281,030	187,234	277,355	181,727	271,916
Likelihood ratio chi^2 (p-values)	43.16 (< 0.001)	20.63 (< 0.001)	49.52 (< 0.001)	75.63 (< 0.001)	113.39 (< 0.001)	111.69 (< 0.001)
C-statistics						
Reference model	0.793 (0.788 to 0.798)	0.842 (0.837 to 0.847)	0.803 (0.797 to 0.808)	0.848 (0.844 to 0.853)	0.773 (0.768 to 0.777)	0.816 (0.813 to 0.820)
Reference + LDL-C model	0.794 (0.790 to 0.799)	0.842 (0.837 to 0.847)	0.804 (0.798 to 0.809)	0.849 (0.845 to 0.854)	0.774 (0.769 to 0.778)	0.818 (0.814 to 0.821)
ΔC-statistics	0.001	0.000	0.001	0.001	0.001	0.002
D-statistics						
Reference model	1.698	2.124	1.984	2.375	1.695	2.059
Reference + LDL-C model	1.696	2.117	1.995	2.391	1.709	2.073
Calibration slope*						
Reference model	1.003 (0.968 to 1.038)	1.003 (0.974 to 1.033)	1.002 (0.980 to 1.024)	1.002 (0.983 to 1.020)	1.002 (0.982 to 1.021)	1.001 (0.985 to 1.018)
Reference + LDL-C model	1.003 (0.969 to 1.038)	1.003 (0.974 to 1.033)	1.002 (0.981 to 1.024)	1.001 (0.983 to 1.020)	1.002 (0.982 to 1.021)	1.001 (0.985 to 1.018)
Adjusted R^2_D						
Reference model	0.408 (0.398 to 0.420)	0.518 (0.509 to 0.530)	0.484 (0.473 to 0.497)	0.574 (0.565 to 0.583)	0.406 (0.398 to 0.416)	0.503 (0.496 to 0.511)
Reference + LDL-C model	0.407 (0.397 to 0.419)	0.517 (0.506 to 0.529)	0.487 (0.477 to 0.498)	0.577 (0.568 to 0.586)	0.411 (0.402 to 0.420)	0.506 (0.498 to 0.514)
Four-category NRI**						
NRI event	-0.007 (-0.017 to 0.002)	-0.007 (-0.011 to 0.007)	0.007 (-0.001 to 0.014)	0.008 (0.001 to 0.017)	0.005 (-0.002 to 0.010)	-0.003 (-0.006 to 0.004)
NRI nonevent	0.007 (0.003 to 0.011)	0.001 (0.001 to 0.002)	-0.001 (-0.003 to -0.001)	-0.002 (-0.002 to -0.001)	0.001 (-0.001 to 0.003)	0.001 (<0.000 to 0.002)
NRI	0.001 (-0.010 to 0.010)	-0.006 (-0.010 to 0.009)	0.006 (-0.003 to 0.013)	0.007 (-0.001 to 0.015)	0.006 (-0.001 to 0.011)	-0.001 (-0.005 to 0.005)
Category-free (cf) NRI**						
cfNRI event	0.002 (-0.020 to 0.027)	-0.052 (-0.083 to -0.024)	0.113 (0.086 to 0.137)	0.124 (0.094 to 0.146)	0.097 (0.079 to 0.117)	0.116 (0.099 to 0.136)
cfNRI nonevent	0.099 (0.087 to 0.111)	0.157 (0.143 to 0.173)	-0.048 (-0.061 to -0.035)	-0.086 (-0.100 to -0.072)	-0.038 (-0.048 to -0.028)	-0.066 (-0.076 to -0.055)
cfNRI	0.101 (0.070 to 0.134)	0.105 (0.066 to 0.142)	0.065 (0.032 to 0.095)	0.038 (0.003 to 0.067)	0.059 (0.034 to 0.086)	0.050 (0.027 to 0.075)

*Based on heuristic shrinkage-calibration slope, C-statistics. **NRI was adjusted for 10-year risk using Kaplan-Meier estimation. **Abbreviations:** LDL-C; Low-Density Lipoprotein-Cholesterol, NRI; Net Reclassification Improvement.

Table S 7-7 Comparisons of point estimation (hazard ratio) and performance of the risk predictive models for MI, HF, and AF with and without HDL-C variable (n=446,613)

Covariates in the model	Myocardial infarction (event = 8,480) With HDL-C	Without HDL-C	Heart failure (event = 13,228) With HDL-C	Without HDL-C	Atrial fibrillation (event = 23,454) With HDL-C	Without HDL-C
HDL-C (per SD increase)#	0.8464 (0.0125)***	-	0.9944 (0.0107)	-	1.0087 (0.0080)	-
Age	0.2512 (0.0254)***	0.2461 (0.0249)***	0.9755 (0.0917)	0.9743 (0.0915)	3.0300 (0.2268)***	3.0371 (0.2272)***
Age^2	5.9258 (0.5518)***	5.9795 (0.5568)***	2.8137 (0.2280)***	2.8156 (0.2282)***	0.9685 (0.0628)	0.9670 (0.0627)
IMD 2nd quintile vs. 1st quintile	0.9968 (0.0354)	1.0028 (0.0356)	1.0183 (0.0292)	1.0185 (0.0292)	0.9518 (0.0195)*	0.9514 (0.0195)*
IMD 3rd quintile vs. 1st quintile	0.9816 (0.0358)	0.9892 (0.0361)	1.1175 (0.0323)***	1.1178 (0.0323)***	1.0153 (0.0212)	1.0149 (0.0212)
IMD 4th quintile vs. 1st quintile	1.0875 (0.0377)*	1.0919 (0.0378)*	1.1594 (0.0322)***	1.1595 (0.0322)***	0.9371 (0.0193)**	0.9369 (0.0193)**
IMD 5th quintile vs. 1st quintile	1.1438 (0.0393)***	1.1551 (0.0396)***	1.1677 (0.0328)***	1.1681 (0.0328)***	0.9885 (0.0205)	0.9880 (0.0205)
Ex-smokers vs. non-smokers	1.1831 (0.0298)***	1.1786 (0.0296)***	1.2003 (0.0236)***	1.2002 (0.0236)***	1.1092 (0.0162)***	1.1096 (0.0162)***
Current smokers vs. non-smokers	1.6996 (0.0514)***	1.7249 (0.0521)***	1.6857 (0.0458)***	1.6863 (0.0458)***	1.1843 (0.0261)***	1.1838 (0.0261)***
Systolic blood pressure (SBP)	1.0902 (0.0168)***	1.0836 (0.0167)***	1.1103 (0.0132)***	1.1101 (0.0132)***	1.0500 (0.0096)***	1.0503 (0.0096)***
Diastolic blood pressure (DBP)	0.9514 (0.0147)**	0.9450 (0.0146)***	0.9050 (0.0111)***	0.9048 (0.0111)***	0.9660 (0.0089)***	0.9663 (0.0089)***
BMI	0.9392 (0.0129)***	0.9652 (0.0130)**	1.3460 (0.0125)***	1.3471 (0.0124)***	1.2300 (0.0092)***	1.2282 (0.0091)***
Ln Triglyceride (TG)	0.9725 (0.0128)*	1.0301 (0.0125)*	0.9264 (0.0101)***	0.9284 (0.0093)***	0.8769 (0.0072)***	0.8739 (0.0066)***
LDL-C	1.0631 (0.0114)***	1.0646 (0.0114)***	0.9023 (0.0082)***	0.9024 (0.0082)***	0.9064 (0.0062)***	0.9062 (0.0062)***
Ln C-Reactive Protein (CRP)	1.1317 (0.0118)***	1.1327 (0.0118)***	1.2683 (0.0102)***	1.2683 (0.0102)***	1.1735 (0.0072)***	1.1734 (0.0072)***
Diabetes type I vs. no diabetes	1.1848 (0.1844)	1.1474 (0.1786)	1.6997 (0.2108)***	1.6982 (0.2106)***	0.8428 (0.1083)	0.8440 (0.1084)
Diabetes type II vs. no diabetes	1.1788 (0.0394)***	1.2179 (0.0406)***	1.3975 (0.0353)***	1.3993 (0.0352)***	0.9903 (0.0217)	0.9883 (0.0216)
Other diabetes vs. no diabetes	0.6412 (0.0434)***	0.6571 (0.0444)***	0.8541 (0.0450)**	0.8551 (0.0450)**	0.4634 (0.0242)***	0.4626 (0.0241)***
Blood pressure medication use	0.9636 (0.0228)	0.9662 (0.0229)	1.3637 (0.0268)***	1.3638 (0.0268)***	1.2846 (0.0186)***	1.2843 (0.0186)***
Statins use	12.236 (0.5397)***	12.153 (0.5356)***	1.6332 (0.0339)***	1.6327 (0.0339)***	1.3899 (0.0207)***	1.3904 (0.0207)***
Model performance						
Likelihood ratio chi^2 (P-value)	130.57 (<0.001)		0.27 (0.602)		1.19 (0.274)	

[#]1 SD of HDL-C ~ 0.43 mmol/L (16.63 mg/dL), All continuous variables were reported HR as per SD increase as follows: 1 SD of age = 13.29 years, 1 SD of age^2 = 1,545.16 years^2, 1 SD of SBP = 16.93 mmHg, 1 SD of DBP = 9.35 mmHg, 1 SD of BMI = 5.57 kg/m^2, 1 SD of TG = 1.03 mmol/L, 1 SD of LDL-C = 0.94 mmol/L, and 1 SD of CRP = 21.94 mg/L. *, **, *** represent P-values of < 0.05, < 0.01, and < 0.001, respectively. Values are hazard ratio (standard errors). All models are stratified by gender. **Abbreviations:** LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, BMI; body mass index.

Table S 7-8 Performance statistic between the model with and without HDL-C (reference model) stratified by genders

Endpoints	Myocardial infarction		Heart failure		Atrial fibrillation	
	Male	Female	Male	Female	Male	Female
Events (n)	4,911	3,569	6,251	6,977	11,394	12,060
Nonevents (n)	175,014	263,119	173,674	259,711	168,531	254,628
Likelihood ratio chi^2 (p-values)	124.70 (<0.001)	27.94 (<0.001)	0.99 (0.319)	3.04 (0.081)	13.25 (0.0003)	6.83 (0.009)
C-statistics						
Reference model	0.791 (0.786 to 0.795)	0.843 (0.838 to 0.848)	0.806 (0.800 to 0.811)	0.850 (0.845 to 0.854)	0.776 (0.771 to 0.780)	0.819 (0.815 to 0.823)
Reference + HDL-C model	0.796 (0.791 to 0.801)	0.844 (0.839 to 0.849)	0.806 (0.800 to 0.811)	0.850 (0.845 to 0.854)	0.776 (0.771 to 0.780)	0.819 (0.815 to 0.823)
ΔC-statistics	0.005	0.001	0.000	0.000	0.000	0.000
D-statistics						
Reference model	1.678	2.121	2.010	2.398	1.723	2.081
Reference + HDL-C model	1.709	2.129	2.009	2.399	1.722	2.083
Calibration slope*						
Reference model	1.003 (0.967 to 1.040)	1.003 (0.973 to 1.034)	1.002 (0.980 to 1.025)	1.002 (0.982 to 1.021)	1.002 (0.982 to 1.022)	1.001 (0.984 to 1.018)
Reference + HDL-C model	1.003 (0.968 to 1.039)	1.004 (0.973 to 1.034)	1.002 (0.980 to 1.025)	1.002 (0.983 to 1.021)	1.002 (0.982 to 1.022)	1.001 (0.984 to 1.018)
Adjusted R^2_D						
Reference model	0.402 (0.392 to 0.414)	0.518 (0.507 to 0.530)	0.491 (0.480 to 0.504)	0.578 (0.570 to 0.588)	0415 (0.406 to 0.425)	0.508 (0.500 to 0.516)
Reference + HDL-C model	0.411 (0.401 to 0.423)	0.520 (0.509 to 0.533)	0.491 (0.480 to 0.502)	0.579 (0.570 to 0.588)	0.415 (0.405 to 0.424)	0.509 (0.501 to 0.517)
Four-category NRI**						
NRI event	0.009 (-0.001 to 0.026)	0.003 (-0.005 to 0.015)	0.000 (-0.004 to 0.004)	-0.002 (-0.004 to 0.005)	-0.002 (-0.005 to 0.003)	-0.002 (-0.004 to 0.002)
NRI nonevent	0.011 (0.006 to 0.015)	-0.001 (-0.002 to -0.001)	0.000 (-0.001 to 0.001)	0.000 (-0.001 to 0.000)	0.001 (-0.000 to 0.002)	-0.000 (-0.000 to 0.001)
NRI	0.020 (0.009 to 0.038)	0.001 (-0.006 to 0.014)	0.000 (-0.004 to 0.005)	-0.002 (-0.004 to 0.005)	-0.001 (-0.005 to 0.004)	-0.002 (-0.004 to 0.002)
Category-free (cf) NRI**						
cfNRI event	0.214 (0.187 to 0.243)	0.133 (0.099 to 0.170)	-0.130 (-0.150 to 0.148)	0.105 (-0.099 to 0.128)	-0.121 (-0.137 to -0.106)	0.117 (0.099 to 0.136)
cfNRI nonevent	-0.058 (-0.073 to -0.042)	-0.077 (-0.094 to -0.059)	0.132 (-0.127 to 0.148)	-0.109 (-0.122 to 0.123)	0.141 (0.128 to 0.151)	-0.097 (-0.108 to -0.085)
cfNRI	0.157 (0.123 to 0.192)	0.056 (0.015 to 0.101)	0.002 (-0.019 to 0.035)	-0.003 (-0.032 to 0.031)	0.020 (-0.004 to 0.038)	0.020 (-0.002 to 0.042)

*Based on heuristic shrinkage-calibration slope, C-statistics. **NRI was adjusted for 10-year risk using Kaplan-Meier estimation. **Abbreviations:** HDL-C; How-Density Lipoprotein-Cholesterol, NRI; Net Reclassification Improvement.

Table S 7-9 Comparisons of point estimation (hazard ratio) and performance of the risk predictive models for MI, HF, and AF with and without TG variable (n=442,530)

Covariates in the model	Myocardial infarction (event = 8,309)		Heart failure (event = 12,910)		Atrial fibrillation (event = 22,950)	
	With TG	Without TG	With TG	Without TG	With TG	Without TG
TG (per SD decrease)[#]	1.0262 (0.0130)	-	1.0775 (0.0102)***	-	1.1369 (0.0073)***	-
Age	0.2587 (0.0264)***	0.2591 (0.0265)***	0.9521 (0.0901)	0.9499 (0.0900)	2.9545 (0.2221)***	2.9428 (0.2216)***
Age^2	5.7802 (0.5440)***	5.7815 (0.5442)***	2.8780 (0.2349)***	2.8885 (0.2359)***	0.9880 (0.0644)	0.9929 (0.0648)
IMD 2nd quintile vs. 1st quintile	1.0016 (0.0359)	1.0021 (0.0360)	1.0343 (0.0301)	1.0351 (0.0301)	0.9517 (0.0197)*	0.9533 (0.0197)*
IMD 3rd quintile vs. 1st quintile	0.9789 (0.0364)	0.9793 (0.0364)	1.1238 (0.0332)***	1.1234 (0.0332)***	1.0216 (0.0217)	1.0220 (0.0217)
IMD 4th quintile vs. 1st quintile	1.0957 (0.0385)**	1.0955 (0.0385)**	1.1629 (0.0329)***	1.1608 (0.0329)***	0.9373 (0.0196)**	0.9350 (0.0195)**
IMD 5th quintile vs. 1st quintile	1.1573 (0.0402)***	1.1561 (0.0402)***	1.1960 (0.0341)***	1.1918 (0.0340)***	0.9922 (0.0209)	0.9869 (0.0208)
Ex-smokers vs. non-smokers	1.1846 (0.0301)***	1.1823 (0.0301)***	1.2169 (0.0242)***	1.2105 (0.0241)***	1.1101 (0.0164)***	1.0998 (0.0163)***
Current smokers vs. non-smokers	1.7127 (0.0521)***	1.7062 (0.0518)***	1.7086 (0.0468)***	1.6882 (0.0462)***	1.1798 (0.0262)***	1.1556 (0.0257)***
Systolic blood pressure (SBP)	1.0961 (0.0170)***	1.0953 (0.0170)***	1.1035 (0.0133)***	1.1016 (0.0133)***	1.0484 (0.0096)***	1.0451 (0.0096)***
Diastolic blood pressure (DBP)	0.9479 (0.0148)**	0.9465 (0.0148)***	0.9107 (0.0113)***	0.9070 (0.0112)***	0.9682 (0.0091)**	0.9619 (0.0094)***
BMI	0.9385 (0.0130)***	0.9354 (0.0129)***	1.3459 (0.0127)***	1.3366 (0.0126)***	1.2289 (0.0093)***	1.2110 (0.0092)***
HDL-C	0.8423 (0.0126)***	0.8515 (0.0118)***	1.0011 (0.0109)	1.0303 (0.0103)**	1.0092 (0.0081)	1.0588 (0.0077)***
LDL-C	1.0709 (0.0116)***	1.0690 (0.0115)***	0.9019 (0.0083)***	0.8967 (0.0082)***	0.9070 (0.0063)***	0.8968 (0.0061)***
Ln C-Reactive Protein (CRP)	1.1304 (0.0119)***	1.1301 (0.0119)***	1.2681 (0.0103)***	1.2671 (0.0103)***	1.1744 (0.0073)***	1.1721 (0.0073)***
Diabetes type I vs. no diabetes	1.0346 (0.1787)	1.0371 (0.1791)	1.6231 (0.2146)***	1.6166 (0.2137)***	0.7939 (0.1094)	0.7908 (0.1090)
Diabetes type II vs. no diabetes	1.2077 (0.0408)***	1.2048 (0.0407)***	1.3996 (0.0360)***	1.3883 (0.0357)***	0.9905 (0.0222)	0.9766 (0.0219)
Other diabetes vs. no diabetes	0.6350 (0.0447)***	0.6342 (0.0447)***	0.7937 (0.0449)***	0.7882 (0.0446)***	0.4569 (0.0250)***	0.4521 (0.0247)***
Blood pressure medication use	0.9855 (0.0235)	0.9843 (0.0235)	1.3954 (0.0277)***	1.3884 (0.0275)***	1.2945 (0.0189)***	1.2834 (0.0187)***
Statins use	11.917 (0.5254)***	11.807 (0.5173)***	1.6293 (0.0341)***	1.5948 (0.0330)***	1.3866 (0.0208)***	1.3355 (0.0198)***
Model performance						
Likelihood ratio chi^2 (P-value)	3.76 (0.052)		45.66 (<0.001)		238.41 (<0.001)	

Note: #1 SD of TG ~ 1.14 mmol/L (100.97 mg/dL), All continuous variables were reported HR as per SD increase as follows: 1 SD of age = 13.30 years, 1 SD of age^2 = 1,542.61 years^2, 1 SD of SBP = 17.00 mmHg, 1 SD of DBP = 9.36 mmHg, 1 SD of BMI = 5.58 kg/m^2, 1 SD of LDL-C = 0.94 mmol/L, 1 SD of HDL-C = 0.43 mmol/L, and 1 SD of CRP = 22.15 mg/L. *, **, *** represent P-values of < 0.05, < 0.01, and < 0.001, respectively. Values are hazard ratio (standard errors). All models are stratified by gender. **Abbreviations:** LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, BMI; body mass index.

Table S 7-10 Performance statistic between the model with and without TG (reference model) stratified by genders

Endpoints	Myocardial infarction		Heart failure		Atrial fibrillation	
	Male	Female	Male	Female	Male	Female
Events (n)	4,793	3,516	6,096	6,814	11,146	11,804
Nonevents (n)	173,305	260,916	172,002	257,618	166,952	252,628
Likelihood ratio chi^2 (p-values)	31.34 (<0.001)	2.21 (0.137)	69.18 (<0.001)	6.18 (0.013)	137.81 (<0.001)	153.16 (<0.001)
C-statistics						
Reference model	0.798 (0.793 to 0.802)	0.844 (0.839 to 0.849)	0.806 (0.800 to 0.811)	0.850 (0.845 to 0.855)	0.774 (0.769 to 0.778)	0.818 (0.814 to 0.822)
Reference + TG model	0.799 (0.794 to 0.804)	0.844 (0.839 to 0.849)	0.807 (0.801 to 0.812)	0.850 (0.845 to 0.855)	0.775 (0.771 to 0.780)	0.819 (0.815 to 0.823)
ΔC-statistics	0.001	0.000	0.001	0.000	0.001	0.001
D-statistics						
Reference model	1.716	2.132	2.010	2.412	1.707	2.081
Reference + TG model	1.728	2.131	2.021	2.412	1.719	2.090
Calibration slope*						
Reference model	1.003 (0.967 to 1.039)	1.003 (0.973 to 1.034)	1.002 (0.980 to 1.025)	1.002 (0.982 to 1.021)	1.002 (0.982 to 1.022)	1.001 (0.984 to 1.018)
Reference + TG model	1.003 (0.968 to 1.039)	1.004 (0.973 to 1.034)	1.003 (0.980 to 1.025)	1.002 (0.982 to 1.021)	1.002 (0.982 to 1.022)	1.001 (0.984 to 1.018)
Adjusted R^2_D						
Reference model	0.413 (0.403 to 0.426)	0.520 (0.508 to 0.531)	0.491 (0.479 to 0.503)	0.581 (0.572 to 0.590)	0.410 (0.400 to 0.420)	0.508 (0.501 to 0.516)
Reference + TG model	0.416 (0.406 to 0.430)	0.520 (0.508 to 0.532)	0.494 (0.483 to 0.506)	0.581 (0.573 to 0.590)	0.414 (0.405 to 0.423)	0.510 (0.502 to 0.519)
Four-category NRI**						
NRI event	0.002 (-0.009 to 0.010)	0.000 (-0.004 to 0.008)	-0.001 (-0.007 to 0.011)	-0.000 (-0.005 to 0.005)	0.003 (-0.004 to 0.009)	0.004 (-0.002 to 0.010)
NRI nonevent	0.004 (0.001 to 0.007)	0.000 (-0.000 to 0.000)	0.001 (0.000 to 0.003)	0.000 (-0.000 to 0.001)	0.006 (0.004 to 0.008)	0.004 (0.002 to 0.005)
NRI	0.006 (-0.005 to 0.015)	0.000 (-0.004 to 0.008)	-0.000 (-0.006 to 0.013)	0.000 (-0.005 to 0.006)	0.009 (0.001 to 0.016)	0.007 (0.001 to 0.015)
Category-free (cf) NRI**						
cfNRI event	0.048 (0.018 to 0.072)	-0.022 (-0.055 to 0.040)	0.082 (0.057 to 0.107)	0.044 (0.020 to 0.071)	0.075 (0.056 to 0.095)	0.080 (0.062 to 0.099)
cfNRI nonevent	0.005 (-0.009 to 0.020)	0.081 (-0.070 to 0.098)	0.007 (-0.008 to 0.023)	-0.076 (-0.091 to -0.061)	0.015 (0.004 to 0.025)	-0.014 (-0.025 to -0.003)
cfNRI	0.053 (0.017 to 0.085)	0.059 (-0.038 to 0.101)	0.088 (0.055 to 0.123)	-0.032 (-0.061 to 0.005)	0.090 (0.066 to 0.116)	0.066 (0.043 to 0.088)

*Based on heuristic shrinkage-calibration slope, C-statistics. **NRI was adjusted for 10-year risk using Kaplan-Meier estimation. **Abbreviations:** TG; Triglyceride, NRI; Net Reclassification Improvement.

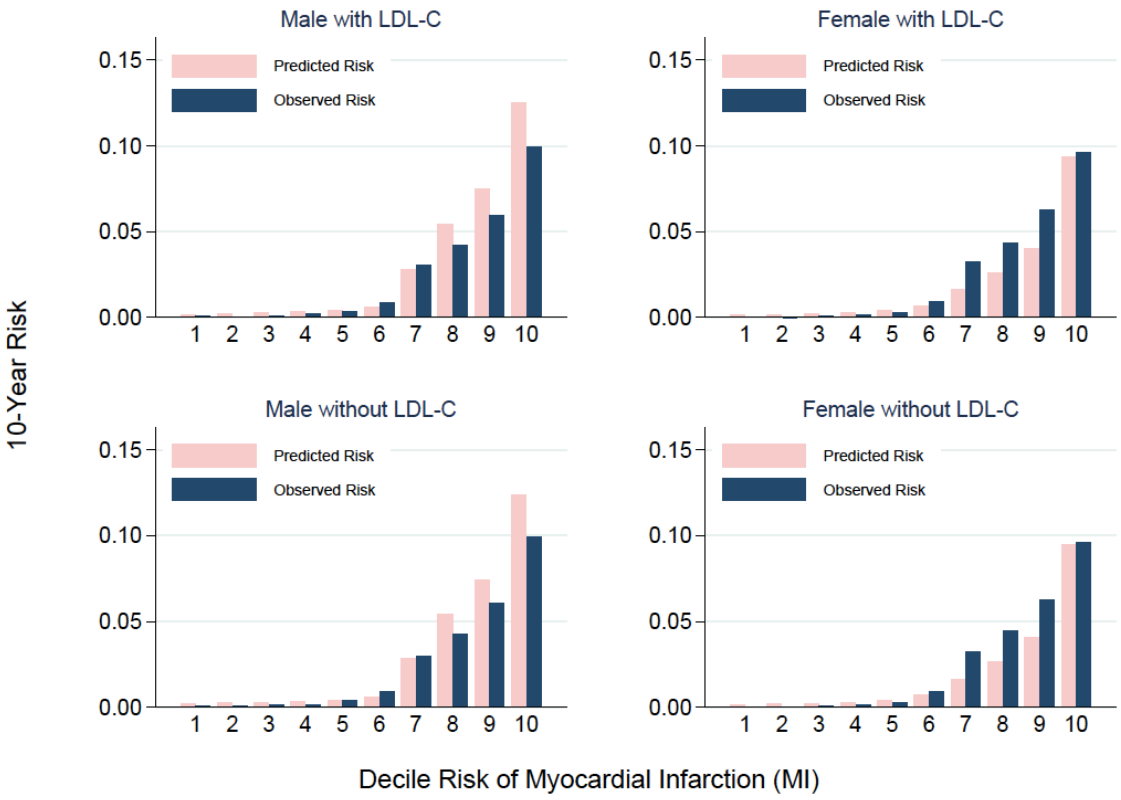


Figure S 7-4 Calibration by deciles of MI risk function by genders and by with or without LDL-C in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident myocardial infarction in 10 year.

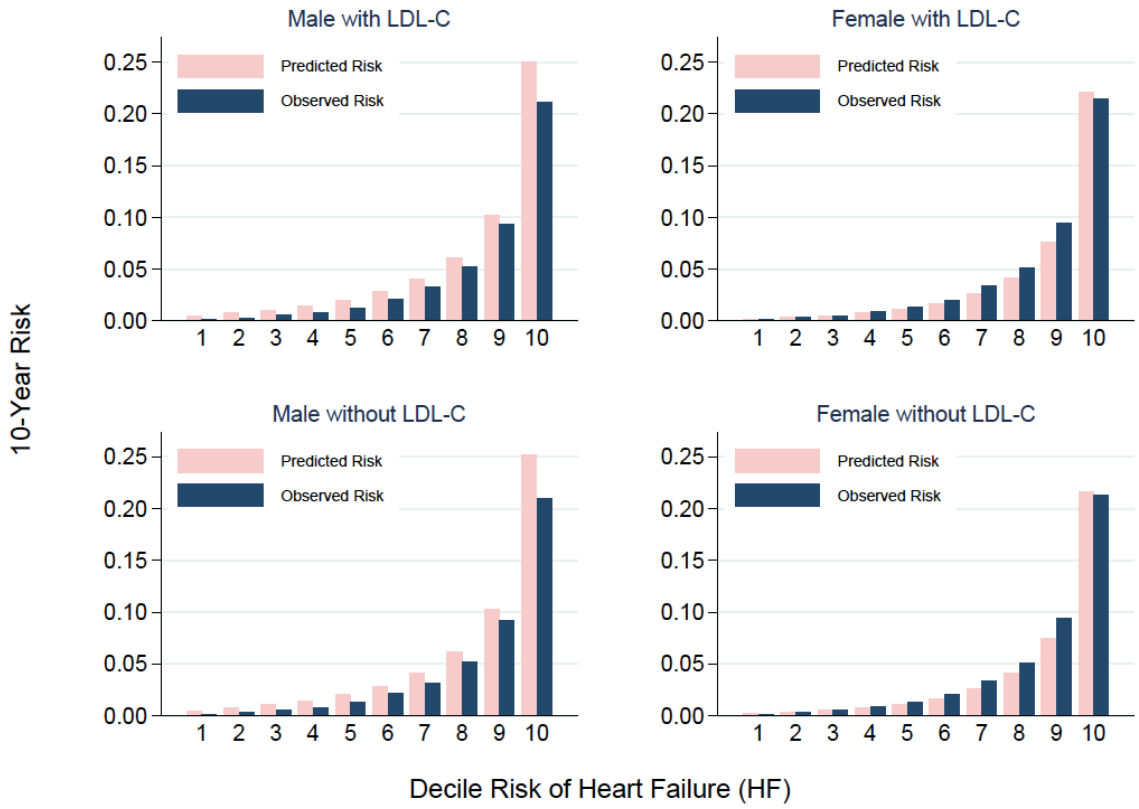


Figure S 7-5 Calibration by deciles of HF risk function by genders and by with or without LDL-C in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident heart failure in 10 years.

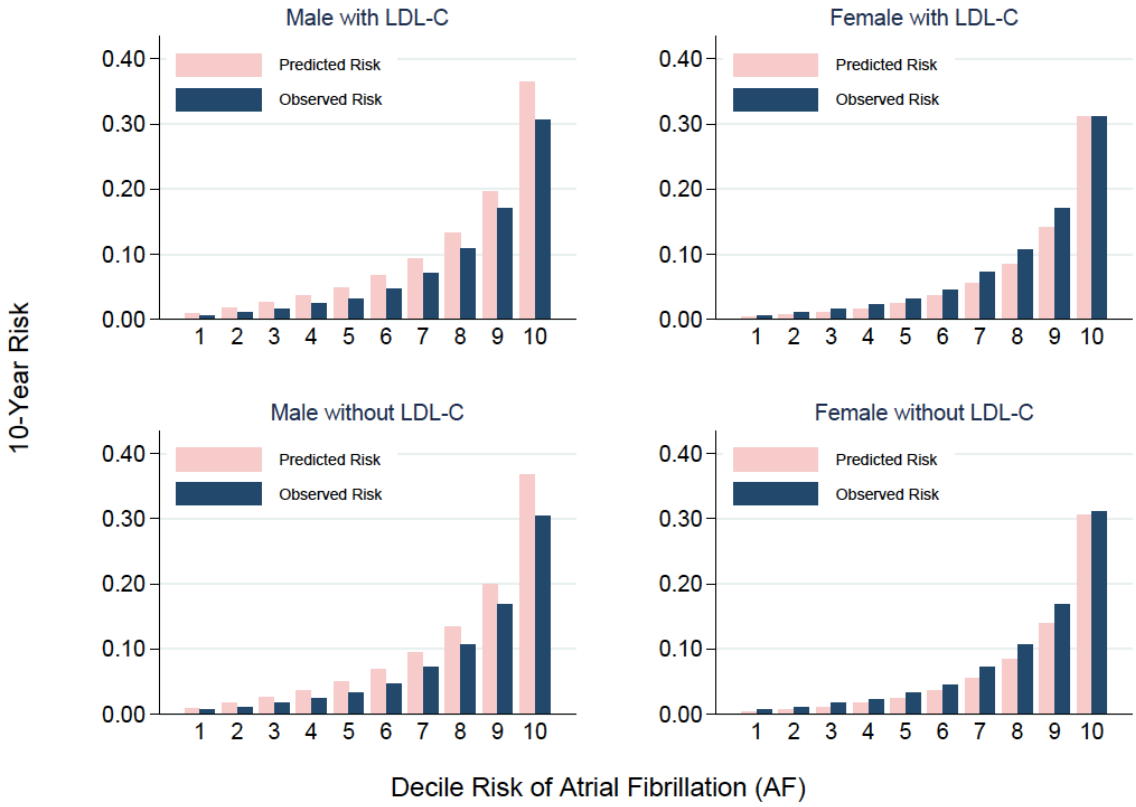


Figure S 7-6 Calibration by deciles of AF risk function by genders and by with or without LDL-C in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident atrial fibrillation in 10 years.

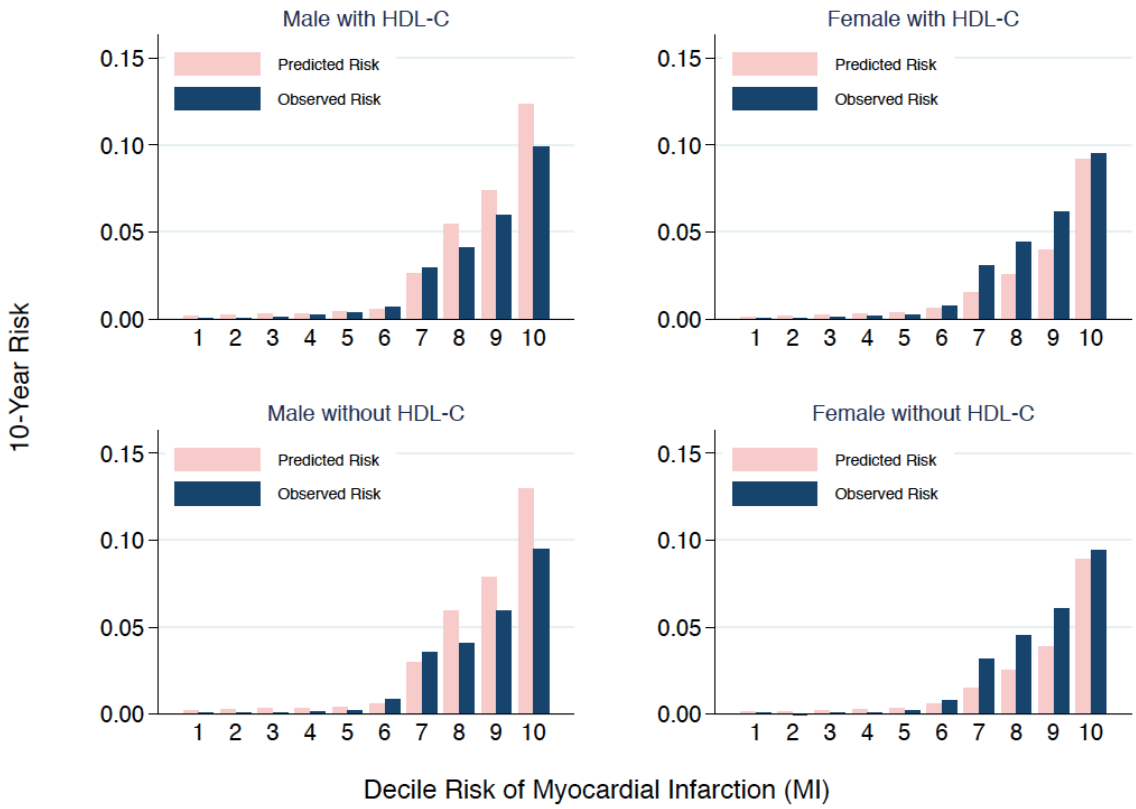


Figure S 7-7 Calibration by deciles of MI risk function by genders and by with or without HDL-C in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident myocardial infarction in 10 years.

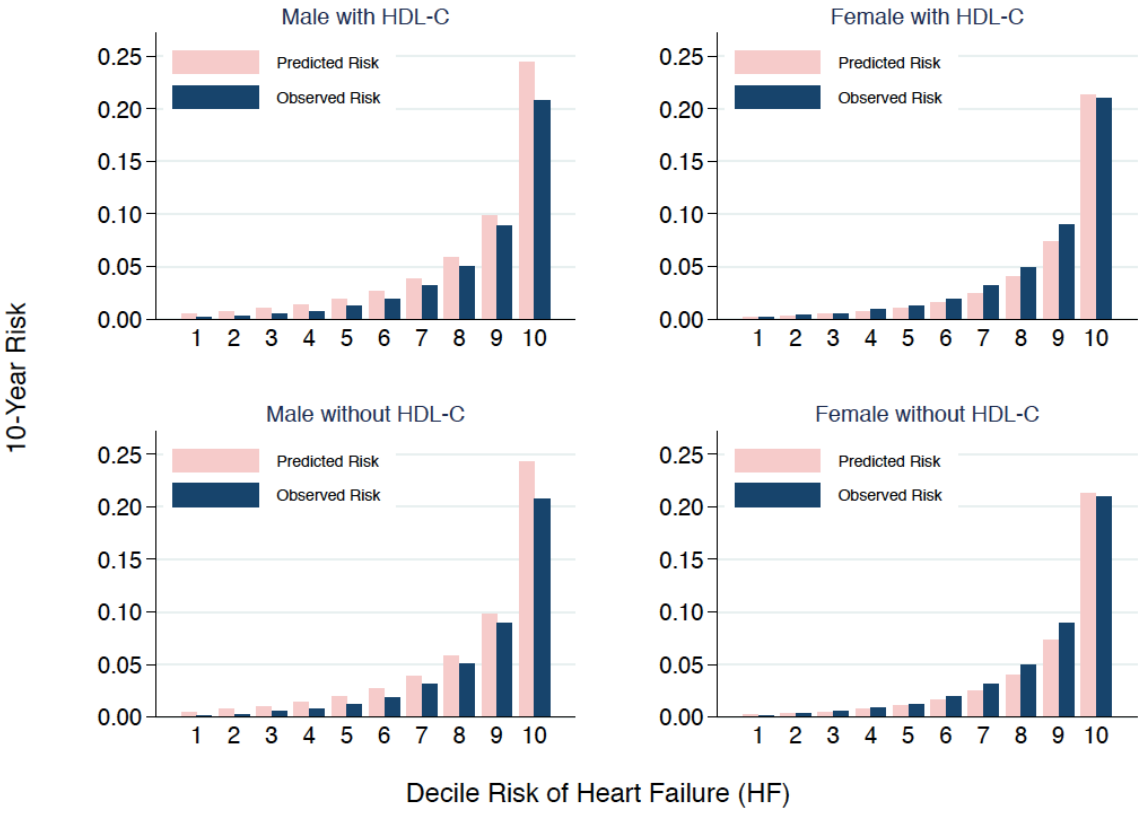


Figure S 7-8 Calibration by deciles of HF risk function by genders and by with or without HDL-C in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident heart failure in 10 years.

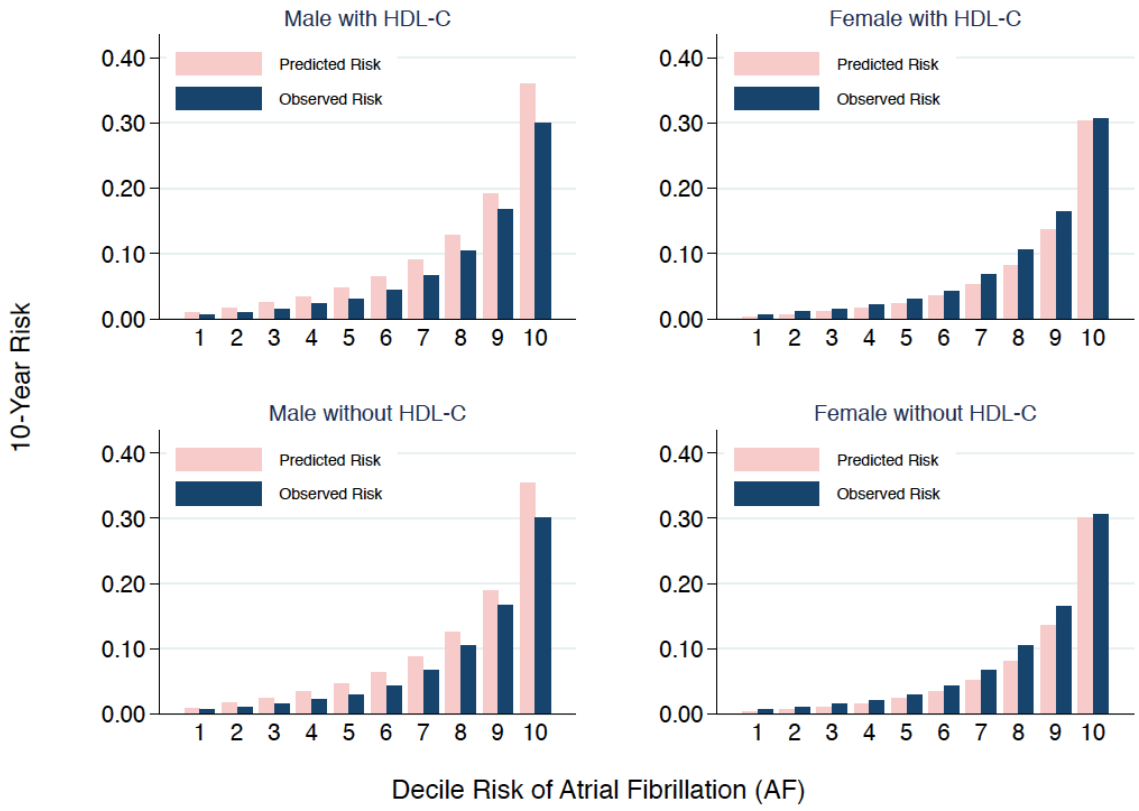


Figure S 7-9 Calibration by deciles of AF risk function by genders and by with or without HDL-C in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident atrial fibrillation in 10 years.

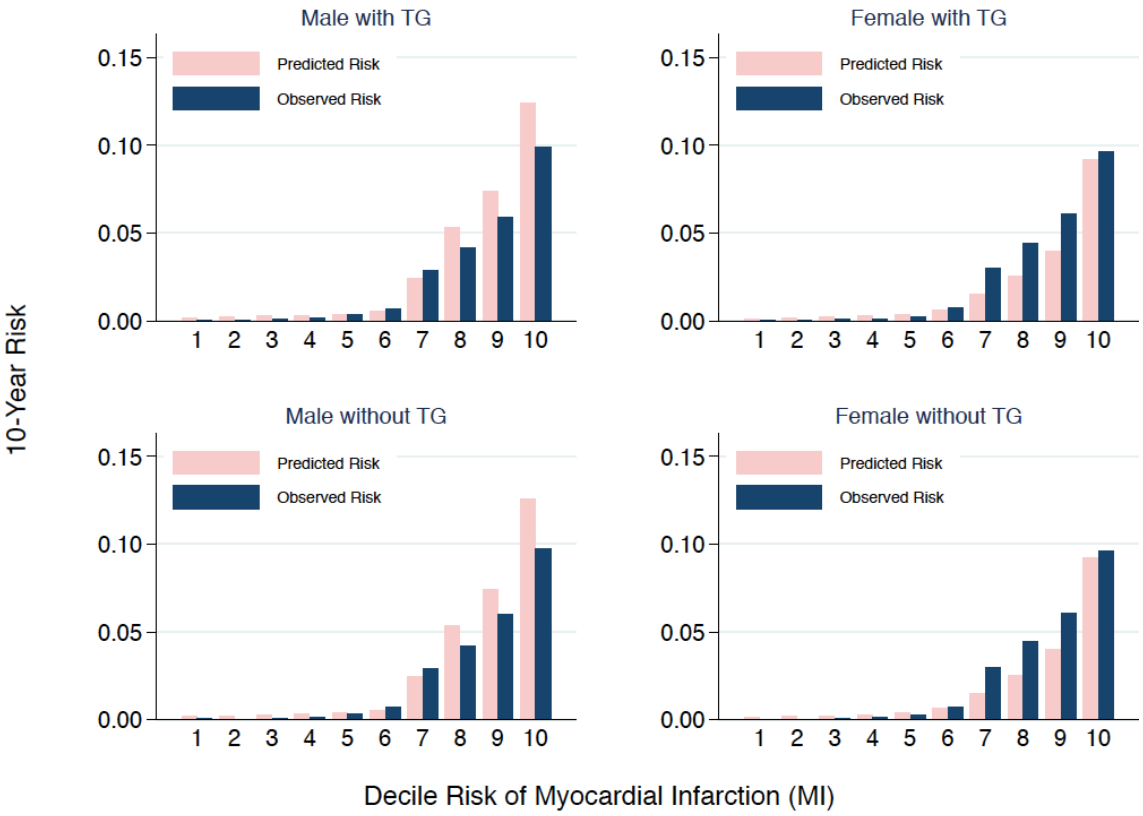


Figure S 7-10 Calibration by deciles of MI risk function by genders and by with or without TG in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident myocardial infarction in 10 years.

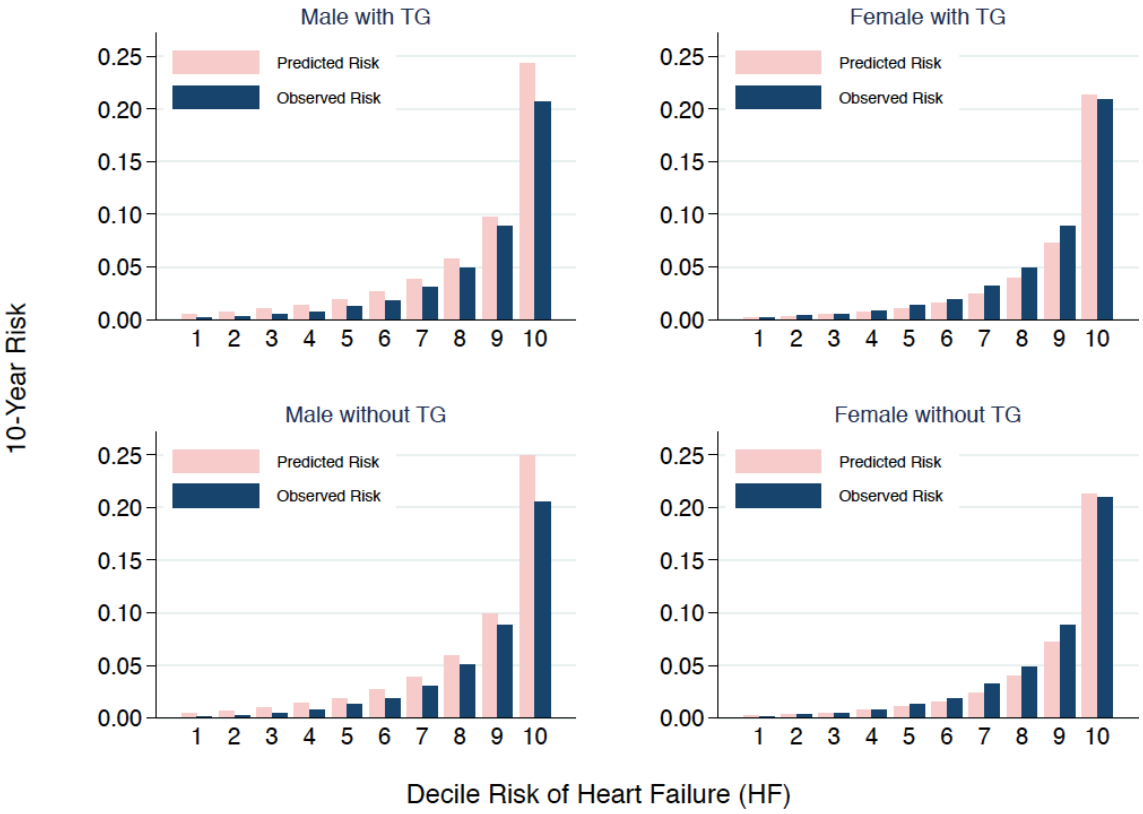


Figure S 7-11 Calibration by deciles of HF risk function by genders and by with or without TG in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident heart failure in 10 years.

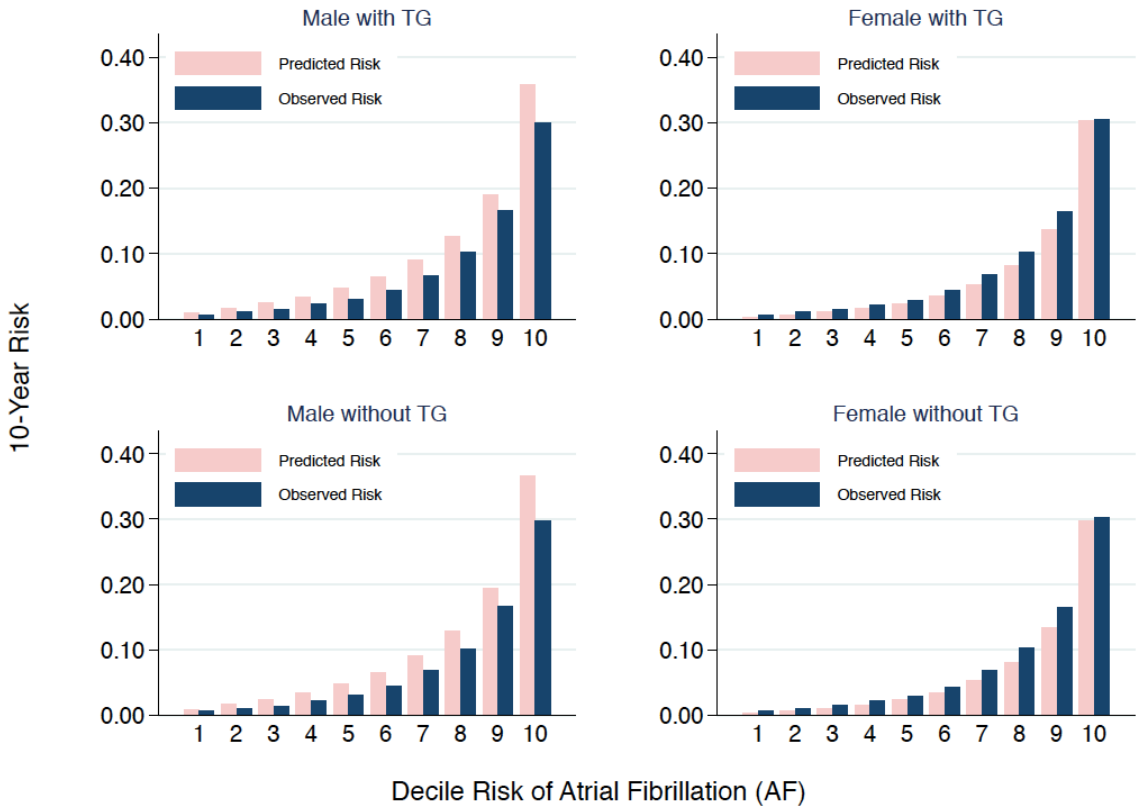


Figure S 7-12 Calibration by deciles of AF risk function by genders and by with or without TG in a model

Note: Darker bars represent observed (Kaplan-Meier) and lighter bars represent predicted risks of incident atrial fibrillation in 10 years

CHAPTER 8 CONCLUSIONS

The research of this PhD in investigating the potential causal relevance of blood lipids for two major cardiovascular disorders, heart failure (HF) and atrial fibrillation (AF) is summarised in Table 8-1. HF and AF are currently representing a growing global public health burden in contrast with the declining trends in the incidence of myocardial infarction (MI) and stroke. The nine clinical questions posed in Chapter 1, that my research aimed to investigate, are now answered below.

1. *How commonly are HF and AF firstly diagnosed in comparison to other cardiovascular diseases?*

Very commonly. According to Chapter 3, we conducted the first large scale, contemporary study (1997 to 2016) answering this question and we were surprised with the findings. About one third of all first diagnoses of major CVDs were of either HF or AF in both genders. This highlights the importance of focusing efforts toward the primary prevention of these diseases.

2. *Is there clear evidence of any specific lipid-lowering agent being effective in the risk reduction of HF and AF?*

In Chapter 2, according the meta-analysis of lipid-lowering trials, we demonstrated that only statins have shown significant evidence in decreasing the risk of HF, whereas other lipid-lowering agents did not. For AF, trials of all lipid-lowering agents did not show any risk reduction effect. To our knowledge, this is the first time that other classes of lipid-lowering agents (not just statins) have been co-investigated.

3. *Is there clear evidence that lipid-modulating strategy is effective in the primary prevention of HF and AF?*

No. According to Chapter 2, there are only a few trials conducted that recruited patients without established CVDs, and of those studies that exist, none have shown any clear evidence that lipid-modulating strategy has primary preventative benefits for HF and AF.

4. *Are blood lipid levels associated with the incidence of HF and AF?*

Yes, strongly. These associations are mostly paradoxically inverse with the exception of the association between HDL-C and incident HF and AF in which a U-shaped pattern has been observed (Figure 8-1). These associations have not been previously reported for the incidence of HF and remain robust across a range of subgroup and sensitivity analyses (Table 8-1 and Chapter 4-6).

5. *Are blood lipid levels associated with the incidence of both diseases, when studied at lower and higher levels than in previous observational studies or clinical trials?*

Interestingly, from Chapter 4-6, we found that the positive association of LDL-C with MI as well as the inverse (paradoxical) associations with HF and AF continues at the lower levels of LDL-C. In contrast, the inverse association of HDL-C with MI does not continue at the higher levels of HDL-C. We also observed the U-shaped pattern of the association between HDL-C and the risk of both HF and AF, whereas the risk of MI is relatively flat across the TG strata (Figure 8-1).

Moreover, compared to trials where lipids might reach to the levels of 1.2 mmol/L (48 mg/dL) of LDL-C (ODYSSEY Longterm trial), 1.9 mmol/L (72 mg/dL) of HDL-C (ILLUSTRATE trial), and 1.3 mmol/L (115 mg/dL) of TG (VA-HIT trial), our results reflected wider range of lipid levels: 1.3-5.7 mmol/L (50-220 mg/dL) for LDL-C, 0.7-2.7 mmol/L (27-104 mg/dL) for HDL-C, and 0.4-5.8 mmol/L (35-514 mg/dL) for TG. These results may collectively raise further questions about the value of extreme lipid levels for the primary prevention of cardiovascular disease.

6. *Are blood lipids causally relevant to the risk of HF and AF?*

Mostly no. Our trials and genetic evidence suggest that most of the observational findings may be biased and misleading due to residual confounders and reverse causation. Surprisingly, this is the first time that the potential casual relevance of TG to the risk of AF has been suggested (Table 8-1). Moreover, the observed U-shaped association between HDL-C and the risk of HF (Figure 8-1) needs further investigation due to the limitations of our

Mendelian randomisation approach used to synthesise the genetic evidence (Chapter 4-6).

Importantly, excluding intercurrent MI did not change the main findings, or even strengthen the associations in some cases, such as in TG and AF. This implies that the association between blood lipids and the risk of HF and AF is independent of MI. In other words, our findings suggested that MI is not a major intermediate of the association between blood lipids and the risk of HF and AF (Chapter 4-6).

7. *Are our findings of MI outcomes, used as a positive control, consistent with those from previous studies?*

Yes. Regarding LDL-C, our robust direct association of the results across three study designs remain consistent with the well-established knowledge. In terms of HDL-C, our cohort showed an inverse association, which is consistent with previous reporting carried out by the Emerging Risk Factors Collaboration (ERFC). However, the observed inverse association of HDL-C with MI are not supported by trials and genetic study. Recently, the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) concluded that Mendelian randomisation and trials did not provide compelling evidence that HDL-C is causally associated with the risk of atherosclerotic cardiovascular disease (ASCVD). In addition, our MR study further suggests the potential pleiotropy that might explain the false positive findings of some previous genetic studies.

Our observational results from TG are concordant with those from ERFC – a positive association in age and sex adjusted model, which significantly was attenuated after multivariable adjustment. Additionally, in our MR study on TG, we found conflicting results between the UK Biobank and HERMES consortium. However, we detected the potential horizontal pleiotropy, suggesting a role of other lipid traits that might mediate the association. Overall, we have reproduced the comparable results of MI outcomes with an existing piece of evidence.

8. *Do the patients who are at low risk of atherosclerotic disease, such as MI, also have a low risk of HF and AF?*

No. As detailed in Chapter 7, we surprisingly found that up to one third of patients who are at a very low risk of MI (i.e., 10-years risk < 5%) still have a borderline to high risk of HF and AF (i.e., 10-year risk 5% to 20%). This warrants the need for further research in this area, and patients in this group cannot be ignored. Furthermore, since we found that decreases in TG levels may be causally related with an increased risk of AF, individuals with low TG levels should be monitored for the occurrence of AF.

9. *Do blood lipids add value to the risk prediction of HF and AF?*

Mostly no, with the exception of TG that might add modest benefits to the risk prediction of AF in both genders (Chapter 7).

These findings have important clinical implications, as they provide the first evidence that:

- Primary prevention of HF and AF should be a public health priority because these diseases are the common first precursors to major cardiovascular disease.
- Available trials and genetic evidence do not support the role of LDL-C in the primary prevention of HF and AF – this may come as a surprise to many clinicians. However, TG may have a role in the primary prevention of AF, whereas the role of HDL-C is more complex and requires further investigation.
- Since the causal role of TG in AF would need further academic exploration, patients with low TG levels (i.e., TG levels < 1.09 mmol/L or < 96 mg/dL) should be monitored for the occurrence of AF, as our findings suggested that these patients may have an increased risk of developing AF by at least 9%, compared with individuals who had lower levels of TG.
- More specific risk prediction to identify people at high risk of HF and AF, which might differ from the MI risk, may have a role in:

    - Tailoring appropriate primary preventive strategies for HF and AF (e.g. the choice of blood pressure lowering drugs may differ between hypertensive patients who have a low risk of HF and those who have a high risk of HF);
    - Selecting patients for new trials aimed at the primary prevention of HF and AF.
- We have shown the use of electronic health records (EHRs) to develop the risk prediction model for HF and AF, and we have obtained validated results. This should empower the use of EHRs in healthcare research.
- Lastly, we make two reflections on modern epidemiology. First it “struck lucky” with LDL-C and MI. If the initial public health epidemic and focus of enquiry in the 1970s onwards has been HF or AF. Observational epidemiology might have directed efforts away from LDL-C as a treatment target. Second, overall we illustrate how potentially misleading observational evidence on its own can be – a note of caution to these areas of primary prevention where this is the mainstay of evidence.

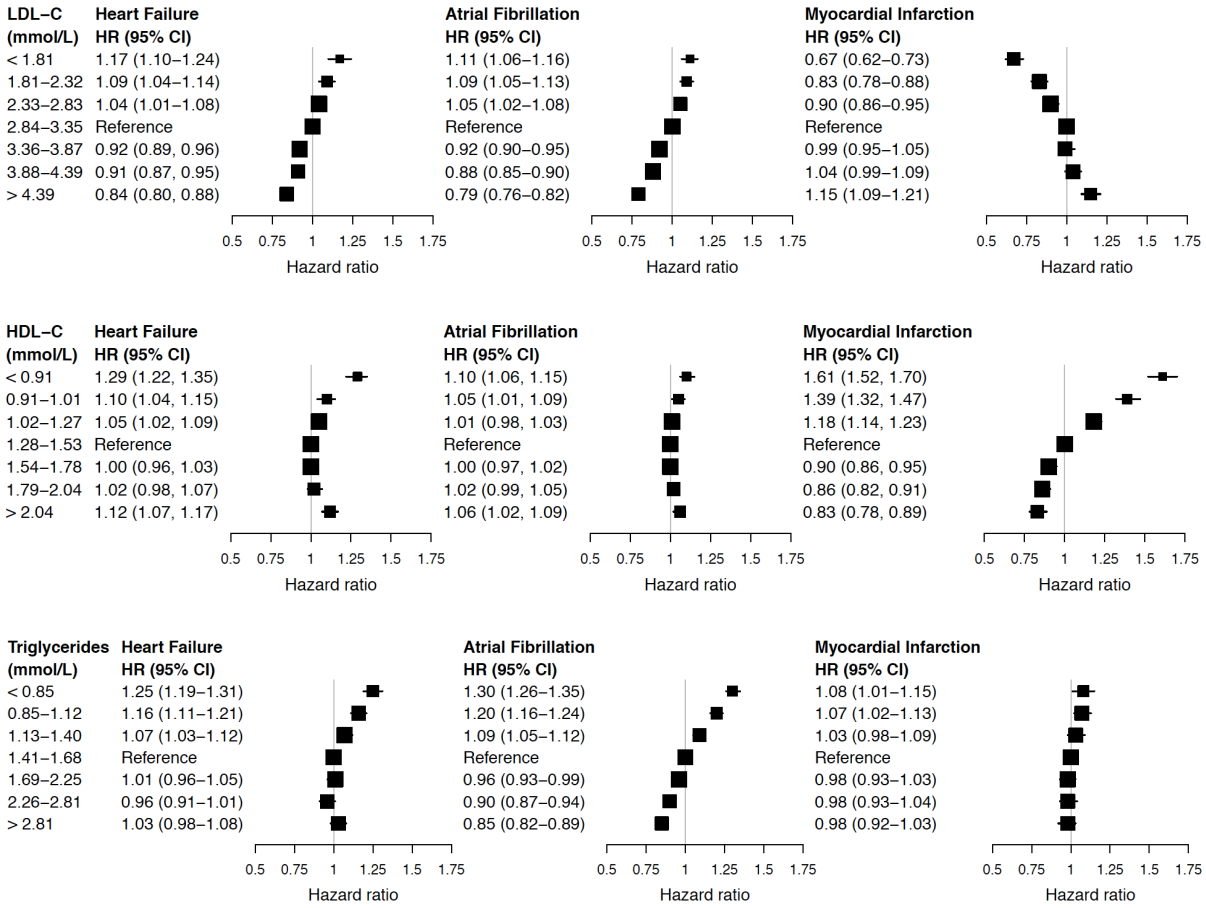


Figure 8-1 Summary of observational findings of lipids strata with the risk of HF, AF, and MI

Table 8-1 Summary of the contribution of this PhD in comparison to previously published evidence

Evidence	Heart failure		Atrial fibrillation		Myocardial infarction	
	Previous Evidence	This PhD	Previous Evidence	This PhD	Previous Evidence	This PhD
LDL-C						
Cohort	↔↑	↓	↓↔↑	↓	↑	↑
Meta-regression of trials	0	↔	0	↔	↑	↑
Genetic (MR)	0	↔	↔	↔	↑	↑
HDL-C						
Cohort	↓↔	U	↓↔	U	↓	↓
Meta-regression of trials	0	↑[a]	0	↔	↔	↑[a]
Genetic (MR)	0	↔	↔	↔	↓↔	↔
TG						
Cohort	↔↑	↓	↔	↓	↔	↑[b]
Meta-regression of trials	0	↔	0	↔	0	↔
Genetic (MR)	0	↔↑[c]	↔	↓	↔↑	↔

Symbol	Interpretation
↓	Inverse (indirect, negative) association (i.e., lower lipids, higher risk of disease)
↔	Null association
↑	Direct (positive) association (i.e., lower lipids, lower risk of disease)
U	U shaped association (i.e., lower and higher lipids, higher risk of disease)
↔↑	Conflicting evidence denoted by two or more symbols
0	No previous evidence

Note:

[a] Direct (positive) association in univariable model, which became null in multivariable model.

[b] Weakly positive association per standard deviation (continuous) increase in TG levels (log scale), but relatively flat (null) association across TG strata (categorical) (see Figure 8-1)

[c] Null association in the UK Biobank but positive association in the HERMES consortium

Abbreviations: HDL-C; High-density lipoprotein cholesterol, LDL-C; Low-density lipoprotein cholesterol, MR; Mendelian randomisation, NA; Not available, TG; Triglyceride

Bibliography

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2016;37(27):2129–200.

2. Butler J, Fonarow GC, Zile MR, Lam CS, Roessig L, Schelbert EB, et al. Developing therapies for heart failure with preserved ejection fraction: current state and future directions. JACC Heart Fail. 2014;2(2):97–112.

3. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, et al. 2013 ACCF/AHA Guideline for the Management of Heart Failure. J Am Coll Cardiol. 2013;62(16):e147–239.

4. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2014;64(21):e1-76.

5. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J. 2016;37(38):2893–962.

6. Braunwald E. Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med. 1997;337(19):1360–9.

7. Hu C-Y, Wang C-Y, Li J-Y, Ma J, Li Z-Q. Relationship between atrial fibrillation and heart failure. Eur Rev Med Pharmacol Sci. 2016;20(21):4593–600.

8. Prabhu S, Voskoboinik A, Kaye DM, Kistler PM. Atrial fibrillation and heart failure — cause or effect? Hear Lung Circ. 2017;26(9):967–74.

9. Chugh SS, Roth GA, Gillum RF, Mensah GA. Global burden of atrial fibrillation in developed and developing nations. Glob Heart. 2014;9(1):113–9.

10. McMurray JJ, Petrie MC, Murdoch DR, Davie AP. Clinical epidemiology of heart failure: public and private health burden. Eur Heart J. 1998;19 Suppl P:P9-16.

11. Conrad N, Judge A, Tran J, Mohseni H, Hedgecott D, Crespillo AP, et al. Temporal trends and patterns in heart failure incidence: a population-based

study of 4 million individuals. Lancet. 2018;391(10120):572–80.

12. Owan TE, Redfield MM. Epidemiology of diastolic heart failure. Prog Cardiovasc Dis. 2005;47(5):320–32.

13. National Heart Lung and Blood Institute. Morbidity and Mortality Chartbook on Cardiovascular, Lung and blood Diseases. 1996. NIH Bethesda MD.

14. Askoxylakis V, Thieke C, Pleger ST, Most P, Tanner J, Lindel K, et al. Long-term survival of cancer patients compared to heart failure and stroke: a systematic review. BMC Cancer. 2010;10:105.

15. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. Circulation. 2013;127(1):e6–245.

16. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. Circulation. 2014;129(8):837–47.

17. Naccarelli G V, Varker H, Lin J, Schulman KL. Increasing prevalence of atrial fibrillation and flutter in the United States. Am J Cardiol. 2009;104(11):1534–9.

18. Ball J, Carrington MJ, McMurray JJ V, Stewart S. Atrial fibrillation: profile and burden of an evolving epidemic in the 21st century. Int J Cardiol. 2013;167(5):1807–24.

19. Majeed A, Moser K, Carroll K. Trends in the prevalence and management of atrial fibrillation in general practice in England and Wales, 1994-1998: analysis of data from the general practice research database. Heart. 2001;86(3):284–8.

20. Heeringa J, van der Kuip DAM, Hofman A, Kors JA, van Herpen G, Stricker BHC, et al. Prevalence, incidence and lifetime risk of atrial fibrillation: the Rotterdam study. Eur Heart J. 2006;27(8):949–53.

21. Kannel WB, Wolf PA, Benjamin EJ, Levy D. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: population-based estimates. Am J Cardiol. 1998;82(8A):2N-9N.

22. Stewart S, Hart CL, Hole DJ, McMurray JJ V. A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. Am J Med. 2002;113(5):359–64.

23. Ott A, Breteler MM, de Bruyne MC, van Harskamp F, Grobbee DE, Hofman A. Atrial fibrillation and dementia in a population-based study. The Rotterdam

Study. Stroke. 1997;28(2):316–21.

24. Kim MH, Johnston SS, Chu B-C, Dalal MR, Schulman KL. Estimation of Total Incremental Health Care Costs in Patients With Atrial Fibrillation in the United States. Circ Cardiovasc Qual Outcomes. 2011 May;4(3):313–20.

25. Bozkurt B, Aguilar D, Deswal A, Dunbar SB, Francis GS, Horwich T, et al. Contributory Risk and Management of Comorbidities of Hypertension, Obesity, Diabetes Mellitus, Hyperlipidemia, and Metabolic Syndrome in Chronic Heart Failure: A Scientific Statement From the American Heart Association. Circulation. 2016;134(23):e535–78.

26. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DEJ, Colvin MM, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of Amer. J Card Fail. 2017;23(8):628–51.

27. Gorenek Chair B, Pelliccia Co-Chair A, Benjamin EJ, Boriani G, Crijns HJ, Fogel RI, et al. EHRA EACPR position paper on how to prevent atrial fibrillation. Eur J Prev Cardiol. 2017;24(1):4–40.

28. January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland JCJ, et al. 2019 AHA/ACC/HRS Focused Update of the 2014 AHA/ACC/HRS Guideline for the Management of Patients With Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart R. J Am Coll Cardiol. 2019;74(1):104–32.

29. Healey JS, Parkash R, Pollak T, Tsang T, Dorian P. Canadian Cardiovascular Society atrial fibrillation guidelines 2010: etiology and initial investigations. Can J Cardiol. 2011;27(1):31–7.

30. Macle L, Cairns J, Leblanc K, Tsang T, Skanes A, Cox JL, et al. 2016 Focused Update of the Canadian Cardiovascular Society Guidelines for the Management of Atrial Fibrillation. Can J Cardiol. 2016;32(10):1170–85.

31. Alonso A, Yin X, Roetker NS, Magnani JW, Kronmal RA, Ellinor PT, et al. Blood lipids and the incidence of atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis and the Framingham Heart Study. J Am Heart Assoc. 2014;3(5):e001211.

32. Lopez FL, Agarwal SK, Maclehose RF, Soliman EZ, Sharrett AR, Huxley RR, et al. Blood lipid levels, lipid-lowering medications, and the incidence of atrial fibrillation: the atherosclerosis risk in communities study. Circ Arrhythm Electrophysiol. 2012;5(1):155–62.

33. Savelieva I, Kakouros N, Kourliouros A, Camm AJ. Upstream therapies for management of atrial fibrillation: review of clinical evidence and implications for European Society of Cardiology guidelines. Part I: primary prevention. Europace. 2011;13(3):308–28.

34. Quehenberger O, Dennis EA. The Human Plasma Lipidome. N Engl J Med. 2011;365(19):1812–23.

35. Trieb M, Kornej J, Knuplez E, Hindricks G, Thiele H, Sommer P, et al. Atrial fibrillation is associated with alterations in HDL function, metabolism, and particle number. Basic Res Cardiol. 2019;114(4):27.

36. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2019 Aug 31;

37. Atherton JJ, Sindone A, De Pasquale CG, Driscoll A, MacDonald PS, Hopper I, et al. National Heart Foundation of Australia and Cardiac Society of Australia and New Zealand: Guidelines for the Prevention, Detection, and Management of Heart Failure in Australia 2018. Vol. 27, Heart, lung & circulation. Australia; 2018. p. 1123–208.

38. Choi HY, Hafiane A, Schwertani A, Genest J. High-Density Lipoproteins: Biology, Epidemiology, and Clinical Management. Can J Cardiol. 2017 Mar;33(3):325–33.

39. Nagao M, Nakajima H, Toh R, Hirata K, Ishida T. Cardioprotective Effects of High-Density Lipoprotein Beyond its Anti-Atherogenic Action. J Atheroscler Thromb. 2018;25(10):985–93.

40. Paik J, Duggan S. Volanesorsen: First Global Approval. Drugs. 2019;79(12):1349–54.

41. Santhanakrishnan R, Wang N, Larson MG, Magnani JW, McManus DD, Lubitz SA, et al. Atrial Fibrillation Begets Heart Failure and Vice Versa: Temporal Associations and Differences in Preserved Versus Reduced Ejection Fraction. Circulation. 2016;133(5):484–92.

42. Hohendanner F, Heinzel FR, Blaschke F, Pieske BM, Haverkamp W, Boldt HL,

et al. Pathophysiological and therapeutic implications in patients with atrial fibrillation and heart failure. Heart Fail Rev. 2018;23(1):27–36.

43. Patti G, Chello M, Candura D, Pasceri V, D'Ambrosio A, Covino E, et al. Randomized trial of atorvastatin for reduction of postoperative atrial fibrillation in patients undergoing cardiac surgery: results of the ARMYDA-3 (Atorvastatin for Reduction of MYocardial Dysrhythmia After cardiac surgery) study. Circulation. 2006;114(14):1455–61.

44. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008;27(8):1133–63.

45. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Annu Rev Psychol. 2007;58:593–614.

46. VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. Annu Rev Public Health. 2016;37:17–32.

47. Bentler PM, Stein JA. Structural equation models in medical research. Stat Methods Med Res. 1992;1(2):159–81.

48. Zhu Z, Zheng Z, Zhang F, Wu Y, Trzaskowski M, Maier R, et al. Causal associations between risk factors and common diseases inferred from GWAS summary data. Nat Commun. 2018;9(1):224.

49. Seferovic PM, Ponikowski P, Anker SD, Bauersachs J, Chioncel O, Cleland JGF, et al. Clinical practice update on heart failure 2019: pharmacotherapy, procedures, devices and patient management. An expert consensus meeting report of the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail [Internet]. 2019 Aug 30; Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ejhf.1531

50. Brouwers FP, De Boer RA, Van Der Harst P, Voors AA, Gansevoort RT, Bakker SJ, et al. Incidence and epidemiology of new onset heart failure with preserved vs. reduced ejection fraction in a community-based cohort: 11-year follow-up of PREVEND. Eur Heart J. 2013;34(19):1424–31.

51. He J, Ogden LG, Bazzano LA, Vupputuri S, Loria C, Whelton PK. Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. Arch Intern Med. 2001;161(7):996–1002.

52. Uijl A, Koudstaal S, Direk K, Denaxas S, Groenwold RHH, Banerjee A, et al. Risk factors for incident heart failure in age- and sex-specific strata: a

population-based cohort using linked electronic health records. Eur J Heart Fail. 2019;1–10.

53. Krahn AD, Manfreda J, Tate RB, Mathewson FA, Cuddy TE. The natural history of atrial fibrillation: incidence, risk factors, and prognosis in the Manitoba Follow-Up Study. Am J Med. 1995;98(5):476–84.

54. Kardys I, Knetsch AM, Bleumink GS, Deckers JW, Hofman A, Stricker BHC, et al. C-reactive protein and risk of heart failure. The Rotterdam Study. Am Heart J. 2006;152(3):514–20.

55. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, et al. Inflammation as a Risk Factor for Atrial Fibrillation. Circulation. 2003;108(24):3006–10.

56. Osuna PM, Udovcic M, Sharma MD. Hyperthyroidism and the Heart. Methodist Debakey Cardiovasc J. 2017;13(2):60–3.

57. Lane DA, Skjøth F, Lip GYH, Larsen TB, Kotecha D. Temporal trends in incidence, prevalence, and mortality of atrial fibrillation in primary care. J Am Heart Assoc. 2017;6(5):1–9.

58. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur J Heart Fail. 2016;18(8):891–975.

59. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Colvin MM, et al. 2016 ACC/AHA/HFSA focused update on new pharmacological therapy for heart failure: An update of the 2013 ACCF/AHA guideline for the management of heart failure: A report of the American College of Cardiology/American Heart Association Task Force on Clinic. Circulation. 2016;134(13):e282-93.

60. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: A systematic review and meta-analysis. JAMA. 2016;316(12):1289.

61. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: A meta-analysis of data from 170 000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670–81.

62. White J, Swerdlow DI, Preiss D, Fairhurst-Hunter Z, Keating BJ, Asselbergs FW, et al. Association of lipid fractions with risks for coronary artery disease

and diabetes. JAMA Cardiol. 2016;1(6):692–9.

63. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6(7):e1000097.

64. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol. 2009;62(10):e1-34.

65. Rahimi K, Emberson J, McGale P, Majoni W, Merhi A, Asselbergs FW, et al. Effect of statins on atrial fibrillation: collaborative meta-analysis of published and unpublished evidence from randomised controlled trials. BMJ. 2011;342:d1250–d1250.

66. Preiss D, Campbell RT, Murray HM, Ford I, Packard CJ, Sattar N, et al. The effect of statin therapy on heart failure events: a collaborative meta-analysis of unpublished data from major randomized trials. Eur Heart J. 2015;36(24):1536–46.

67. Navarese EP, Robinson JG, Kowalewski M, Kołodziejczak M, Andreotti F, Bliden K, et al. Association between baseline LDL-C level and total and cardiovascular mortality after LDL-C lowering a systematic review and meta-analysis. JAMA. 2018;319(15):1566–79.

68. Sciacqua A, Perticone M, Tripepi G, Tassone EJ, Cimellaro A, Mazzaferro D, et al. CHADS2 and CHA2DS2-VASc scores are independently associated with incident atrial fibrillation: the Catanzaro Atrial Fibrillation Project. Intern Emerg Med. 2015;10(7):815–21.

69. Watanabe H, Tanabe N, Yagihara N, Watanabe T, Aizawa Y, Kodama M. Association between lipid profile and risk of atrial fibrillation. Circulation. 2011;75(12):2767–74.

70. Mourtzinis G, Kahan T, Bengtsson Boström K, Schiöler L, Cedstrand Wallin L, Hjerpe P, et al. Relation between lipid profile and new-onset atrial fibrillation in patients with systemic hypertension (from the Swedish Primary Care Cardiovascular Database [SPCCD]). Am J Cardiol. 2018;122(1):102–7.

71. Velagaleti RS, Massaro J, Vasan RS, Robins SJ, Kannel WB, Levy D. Relations of lipid concentrations to heart failure incidence: the Framingham Heart Study. Circulation. 2009;120(23):2345–51.

72. Bibbins-Domingo K, Pletcher MJ, Lin F, Vittinghoff E, Gardin JM, Arynchyn A, et al. Racial differences in incident heart failure among young adults. N Engl J Med. 2009;360(12):1179–90.

73. Li X, Gao L, Wang Z, Guan B, Guan X, Wang B, et al. Lipid profile and incidence of atrial fibrillation: A prospective cohort study in China. Clin Cardiol. 2018;41(3):314–20.

74. Smith JG, Newton-Cheh C, Almgren P, Struck J, Morgenthaler NG, Bergmann A, et al. Assessment of Conventional Cardiovascular Risk Factors and Multiple Biomarkers for the Prediction of Incident Heart Failure and Atrial Fibrillation. J Am Coll Cardiol. 2010;56(21):1712–9.

75. Knuiman M, Briffa T, Divitini M, Chew D, Eikelboom J, McQuillan B. A cohort study examination of established and emerging risk factors for atrial fibrillation: The Busselton Health Study. Eur J Epidemiol. 2014;29(3):181–90.

76. Mora S, Akinkuolie AO, Sandhu RK, Conen D, Albert CM. Paradoxical association of lipoprotein measures with incident atrial fibrillation. Circ Arrhythm Electrophysiol. 2014;7(4):612–9.

77. Dhingra R, Sesso HD, Kenchaiah S, Gaziano JM. Differential effects of lipids on the risk of heart failure and coronary heart disease: The Physicians' Health Study. Am Heart J. 2008;155(5):869–75.

78. Ebong IA, Goff DCJ, Rodriguez CJ, Chen H, Sibley CT, Bertoni AG. Association of lipids with incident heart failure among adults with and without diabetes mellitus: Multiethnic Study of Atherosclerosis. Circ Heart Fail. 2013;6(3):371–8.

79. Officers A, Coordinators for the ACRGTA, Lipid-Lowering Treatment to Prevent Heart Attack T. Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). JAMA. 2002;288(23):2998–3007.

80. Koren MJ, Hunninghake DB. Clinical outcomes in managed-care patients with coronary heart disease treated aggressively in lipid-lowering disease management clinics: the ALLIANCE study. J Am Coll Cardiol. 2004;44(9):1772–9.

81. Cannon CP, Shah S, Dansky HM, Davidson M, Brinton EA, Gotto AM, et al. Safety of Anacetrapib in Patients with or at High Risk for Coronary Heart

Disease. N Engl J Med. 2010;363(25):2406–15.

82. Kjekshus J, Apetrei E, Barrios V, Böhm M, Cleland JGF, Cornel JH, et al. Rosuvastatin in Older Patients with Systolic Heart Failure. N Engl J Med. 2007;357(22):2248–61.

83. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, et al. Efficacy and safety of Alirocumab in reducing lipids and cardiovascular events. N Engl J Med. 2015;372(16):1489–99.

84. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, et al. Efficacy and safety of Evolocumab in reducing lipids and cardiovascular events. N Engl J Med. 2015;372(16):1500–9.

85. Study of the Effectiveness of Additional Reductions in C, Homocysteine Collaborative G. Intensive lowering of LDL cholesterol with 80 mg versus 20 mg simvastatin daily in 12 064 survivors of myocardial infarction: a double-blind randomised trial. Lancet. 2010;376(9753):1658–69.

86. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med. 1996;335(14):1001–9.

87. Results of the low-dose (20 mg) pravastatin GISSI Prevenzione trial in 4271 patients with recent myocardial infarction: do stopped trials contribute to overall knowledge? GISSI Prevenzione Investigators (Gruppo Italiano per lo Studio della Sopravvivenza n. Ital Heart J. 2000;1(12):810–20.

88. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes. N Engl J Med. 2004;350(15):1495–504.

89. Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, Barton J. Effects of n−3 Fatty Acid Supplements in Diabetes Mellitus. N Engl J Med. 2018;379(16):1540–50.

90. Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, et al. High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. JAMA. 2005;294(19):2437–45.

91. Nakamura H, Arakawa K, Itakura H, Kitabatake A, Goto Y, Toyota T, et al. Primary prevention of cardiovascular disease with pravastatin in Japan (MEGA

Study): a prospective randomised controlled trial. Lancet. 2006;368(9542):1155–63.

92. Norby FL, Eryd SA, Niemeijer MN, Rose LM, Smith A V, Yin X, et al. Association of lipid-related genetic variants with the incidence of atrial fibrillation: The AFGen consortium. PLoS One. 2016;11(3):e0151932–e0151932.

93. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA. 1986;256(20):2823–8.

94. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann Intern Med. 1971;74(1):1–12.

95. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009;302(18):1993–2000.

96. Hourcade-Potelleret F, Laporte S, Lehnert V, Delmar P, Benghozi R, Torriani U, et al. Clinical benefit from pharmacological elevation of high-density lipoprotein cholesterol: meta-regression analysis. Heart. 2015;101(11):847-853.

97. Vitali C, Khetarpal SA, Rader DJ. HDL Cholesterol Metabolism and the Risk of CHD: New Insights from Human Genetics. Curr Cardiol Rep. 2017;19(12):132.

98. Dron JS, Hegele RA. Genetics of Triglycerides and the Risk of Atherosclerosis. Curr Atheroscler Rep. 2017;19(7):31.

99. Chaiyasothi T, Nathisuwan S, Dilokthornsakul P, Vathesatogkit P, Thakkinstian A, Reid C, et al. Effects of Non-statin Lipid-Modifying Agents on Cardiovascular Morbidity and Mortality Among Statin-Treated Patients: A Systematic Review and Network Meta-Analysis. Front Pharmacol. 2019;10:547.

100. Ridker PM, Pare G, Parker AN, Zee RYL, Miletich JP, Chasman DI. Polymorphism in the CETP gene region, HDL cholesterol, and risk of future myocardial infarction: Genomewide analysis among 18 245 initially healthy women from the Women's Genome Health Study. Circ Cardiovasc Genet. 2009;2(1):26–33.

101. Blauw LL, Li-Gao R, Noordam R, de Mutsert R, Trompet S, Berbee JFP, et al.

CETP (Cholesteryl Ester Transfer Protein) Concentration: A Genome-Wide Association Study Followed by Mendelian Randomization on Coronary Artery Disease. Circ Genomic Precis Med. 2018;11(5):e002034.

102. Jansen H, Samani NJ, Schunkert H. Mendelian randomization studies in coronary artery disease. Eur Heart J. 2014;35(29):1917–24.

103. Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, et al. Association of Triglyceride-Lowering LPL Variants and LDL-C-Lowering LDLR Variants With Risk of Coronary Heart Disease. JAMA. 2019;321(4):364–73.

104. Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, et al. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. J Am Coll Cardiol. 2013;62(8):732–9.

105. Maggioni AP, Fabbri G, Lucci D, Marchioli R, Franzosi MG, Latini R, et al. Effects of rosuvastatin on atrial fibrillation occurrence: Ancillary results of the GISSI-HF trial. Eur Heart J. 2009;30(19):2327–36.

106. Wang J, Sarnola K, Ruotsalainen S, Moilanen L, Lepisto P, Laakso M, et al. The metabolic syndrome predicts incident congestive heart failure: a 20-year follow-up study of elderly Finns. Atherosclerosis. 2010;210(1):237–42.

107. Barkas F, Elisaf M, Korantzopoulos P, Tsiara S, Liberopoulos E. The CHADS 2 and CHA2DS2 -VASc scores predict atrial fibrillation in dyslipidemic individuals: Role of incorporating low high-density lipoprotein cholesterol levels. Int J Cardiol. 2017;241:194–9.

108. Ingelsson E, Ärnlöv J, Sundström J, Zethelius B, Vessby B, Lind L. Novel Metabolic Risk Factors for Heart Failure. J Am Coll Cardiol. 2005;46(11):2054–60.

109. He J, Shlipak M, Anderson A, Roy J, Feldman H, Kallem R, et al. Risk factors for heart failure in patients with chronic kidney disease: The CRIC (Chronic Renal Insufficiency Cohort) study. J Am Heart Assoc. 2017;6(5):e005336.

110. Kodani E, Kaneko T, Fujii H, Nakamura H, Sasabe H, Tamura Y. Prevalence and incidence of atrial fibrillation in the general population based on national health insurance special health checkups: - TAMA MED project-AF -. Circ J. 2019;83(3):524–31.

111. Kim Y-G, Choi K-J, Han S, Hwang K, Kwon C. Metabolic syndrome and the risk of new-onset atrial fibrillation in middle-aged east Asian men. Circ J.

2018;82(7):1763–9.

112. Holme I, Aastveit AH, Hammar N, Jungner I, Walldius G. Lipoprotein components and risk of congestive heart failure in 84,740 men and women in the Apolipoprotein MOrtality RISk study (AMORIS). Eur J Heart Fail. 2009;11(11):1036–42.

113. Varbo A, Nordestgaard BG. Nonfasting Triglycerides, Low-Density Lipoprotein Cholesterol, and Heart Failure Risk. Arterioscler Thromb Vasc Biol. 2018;38(2):464–72.

114. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. JAMA. 1998;279(20):1615–22.

115. Knopp RH, D'Emden M, Smilde JG, Pocock SJ. Efficacy and safety of atorvastatin in the prevention of cardiovascular end points in subjects with type 2 diabetes: the Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in non-insulin-dependent diabetes mellitus (ASPEN). Diabetes Care. 2006;29(7):1478–85.

116. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet. 2004;364(9435):685–96.

117. Rossebø AB, Pedersen TR, Boman K, Brudi P, Chambers JB, Egstrup K, et al. Intensive Lipid Lowering with Simvastatin and Ezetimibe in Aortic Stenosis. N Engl J Med. 2008;359(13):1343–56.

118. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr. AM, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359(21):2195–207.

119. Yusuf S, Bosch J, Dagenais G, Zhu J, Xavier D, Liu L, et al. Cholesterol Lowering in Intermediate-Risk Persons without Cardiovascular Disease. N Engl J Med. 2016 May 26;374(21):2021–31.

120. Ford I, Murray H, McCowan C, Packard CJ. Long-Term Safety and Efficacy of Lowering Low-Density Lipoprotein Cholesterol With Statin Therapy. Circulation. 2016 Mar 15;133(11):1073–80.

121. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med. 1995;333(20):1301–7.

122. Heart Protection Study Collaborative G. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet. 2002;360(9326):7–22.

123. Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. Lancet. 2002;360(9346):1623–30.

124. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial--Lipid Lowering Arm (ASCOT-LLA): a multicentre randomis. Lancet. 2003;361(9364):1149–58.

125. Wanner C, Krane V, März W, Olschewski M, Mann JFE, Ruf G, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. N Engl J Med. 2005;353(3):238–48.

126. Ginsberg HN, Elam MB, Lovato LC, Crouse JR 3rd, Leiter LA, Linz P, et al. Effects of Combination Lipid Therapy in Type 2 Diabetes Mellitus. N Engl J Med. 2010;362(17):1563–74.

127. The treatment of cerebrovascular disease with clofibrate. Final report of the Veterans Administration Cooperative Study of Atherosclerosis, Neurology Section. Stroke. 1973;4(4):684–93.

128. Adabag AS, Mithani S, Al Aloul B, Collins D, Bertog S, Bloomfield HE. Efficacy of gemfibrozil in the primary prevention of atrial fibrillation in a large randomized controlled trial. Am Heart J. 2009;157(5):913–8.

129. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, et al. Gemfibrozil for the Secondary Prevention of Coronary Heart Disease in Men with Low Levels of High-Density Lipoprotein Cholesterol. N Engl J Med. 1999;341(6):410–8.

130. Serruys PW, de Feyter P, Macaya C, Kokott N, Puel J, Vrolix M, et al. Fluvastatin for prevention of cardiac events following successful first percutaneous coronary intervention: a randomized controlled trial. JAMA.

2002;287(24):3215–22.

131. Amarenco P, Bogousslavsky J, Callahan 3rd A, Goldstein LB, Hennerici M, Rudolph AE, et al. High-dose atorvastatin after stroke or transient ischemic attack. N Engl J Med. 2006;355(6):549–59.

132. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. 2005;352(14):1425–35.

133. de Lemos JA, Blazing MA, Wiviott SD, Lewis EF, Fox KA, White HD, et al. Early intensive vs a delayed conservative simvastatin strategy in patients with acute coronary syndromes: phase Z of the A to Z trial. JAMA. 2004;292(11):1307–16.

134. Athyros VG, Papageorgiou AA, Mercouris BR, Athyrou V V., Symeonidis AN, Basayannis EO, et al. Treatment with Atorvastatin to the National Cholesterol Educational Program Goal Versus “Usual” Care in Secondary Coronary Heart Disease Prevention. Curr Med Res Opin. 2002;18(4):220–8.

135. Nissen SE, Tardif JC, Nicholls SJ, Revkin JH, Shear CL, Duggan WT, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. N Engl J Med. 2007;356(13):1304-1316.

136. Derosa G, Cicero AEG, Bertone G, Piccinni MN, Ciccarelli L, Roggeri DE. Comparison of fluvastatin + fenofibrate combination therapy and fluvastatin monotherapy in the treatment of combined hyperlipidemia, type 2 diabetes mellitus, and coronary heart disease: a 12-month, randomized, double-blind, controlled trial. Clin Ther. 2004;26(10):1599–607.

137. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007;357(21):2109-2122.

138. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, et al. Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome. N Engl J Med. 2012;367(22):2089–99.

139. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med. 2019;380(1):11–22.

140. Chen F, Wallendszus K, Collins R, Trial C, Unit S, Kingdom U, et al. Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. N Engl J Med.

2017;377:1217–27.

141. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. N Engl J Med. 2018 Nov 29;379(22):2097–107.

142. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376(18):1713–22.

143. Ray KK, Bays HE, Catapano AL, Lalwani ND, Bloedon LT, Sterling LR, et al. Safety and Efficacy of Bempedoic Acid to Reduce LDL Cholesterol. N Engl J Med. 2019;380(11):1022–32.

144. Probstfield JL, Rifkind BM. The lipid research clinics coronary primary prevention trial: Design, results, and implications. Eur J Clin Pharmacol. 1991;40(1):S69–75.

145. Frick MH, Elo O, Haapa K, Heinonen OP, Heinsalmi P, Helo P, et al. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. N Engl J Med. 1987;317(20):1237–45.

146. Meade T, Zuhrie R, Cook C, Cooper J. Bezafibrate in men with lower extremity arterial disease: randomised controlled trial. BMJ. 2002;325(7373):1139.

147. Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet. 2011;377(9784):2181–92.

148. Holdaas H, Fellstrom B, Jardine AG, Holme I, Nyberg G, Fauchald P, et al. Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. Lancet. 2003;361(9374):2024–31.

149. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet. 2005;366(9500):1849–61.

150. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet. 1994;344(8934):1383–9.

151. Post Coronary Artery Bypass Graft Trial I. The effect of aggressive lowering of low-density lipoprotein cholesterol levels and low-dose anticoagulation on obstructive changes in saphenous-vein coronary-artery bypass grafts. N Engl J Med. 1997;336(3):153–62.

152. Long-Term Intervention with Pravastatin in Ischaemic Disease Study G. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. N Engl J Med. 1998;339(19):1349–57.

153. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease. Circulation. 2000;102(1):21–7.

154. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med. 2015 Jun 18;372(25):2387–97.

155. Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014;371(3):203–12.

156. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med. 2011;365(24):2255–67.

157. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, et al. Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. N Engl J Med. 2017;376(20):1933–42.

158. Ridker PM, Revkin J, Amarenco P, Brunell R, Curto M, Civeira F, et al. Cardiovascular efficacy and safety of Bococizumab in high-risk patients. N Engl J Med. 2017;376(16):1527–39.

159. Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. Lancet. 2010;375(9726):1634–9.

160. Ference BA, Yoo W, Alesh I, Mahajan N, Mirowska KK, Mewada A, et al. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. J Am Coll Cardiol. 2012;60(25):2631–9.

161. Haase CL, Tybjaerg-Hansen A, Qayyum AA, Schou J, Nordestgaard BG,

Frikke-Schmidt R. LCAT, HDL cholesterol and ischemic cardiovascular disease: a Mendelian randomization study of HDL cholesterol in 54,500 individuals. J Clin Endocrinol Metab. 2012;97(2):E248-56.

162. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. Lancet. 2012;380(9841):572–80.

163. Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nat Genet. 2013 Nov;45(11):1345–52.

164. Holmes M V., Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, et al. Mendelian randomization of blood lipids for coronary heart disease. Eur Heart J. 2015;36(9):539–50.

165. Tragante V, Asselbergs FW, Swerdlow DI, Palmer TM, Moore JH, de Bakker PIW, et al. Harnessing publicly available genetic data to prioritize lipid modifying therapeutic targets for prevention of coronary heart disease based on dysglycemic risk. Hum Genet. 2016;135(5):453–67.

166. van Iperen EPA, Sivapalaratnam S, Holmes M V, Hovingh GK, Zwinderman AH, Asselbergs FW. Genetic analysis of emerging risk factors in coronary artery disease. Atherosclerosis. 2016 Nov;254:35–41.

167. Casey JA, Schwartz B, Stewart W, Adler NE. Using Electronic Health Records for Population Health Research: A Review of Methods and Applications. Annu Rev Public Health. 2016;37:61–81.

168. Kuan V, Denaxas S, Gonzalez-Izquierdo A, Direk K, Bhatti O, Husain S, et al. A chronological map of 308 physical and mental health conditions from 4 million individuals in the English National Health Service. Lancet Digit Heal. 2019;1(2):e63–77.

169. Laleman N, Henrard S, van den Akker M, Goderis G, Buntinx F, Van Pottelbergh G, et al. Time trends in statin use and incidence of recurrent cardiovascular events in secondary prevention between 1999 and 2013: a registry-based study. BMC Cardiovasc Disord. 2018;18(1):209.

170. Denaxas SC, George J, Herrett E, Shah AD, Kalra D, Hingorani AD, et al. Data resource profile: Cardiovascular disease research using linked bespoke studies and electronic health records (CALIBER). Int J Epidemiol. 2012;41(6):1625–38.

171. Herrett E, Gallagher AM, Bhaskaran K, Forbes H, Mathur R, van Staa T, et al. Data resource profile: Clinical Practice Research Datalink (CPRD). Int J Epidemiol. 2015;44(3):827–36.

172. Denaxas S, Gonzalez-Izquierdo A, Direk K, Fitzpatrick NK, Fatemifar G, Banerjee A, et al. UK phenomics platform for developing and validating electronic health record phenotypes: CALIBER. J Am Med Informatics Assoc [Internet]. 2019 Jul 22; Available from: https://academic.oup.com/jamia/advance-article/doi/10.1093/jamia/ocz105/5536916

173. Quint JK, Müllerova H, DiSantostefano RL, Forbes H, Eaton S, Hurst JR, et al. Validation of chronic obstructive pulmonary disease recording in the Clinical Practice Research Datalink (CPRD-GOLD). BMJ Open. 2014;4(7):1–8.

174. Rothnie KJ, Müllerová H, Hurst JR, Smeeth L, Davis K, Thomas SL, et al. Validation of the recording of acute exacerbations of COPD in UK primary care electronic healthcare records. PLoS One. 2016;11(3):1–14.

175. Herrett E, Shah AD, Boggon R, Denaxas S, Smeeth L, van Staa T, et al. Completeness and diagnostic validity of recording acute myocardial infarction events in primary care, hospital care, disease registry, and national mortality records: cohort study. BMJ. 2013;346:f2350–f2350.

176. Denaxas SC, Fatemifar G, Patel R, Hemingway H. Deriving research-quality phenotypes from national electronic health records to advance precision medicine: a UK Biobank case-study. In: Proceedings of the BHI-2017 International Conference on Biomedical and Health Informatics.

177. Rapsomaniki E, Thuresson M, Yang E, Blin P, Hunt P, Chung SC, et al. Using big data from health records from four countries to evaluate chronic disease outcomes: A study in 114 364 survivors of myocardial infarction. Eur Heart J. 2016;2(3):172–83.

178. Koudstaal S, Pujades-Rodriguez M, Denaxas S, Gho JMIH, Shah AD, Yu N, et al. Prognostic burden of heart failure recorded in primary care, acute hospital admissions, or both: a population-based linked electronic health record cohort study in 2.1 million people. Eur J Heart Fail. 2017;19(9):1119–27.

179. Morley KI, Wallace J, Denaxas SC, Hunter RJ, Patel RS, Perel P, et al. Defining disease phenotypes using national linked electronic health records: A case study of atrial fibrillation. PLoS One. 2014;9(11):e110900.

180. Allan V, Banerjee A, Shah AD, Patel R, Denaxas S, Casas J-P, et al. Net clinical benefit of warfarin in individuals with atrial fibrillation across stroke risk and across primary and secondary care. Heart. 2017;103(3):210–8.

181. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. Nat Rev Cardiol. 2011;8(1):30–41.

182. D'Agostino RBS, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743–53.

183. Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. Circulation. 2008;118(22):2243–51.

184. Goff DC, Lloyd-jones DM, Bennett G, Coady S, Agostino RBD, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk. Circulation. 2014;129(suppl 2):S49–73.

185. Anwar M, Khan DA, Khan FA. Comparison of friedewald formula and modified friedewald formula with direct homogeneous assay for low density lipoprotein cholesterol estimation. J Coll Physicians Surg Pak. 2014;24(1):8–12.

186. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143–421.

187. Carpenter JR, Kenward MG. Multiple imputation and its application. Chichester, United Kingdom: John Wiley & Sons; 2013.

188. Mathur R, Bhaskaran K, Chaturvedi N, Leon DA, Van Staa T, Grundy E, et al. Completeness and usability of ethnicity data in UK-based primary care and hospital databases. J Public Health (Bangkok). 2014 Dec;36(4):684–92.

189. Hemingway H, Asselbergs FW, Danesh J, Dobson R, Maniadakis N, Maggioni A, et al. Big data from electronic health records for early and late translational cardiovascular research: challenges and potential. Eur Heart J. 2018;39(16):1481–95.

190. Brugts JJ, Yetgin T, Hoeks SE, Gotto AM, Shepherd J, Westendorp RGJ, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. BMJ. 2009;338:b2376.

191. Smolina K, Wright FL, Rayner M, Goldacre MJ. Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: Linked national database study. BMJ. 2012;344(7842):1–9.

192. Gho JMIH, Schmidt AF, Pasea L, Koudstaal S, Pujades-Rodriguez M, Denaxas S, et al. An electronic health records cohort study on heart failure following myocardial infarction in England: incidence and predictors. BMJ Open. 2018;8(3):e018331.

193. Schmitt J, Duray G, Gersh BJ, Hohnloser SH. Atrial fibrillation in acute myocardial infarction: a systematic review of the incidence, clinical features and prognostic implications. Eur Heart J. 2009;30(9):1038–45.

194. Ferreira JP, Santos M. Heart failure and atrial fibrillation: from basic science to clinical practice. Int J Mol Sci. 2015;16(2):3133–47.

195. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370:1453–7.

196. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. PLoS Med. 2007;4(10):e297.

197. Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. PLoS Med. 2015;12(10):e1001885.

198. Dempsey GME, Steiner DCP. Cholesterol and triglyceride concentrations in serum/plasma pairs. Clin Chem. 1977;23(1):60–3.

199. Schnabel RB, Yin X, Gona P, Larson MG, Beiser AS, McManus DD, et al. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. Lancet. 2015;386(9989):154–62.

200. Iguchi Y, Kimura K, Shibazaki K, Aoki J, Kobayashi K, Sakai K, et al. Annual incidence of atrial fibrillation and related factors in adults. Am J Cardiol. 2010;106(8):1129–33.

201. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the management of dyslipidaemias. Eur

Heart J. 2016;37(39):2999-3058l.

202. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet. 2013;45(11):1274–83.

203. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet. 2013;45(1):25–33.

204. Collins R. What makes UK Biobank special? Lancet. 2012;379(9822):1173–4.

205. Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, et al. Genome-wide association study provides new insights into the genetic architecture and pathogenesis of heart failure. bioRxiv [Internet]. 2019 Jan 1;682013. Available from: http://biorxiv.org/content/early/2019/07/10/682013.abstract

206. Marzilli M. Pleiotropic effects of statins: evidence for benefits beyond LDL-cholesterol lowering. Am J Cardiovasc drugs. 2010;10:3–9.

207. Charach G, Argov O, Nochomovitz H, Rogowski O, Charach L, Grosskopf I. A longitudinal 20 years of follow up showed a decrease in the survival of heart failure patients who maintained low LDL cholesterol levels. QJM. 2018;111(5):319–25.

208. Charach G, Rabinovich A, Ori A, Weksler D, Sheps D, Charach L, et al. Low levels of low-density lipoprotein cholesterol: a negative predictor of survival in elderly patients with advanced heart failure. Cardiology. 2014;127(1):45–50.

209. Cuspidi C, Quarti F, Dell'Oro R, Facchetti R, Bombelli M, Sala C, et al. Long-term changes in left ventricular mass echocardiographic findings from a general population. J Hypertens. 2017;35(11):2303–9.

210. Das SR, Drazner MH, Dries DL, Vega GL, Stanek HG, Abdullah SM, et al. Impact of body mass and body composition on circulating levels of natriuretic peptides: results from the Dallas Heart Study. Circulation. 2005;112(14):2163–8.

211. Mueller C, Laule-Kilian K, Scholer A, Nusbaumer C, Zeller T, Staub D, et al. B-type natriuretic peptide for acute dyspnea in patients with kidney disease: insights from a randomized comparison. Kidney Int. 2005;67(1):278–84.

212. Tamura N, Ogawa Y, Chusho H, Nakamura K, Nakao K, Suda M, et al. Cardiac

fibrosis in mice lacking brain natriuretic peptide. Proc Natl Acad Sci U S A. 2000;97(8):4239–44.

213. Rahimi K, Majoni W, Merhi A, Emberson J. Effect of statins on ventricular tachyarrhythmia, cardiac arrest, and sudden cardiac death: a meta-analysis of published and unpublished evidence from randomized trials. Eur Heart J. 2012;33(13):1571–81.

214. Fauchier L, Clementy N, Babuty D. Statin therapy and atrial fibrillation: systematic review and updated meta-analysis of published randomized controlled trials. Curr Opin Cardiol. 2013;28(1):7–18.

215. Wang Z, Zhang Y, Gao M, Wang J, Wang Q, Wang X, et al. Statin therapy for the prevention of atrial fibrillation: a meta-analysis of randomized controlled trials. Pharmacotherapy. 2011;31(11):1051–62.

216. Xu Q, Guan Y-Q, Zhang D, Su G-H. The effects of statin on atrial fibrillation: a meta-analysis of published data from randomized controlled trials. Curr Med Res Opin. 2011;27(9):1771–9.

217. Groves D, Mihos CG, Larrauri-Reyes M, Santana O. The use of statins in the treatment and prevention of atrial fibrillation. Cardiol Rev. 2016;24(5):224–9.

218. Ference BA, Bhatt DL, Catapano AL, Packard CJ, Graham I, Kaptoge S, et al. Association of Genetic Variants Related to Combined Exposure to Lower Low-Density Lipoproteins and Lower Systolic Blood Pressure With Lifetime Risk of Cardiovascular Disease. JAMA [Internet]. 2019 Sep 2;1–11. Available from: https://jamanetwork.com/ journals/jama/fullarticle/2749533

219. Johansson S, Wallander MA, Ruigomez A, Garcia Rodriguez LA. Incidence of newly diagnosed heart failure in UK general practice. Eur J Heart Fail. 2001;3(2):225–31.

220. Ruigomez A, Johansson S, Wallander M-A, Garcia Rodriguez LA. Predictors and prognosis of paroxysmal atrial fibrillation in general practice in the UK. BMC Cardiovasc Disord. 2005;5:20.

221. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ. Validation and validity of diagnoses in the General Practice Research Database: a systematic review. Br J Clin Pharmacol. 2010;69(1):4–14.

222. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. Int J Epidemiol. 2016;45(6):1866–86.

223. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7:e34408.

224. Sofat R, Hingorani AD, Smeeth L, Humphries SE, Talmud PJ, Cooper J, et al. Separating the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. Circulation. 2010;121(1):52-62.

225. Korantzopoulos P, Letsas KP, Tse G, Fragakis N, Goudis CA, Liu T. Inflammation and atrial fibrillation: A comprehensive review. J arrhythmia. 2018;34(4):394–401.

226. Ayoub KF, Pothineni NVK, Rutland J, Ding Z, Mehta JL. Immunity, Inflammation, and Oxidative Stress in Heart Failure: Emerging Molecular Targets. Cardiovasc drugs Ther. 2017;31(5–6):593–608.

227. Bahrami H, Bluemke DA, Kronmal R, Bertoni AG, Lloyd-Jones DM, Shahar E, et al. Novel Metabolic Risk Factors for Incident Heart Failure and Their Relationship With Obesity. The MESA (Multi-Ethnic Study of Atherosclerosis) Study. J Am Coll Cardiol. 2008;51(18):1775–83.

228. Hirata A, Sugiyama D, Watanabe M, Tamakoshi A, Iso H, Kotani K, et al. Association of extremely high levels of high-density lipoprotein cholesterol with cardiovascular mortality in a pooled analysis of 9 cohort studies including 43,407 individuals: The EPOCH–JAPAN study. J Clin Lipidol. 2018;12(3):674–84.

229. Ko DT, Alter DA, Guo H, Koh M, Lau G, Austin PC, et al. High-Density Lipoprotein Cholesterol and Cause-Specific Mortality in Individuals Without Previous Cardiovascular Conditions: The CANHEART Study. J Am Coll Cardiol. 2016;68(19):2073–83.

230. Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: Two prospective cohort studies. Eur Heart J. 2017;38(32):2478–86.

231. Jakob T, Nordmann AJ, Schandelmaier S, Ferreira-Gonzalez I, Briel M. Fibrates for primary prevention of cardiovascular disease events. Cochrane database Syst Rev. 2016;11:CD009753.

232. Wang D, Liu B, Tao W, Hao Z, Liu M. Fibrates for secondary prevention of cardiovascular disease and stroke. Cochrane database Syst Rev.

2015;10:CD009580.

233. Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available: www.handbook.cochrane.org. Accessed 3 March 2019.

234. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjaerg-Hansen A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. Circulation. 2000;101(16):1907–12.

235. Zanoni P, Khetarpal SA, Larach DB, Hancock-Cerutti WF, Millar JS, Cuchel M, et al. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. Science. 2016;351(6278):1166–71.

236. Agarwala AP, Rodrigues A, Risman M, McCoy M, Trindade K, Qu L, et al. High-Density Lipoprotein (HDL) Phospholipid Content and Cholesterol Efflux Capacity Are Reduced in Patients With Very High HDL Cholesterol and Coronary Disease. Arterioscler Thromb Vasc Biol. 2015;35(6):1515–9.

237. Suzuki S. “Cholesterol paradox” in atrial fibrillation [editorial]. Circ J. 2011;75(12):2749–50.

238. O’Brien T, Katz K, Hodge D, Nguyen TT, Kottke BA, Hay ID. The effect of the treatment of hypothyroidism and hyperthyroidism on plasma lipids and apolipoproteins AI, AII and E. Clin Endocrinol (Oxf). 1997;46(1):17–20.

239. Haase CL, Tybjærg-Hansen A, Grande P, Frikke-Schmidt R. Genetically elevated apolipoprotein A-I, high-density lipoprotein cholesterol levels, and risk of ischemic heart disease. J Clin Endocrinol Metab. 2010;95(12):500–10.

240. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. Lancet. 2012;380(9841):572–80.

241. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. Int J Epidemiol. 2016;45(3):908–15.

242. Burgess S, Davies NM, Thompson SG. Instrumental variable analysis with a nonlinear exposure-outcome relationship. Epidemiology. 2014;25(6):877–85.

243. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to

Mendelian randomization. Genet Epidemiol. 2017;41(4):341–52.

244. Brion MA, Shakhbazov K, Peter PM. Calculating statistical power in Mendelian randomization studies. Int J Epidemiol. 2013;42:1497-501.

245. Hippisley-Cox J, Coupland C. Development and validation of risk prediction equations to estimate future risk of heart failure in patients with diabetes: A prospective cohort study. BMJ Open. 2015;5(9):e008503.

246. Zhang L, Liebelt JJ, Madan N, Shan J, Taub CC. Comparison of Predictors of Heart Failure With Preserved Versus Reduced Ejection Fraction in a Multiracial Cohort of Preclinical Left Ventricular Diastolic Dysfunction. Am J Cardiol. 2017;119(11):1815–20.

247. Schnabel RB, Sullivan LM, Levy D, Pencina MJ, Massaro JM, D'Agostino RB, et al. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. Lancet. 2009;373(9665):739–45.

248. Hamada R, Muto S. Simple risk model and score for predicting of incident atrial fibrillation in Japanese. J Cardiol. 2019;73(1):65–72.

249. Chamberlain AM, Agarwal SK, Folsom AR, Soliman EZ, Chambless LE, Crow R, et al. A clinical risk score for atrial fibrillation in a biracial prospective cohort (from the Atherosclerosis Risk in Communities [ARIC] Study). Am J Cardiol. 2011;107(1):85–91.

250. Ho JE, Lyass A, Lee DS, Vasan RS, Kannel WB, Larson MG, et al. Predictors of new-onset heart failure: differences in preserved versus reduced ejection fraction. Circ Heart Fail. 2013;6(2):279–86.

251. Riley RD, Moons KGM, Debray TPA, Snell KIE, Steyerberg EW, Altman DG, et al. Prognostic model research. In: Riley R, van der Windt D, Croft P, Moons GM, editors. Prognosis research in healthcare: concepts, methods, and impact. Oxford University Press; 2019. p. 139–87.

252. Ellis A, Fry R. Regional health inequalities in England. Reg Trends. 2010 Jun;42(1):60–79.

253. Cox DR. Note on Grouping. J Am Stat Assoc 1957; 52: 543–7.

254. Royston P, Altman DG. External validation of a Cox prognostic model: Principles and methods. BMC Med Res Methodol. 2013;13(1).

255. Goldman N, Glei DA. Quantifying the value of biomarkers for predicting mortality. Ann Epidemiol. 2015;25(12):901–4.

256. Leening MJG, Vedder MM, Witteman JCM, Pencina MJ, Steyerberg EW. Net reclassification improvement: computation, interpretation, and controversies: a literature review and clinician's guide. Ann Intern Med. 2014;160(2):122–31.

257. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. J Am Coll Cardiol. 2018; doi: https://doi.org/10.1016/j.jacc.2018.11.

258. Pencina MJ, D'Agostino RBS, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med. 2011;30(1):11–21.

259. Zheng Y, Parast L, Cai T, Brown M. Evaluating incremental values from new predictors with net reclassification improvement in survival analysis. Lifetime Data Anal. 2013;19(3):350–70.

260. Hsu C-H, Taylor JMG. A robust weighted Kaplan-Meier approach for data with dependent censoring using linear combinations of prognostic covariates. Stat Med. 2010;29(21):2215–23.

261. Chao T-F, Liu C-J, Chen S-J, Wang K-L, Lin Y-J, Chang S-L, et al. CHADS2 score and risk of new-onset atrial fibrillation: a nationwide cohort study in Taiwan. Int J Cardiol. 2013;168(2):1360–3.

262. Li Y-G, Pastori D, Farcomeni A, Yang P-S, Jang E, Joung B, et al. A Simple Clinical Risk Score (C2HEST) for Predicting Incident Atrial Fibrillation in Asian Subjects: Derivation in 471,446 Chinese Subjects, With Internal Validation and External Application in 451,199 Korean Subjects. Chest. 2019;155(3):510–8.

263. Hippisley-Cox J, Coupland C, Brindle P, J. H-C, C. C. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: Prospective cohort study. BMJ. 2017;357:2099.

264. Hippisley-Cox J, Coupland C, Vinogradova Y, Robson J, Minhas R, Sheikh A, et al. Predicting cardiovascular risk in England and Wales: prospective derivation and validation of QRISK2. BMJ. 2008 Jun;336(7659):1475–82.

265. Agarwal SK, Chambless LE, Ballantyne CM, Astor B, Bertoni AG, Chang PP, et al. Prediction of incident heart failure in general practice: the Atherosclerosis Risk in Communities (ARIC) Study. Circ Heart Fail. 2012;5(4):422–9.

266. Assmann G, Cullen P, Schulte H. Simple scoring scheme for calculating the

risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. Circulation. 2002;105(3):310–5.

267. Chahal H, Bluemke DA, Wu CO, McClelland R, Liu K, Shea SJ, et al. Heart failure risk prediction in the Multi-Ethnic Study of Atherosclerosis. Heart. 2015;101(1):58–64.

268. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003;24(11):987–1003.

269. Ferrario M, Chiodini P, Chambless LE, Cesana G, Vanuzzo D, Panico S, et al. Prediction of coronary events in a low incidence population. Assessing accuracy of the CUORE Cohort Study prediction equation. Int J Epidemiol. 2005;34(2):413–21.

270. Kannel WB, D'Agostino RB, Silbershatz H, Belanger AJ, Wilson PW, Levy D. Profile for estimating risk of heart failure. Arch Intern Med. 1999;159(11):1197–204.

271. Pencina MJ, D'Agostino RB, Pencina KM, Janssens ACJW, Greenland P. Interpreting incremental value of markers added to risk prediction models. Am J Epidemiol. 2012;176(6):473–81.

272. Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. Lancet. 2003;362(9395):1527–35.

273. Riley RD, Snell KIE, Moons KGM, Debray TPA. Fundamental statistical methods for prognosis research. In: Riley RD, van der Windt DA, Croft P, Moons KG, editors. Prognosis research in healthcare: concepts, methods, and impact. Oxford University Press; 2019. p. 37–68.

274. Pencina MJ, D'Agostino RB, Vasan RS. Statistical methods for assessment of added usefulness of new biomarkers. Clin Chem Lab Med. 2010;48(12):1703–11.

275. Van Houwelingen JC, Le Cessie S. Predictive value of statistical models. Stat Med. 1990;9(11):1303–25.

276. Harrell FEJ, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15(4):361–87.

277. Brentnall AR, Cuzick J. Use of the concordance index for predictors of censored survival data. Stat Methods Med Res. 2018;27(8):2359–73.

278. Pencina MJ, D'Agostino RBS, D'Agostino RBJ, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):112–57.

279. Pickering JW, Endre ZH. New metrics for assessing diagnostic potential of candidate biomarkers. Clin J Am Soc Nephrol. 2012;7(8):1355–64.

280. Alonso A, Krijthe BP, Aspelund T, Stepas KA, Pencina MJ, Moser CB, et al. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium. J Am Heart Assoc. 2013;2(2):e000102.

281. Saliba W, Gronich N, Barnett-Griness O, Rennert G. Usefulness of CHADS2 and CHA2DS2-VASc Scores in the Prediction of New-Onset Atrial Fibrillation: A Population-Based Study. Am J Med. 2016;129(8):843–9.

282. Butler J, Kalogeropoulos A, Georgiopoulou V, Belue R, Rodondi N, Garcia M, et al. Incident heart failure prediction in the elderly: the health ABC heart failure score. Circ Heart Fail. 2008;1(2):125–33.

283. Schnabel RB, Rienstra M, Sullivan LM, Sun JX, Moser CB, Levy D, et al. Risk assessment for incident heart failure in individuals with atrial fibrillation. Eur J Heart Fail. 2013;15(8):843–9.

284. Ho JE, Enserro D, Brouwers FP, Kizer JR, Shah SJ, Psaty BM, et al. Predicting Heart Failure With Preserved and Reduced Ejection Fraction: The International Collaboration on Heart Failure Subtypes. Circ Heart Fail. 2016;9(6).

285. Jacobs L, Efremov L, Ferreira JP, Thijs L, Yang W-Y, Zhang Z-Y, et al. Risk for Incident Heart Failure: A Subject-Level Meta-Analysis From the Heart "OMics" in AGEing (HOMAGE) Study. J Am Heart Assoc. 2017;6(5).

286. Khan SS, Ning H, Shah SJ, Yancy CW, Carnethon M, Berry JD, et al. 10-Year Risk Equations for Incident Heart Failure in the General Population. J Am Coll Cardiol. 2019;73(19):2388–97.

287. Woodward M, Brindle P, Tunstall-Pedoe H. Adding social deprivation and family history to cardiovascular risk assessment: the ASSIGN score from the Scottish Heart Health Extended Cohort (SHHEC). Heart. 2007 Feb;93(2):172–6.

288. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007 Feb;297(6):611–9.

289. Hajifathalian K, Ueda P, Lu Y, Woodward M, Ahmadvand A, Aguilar-Salinas CA, et al. A novel risk score to predict cardiovascular disease risk in national populations (Globorisk): a pooled analysis of prospective cohorts and health examination surveys. lancet Diabetes Endocrinol. 2015 May;3(5):339–55.

290. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analysis. 2011. Available: www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 3 March 2019.

291. Duval S, Tweedie R. A Nonparametric "Trim and Fill" Method of Accounting for Publication Bias in Meta-Analysis. J Am Stat Assoc. 2000;95(449):89–98.

292. Pujades-Rodriguez M, Timmis A, Stogiannis D, Rapsomaniki E, Denaxas S, Shah A, et al. Socioeconomic deprivation and the incidence of 12 cardiovascular diseases in 1.9 million women and men: implications for risk prediction and prevention. PLoS One. 2014;9(8):e104671.

293. Pujades-Rodriguez M, George J, Shah AD, Rapsomaniki E, Denaxas S, West R, et al. Heterogeneous associations between smoking and a wide range of initial presentations of cardiovascular disease in 1937360 people in England: lifetime risks and implications for risk prediction. Int J Epidemiol. 2015;44(1):129–41.

294. Rapsomaniki E, Shah A, Perel P, Denaxas S, George J, Nicholas O, et al. Prognostic models for stable coronary artery disease based on electronic health record cohort of 102 023 patients. Eur Heart J. 2014;35(13):844–52.

295. Rapsomaniki E, Timmis A, George J, Pujades-Rodriguez M, Shah AD, Denaxas S, et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1.25 million people. Lancet. 2014;383(9932):1899–911.

296. Dinesh Shah A, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, et al. Type 2 diabetes and incidence of a wide range of cardiovascular diseases: a cohort study in 1·9 million people. Lancet. 2015;385:S86.

297. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 2015;12(3):1–10.

298. Baron RM, Kenny DA. The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. J Pers Soc Psychol. 1986;51(6):1173–82.

299. Burgess S, Thompson SG. Mendelian randomization : methods for using genetic variants in causal estimation. CRC Press; 2015.

300. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. Am J Clin Nutr. 2016;103(4):965–78.

301. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25.

Supplementary Appendices

Systematic review and meta-analysis (Chapter 2, 4-6)

Study eligibility criteria and search strategy

We searched for longitudinal cohort studies on the association between blood lipids and the incidence of HF and AF among community-dwelling populations. Therefore, our exposure of interest included lipid fractions (i.e., LDL-C, HDL-C, and TG), and our outcome of interest was the first occurrence of HF and AF. For RCTs, we applied the same eligibility criteria as the Cholesterol Treatment Trialists' (CTT) Collaboration on the basis of which only clinical trials with at least 1,000 participants and a minimum follow-up of one year have been selected. The justification for including only major clinical trials is because HF and AF are not the primary endpoints in most of the trials for lipid-regulating agents. In order to observe the change in relative risks for both diseases, therefore, we need trials with relatively large sample sizes (n≥1,000) and relatively long follow-up periods (≥1 year). To better approach our research questions, we broadened our search to cover all lipid-regulating agents (not just statins) and all prevention settings (i.e., primary-, secondary-, and mixed prevention).

The search was extensively performed by implementing both medical subject headings (MeSH or thesaurus search) and text-word searches on the three main databases, including Medline (Ovid: 1946 to present), Embase (Ovid: 1974 to present), and Cochrane CENTRAL in the English language. Due to the substantial number of published papers (>10,000 articles) up until 1 July 2019, the day the last search was run, we further narrowed down our search coverage to the last 19 years (from 1 January 2000 onwards). Details of search terms are described in the supplementary appendix (Table S2 and Table S3). We also screened a reference list for previous reviews, meta-analyses, and all included studies. We then directly contacted the authors of each trial via email to obtain complete information on their reported outcomes (i.e., HF and AF).

An additional search for genetic studies was performed through the Medline database using the following terms: "Mendelian Randomization Analysis

(MeSH)" OR "Genetic Association Studies (MeSH)" OR (mendelian adj1 randomi*).tw, which yielded 51,637 studies. We retrieved 285 studies by searching under the preceding keywords in combination with other search terms for the diseases (HF, AF, and MI) and blood lipids (LDL-C, HDL-C, and TG) (see Table S2). The 283 search results were further reviewed. In addition, a reference list of each genetic study was also screened.

Data collection process and data extraction

We developed a data extraction form for cohort studies and RCTs (please refer to the supplementary appendix), which was further refined accordingly. In the longitudinal cohort studies, extracted items included 1) setting, 2) number of participants, 3) events, 4) demographics of participants (i.e., age, gender), 5) inclusion criteria, 6) lipid ascertainment method, 7) outcome ascertainment method, 8) length of follow-up period, 9) effect size with a corresponding 95% confidence interval, and 10) list of adjusting factors (See data extraction form). From the RCTs, we extracted the following items: 1) year of start enrolment, 2) patients' characteristics (i.e., age, gender, and history of CVD at baseline), 3) intervention, 4) comparator, 5) length of follow-up period, 6) baseline lipid levels in each arm, 7) lipid levels at the end of study, and 8) number of HF and AF cases reported in each arm at the end of study (See data extraction form).

Data extraction form (Cohort study)

Study……Published year …..……
Corresponding author……. E-mail ……

Community setting (e.g., country) ……
Population: Total N ……..……
Numbers of HF (%) …….. AF (%) ……..……
Age …….. years
Male (%) ……
F/U …….. years

Inclusion criteria:
……
……

Exclusion criteria:
……
……

Lipid ascertainment:
❏ Directly measurement
❏ Calculated
❏ Details not given

Outcome ascertainment:
❏ Independent blind assessment or confirmation by reference to health records
❏ Calculated
❏ Details not given

Results
Adjusting factors
……
……

Exposure	Outcome effect size (95%CI)			
	HF		AF	
	Continuous (unit …. per ……..)	Categorical	Continuous (unit …. per ……..)	Categorical
LDL-C				
HDL-C				
HDL-C (male)				
HDL-C (female)				
TG				

Data extraction form (RCT)

Study…… Year of publication……
Corresponding author…… E-mail ……
Population: Total N ……
Type ❑ Primary prevention
❑ Secondary prevention
❑ Mixed population (previous MI or stroke = ……%)
Age …….. years
Year of start enrolment …….
F/U …….. years
Intervention:
……
Comparator:
……

Baseline Lipid	**Active**	**Control**
LDL-C		
HDL-C		
TG		
Non HDL-C		
Total-C (optional)		

Change in lipid @.....years	**Active**	**Control**	**Absolute change**	**% Relative to control**
LDL-C				
HDL-C				
TG				
Non HDL-C				
Total-C (optional)				

Outcomes	**Active (n=)**	**Control (n=)**
Nonfatal MI		
AF		
HF		
DM		
Cataract		
All-deaths		
CV deaths		
CA deaths		
Non CVCA deaths		

Quality assessment

To assess the quality of included studies, we used the standard quality score. To the cohort studies, the Newcastle-Ottawa Scale (NOS)[290] was applied (see the next page for details of key criteria), whereas the Cochrane risk of bias tool[233] was employed to assess the quality of included RCTs. In short, the NOS consists of eight items in three domains (i.e., selection, comparability, and outcome) with the total maximum score of nine. In this review, cohort studies with a score of seven and above will be considered "good quality" since a standard cut-off point has not been universally established. The key NOS criteria in this study are specified and provided in the following section.

With the risk of bias tool, the quality of RCTs will be assessed based on seven items, including 1) random sequence generation, 2) allocation concealment, 3) blinding of participants and personnel, 4) blinding of outcome assessment, 5) incomplete outcome data, 6) selective reporting, and 7) other bias, each of which is graded as "high", "low", or "unclear" risk of bias. Since HF and AF are not the primary outcomes in most of the trials, the outcome used as a measure to assess the quality of RCTs in our report is CVD (i.e., coronary artery disease and stroke). We present the risk of bias by providing both overall assessment results and scores of individual items in each trial, which can be found in the following section.

Newcastle - Ottawa quality assessment scale for cohort studies

Key criteria for assessment

Selection (4 out of 4)

1) Representativeness of the exposed cohort

a) Truly representative of the average middle-age to elderly (e.g., age > 50 – 60 years) with CVD-free and community-dwelling resident *

b) Somewhat representative of the middle-age to elderly (e.g., age > 50 – 60 years) with CVD-free and community-dwelling resident *

c) Selected group of users e.g., nurses, volunteers, or only certain socioeconomic groups/ areas

d) No description of the derivation of the cohort

2) Selection of the non-exposed cohort

a) Drawn from the same community as the exposed cohort *

b) Drawn from a different source

c) No description of the derivation of the non-exposed cohort

3) Ascertainment of exposure (i.e., blood lipids)

a) Secure record (e.g., surgical records, health care records, or used standard laboratory procedure) *

b) Structured interview *

c) Written self-report

d) No description or LDL-C and HDL-C were calculated and not directly measured.

4) Demonstration that outcome of interest (i.e., heart failure or atrial fibrillation) was not present at start of study

a) Yes *

b) No

Comparability (2 out of 2)

1) Comparability of cohorts on the basis of the design or analysis

a) Study controls for 1) age, 2) sex, 3) BMI (or obesity), 4) smoking status, 5) hypertension (or blood pressure), 6) type 2 diabetes (or blood glucose, insulin intolerance), and 7) dyslipidaemia (or blood lipids) *

b) Study controls for any additional factors (i.e., interim CVD) *

Outcome (3 out of 3)

1) Assessment of outcome (i.e., heart failure or atrial fibrillation)

a) Independent blind assessment or confirmation of the outcome by reference to secure records (health records, etc.) *

b) Record linkage (e.g., identified through ICD codes on database records) *

c) Self-report (i.e., no reference to original health records or documented source to confirm the outcome)

d) No description

2) Was follow-up long enough for outcomes to occur

a) Yes (if mean or median follow-up period ⩾ 5 years) *

b) No (if mean or median follow-up period < 5 years)

3) Adequacy of follow up of cohorts

a) Complete follow up – all subjects accounted for *

b) Subjects lost to follow up unlikely to introduce bias small number lost (i.e., ⩾ 80 % follow up) or description provided of those lost *

c) Follow up rate < 80% and no description of those lost

d) No statement

Data synthesis and statistical analysis

The hazard ratio (HR) of incident HF and AF per standard deviation (SD) change in lipid fractions was the primary measure of exposure effects in longitudinal cohort studies, whereas relative risk (RR) of HF and AF in an active arm, compared with controlled individuals, was our measurement of RCTs.

To start with, we produced a qualitative summary of cohort studies to compare the direction of the association between blood lipids (i.e., LDL-C, HDL-C, and TG) and incident HF and AF from each included study. The direction of the reported association was designated as + (positive association), - (negative association), or 0 (null association) as appropriate. Then we extracted the effect size and confidence interval from the fully-adjusted model for further meta-analysis. However, some studies that showed their associations in other units, such as per 1 mg/dL,[107,110] per 10 mg/dL,[68,69] or per 1 mmol/L change,[70,113] or reported their associations with different systems measurement (e.g., odds ratio),[107] are incorporated in the main results, but will

later be excluded from the sensitivity analysis to minimise bias in the main findings.

Particular data manipulation is needed in some studies before results can be meta-analysed. In studies that reported the association of blood lipids as a categorical variable (e.g., quartiles,[73,77] or quintiles[76]), the continuous summary is obtained from linear regression of the log HR for each category on each lipid stratum. This process could be completed, providing that a linear trend of the association across lipids strata was proved (P-value for trend < 0.05) and was provided in the original study. Moreover, we meta-analysed the study that separated results by gender,[112] using a random-effect model to obtain the pooled results of the overall population.

In the meta-analysis, we pooled the results based mainly on a fixed-effect model using the inverse variance method. However, if significantly statistical heterogeneity was observed (i.e., I^2 > 75% or P-value of chi-squared test < 0.1)[233], and the source of heterogeneity was unknown, a random-effect model would be adopted for the pooled estimation.

To conduct a meta-analysis of cohort studies, we further a performed sensitivity analysis by i) varying the model used for pooled estimation (i.e., fixed- vs random effect model), ii) excluding studies that did not report their effect sizes as per continuous change in lipid fractions, and iii) additional excluding studies that did not use the unit of change in lipid fractions as per SD change or those that did not report the effect size as a hazard ratio (HR).

To meta-analyse RCTs, we performed a subgroup analysis of different drug groups (i.e., statins, fibrates, CETP inhibitors, PCSK9 inhibitors, and others) as well as different study populations (i.e., free of CVD at baseline, with CVD at baseline, or mixed populations). A further test for subgroup differences (heterogeneity) based on Q-statistics was also carried out and reported.

To ascertain the publication bias, we created funnel plots (with additional Egger's statistics if there were ≥ ten included studies in the meta-analysis[233]). In case the funnel plot was visually inspected and an apparent asymmetrical shape or P-value from the Egger's test < 0.05, which suggested potential publication bias, we further applied Duval and Tweedie's trim and fill

method.[291] This method takes into account outlier studies by trimming those studies and replacing them with theoretical studies that make the funnel plot more symmetrical and then analyses the pooled results based on these hypothetical results.

All analyses in this section have been done using STATA version 15 (IC version, StataCorp). We used RevMan version 5.3 for the visualisation of quality assessment of RCTs and the creation of study flow diagrams. Furthermore, R version 3.3 with a package "CALIBERdatamanagement" was used to create the forest plot in the main results.

Data sources used in this thesis

CALIBER (Chapter 3-7)

CALIBER (clinical research using linked bespoke studies and electronic health records) is a research platform, which was established to provide access to longitudinal data of linked electronic health records from 1996-2019. The CALIBER platform provides access to longitudinal linked electronic health records (EHRs) across primary care data (Clinical Practice Research Datalink (CPRD)), secondary care data (Hospital Episode Statistics (HES)), and cause-specific mortality (Office for National Statistics (ONS)) in England.[170]

CPRD provides information from primary care practices in England about anthropometric measurements, laboratory tests, clinical diagnoses, prescriptions, and medical procedures, coded with the Read clinical coding scheme.[171]

MINAP is a national registry of patients admitted to hospital with acute coronary syndromes. HES provides information about diagnoses (coded with the tenth revision of the International Classification of Diseases [ICD-10]) and medical procedures (OPCS-4; Office of Population Censuses and Surveys Classification of Surgical Operations and Procedures - 4th revision) related to all elective and emergency hospital admissions across all National Health Service hospitals in England.

Primary care records from CPRD were linked to secondary care admission records from Hospital Episodes Statistics Admitted Patient Care data. Linkage

was available for a subset of English practices from Jan 1, 1998, covering approximately 50% of all CPRD records. The method for the linkage is the deterministic approach using the individual national health service (NHS) number as an identifier across data sources.

Diagnosis codes and endpoints in CALIBER have been validated by independent groups about which there is extensive published evidence of risk factors and various disease endpoint validity in CALIBER.[175,179,292–296] Details of CALIBER, such as methods for the development of reproducible phenotypes and metadata, can be found from https://www.caliberresearch.org/portal.

Eligibility criteria for the included patients in this study:

1. Registered at their general practice between 1st January 1998 and 30th June 2016
2. Had been followed-up with their GP for at least one year before study entry
3. Age 18 years or older at study entry
4. Had no previous diagnosis of cardiovascular diseases, including heart failure, atrial fibrillation, ventricular fibrillation, myocardial infarction, unstable angina, stable angina, ischaemic stroke, transient ischaemic attack, subarachnoid haemorrhage, intracerebral haemorrhage, abdominal aortic aneurysm, and peripheral arterial disease, or had previously undergone coronary revascularisation at the date of baseline LDL-C (Chapter 4), HDL-C (Chapter 5), and TG (Chapter 6) measurement

UK Biobank (Chapter 4-6)

UK Biobank is a very large and detailed prospective study with over 500,000 participants aged 40–69 years when recruited in 2006–2010. The study has collected and continued to collect extensive phenotypic and genotypic detail about its participants, including data from questionnaires, physical measures, sample assays, accelerometry, multimodal imaging, genome-wide genotyping

and longitudinal follow-up for a wide range of health-related outcomes. Data are available for open access, without the need for collaboration, to any researcher who wishes to use it to conduct health-related research for the benefit of the public.[297] UK Biobank's publicly available data can be found at http://www. ukbiobank.ac.uk/.

GLGC

GLGC (global lipids genetics consortium) is a study aiming to identify new loci and refining known loci influencing levels of low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and total cholesterol, which examined 188,577 individuals using genome-wide and custom genotyping arrays.[202] It collected summary statistics for Metabochip SNPs from 45 studies. Of these, 37 studies consisted primarily of individuals of European ancestry (90.1%), including both population-based studies and case-control studies of CAD and T2D. Another 8 studies consisted primarily of individuals with non-European ancestry, including 2 studies of individuals of South Asian descent (2.6%), East Asian descent (4.7%), and African ancestry (2.6%). Blood lipid levels were typically measured after >8 h of fasting. Individuals known to be on lipid-lowering medication were excluded when possible. LDL cholesterol levels were directly measured in ten studies (24% of total study individuals) and were estimated using the Friedewald formula in the remaining studies. The data source is publicly available and can be found at http://csg.sph.umich.edu/willer/public/ lipids2013/.

CARDIoGRAMplus4CD (Chapter 4-6)

The CARDIoGRAMplusC4D (coronary artery disease genome wide replication and meta-analysis plus the coronary artery disease genetics) consortium represents a collaborative effort to combine data from multiple large-scale genetic studies to identify risk loci for coronary artery disease and myocardial infarction.[203] CARDIoGRAMplusC4D Metabochip is a two-stage meta-analysis of Metabochip and GWAS studies of European and South Asian descent involving 63,746 coronary artery disease (CAD) or myocardial infarction (MI) cases and 130,681 controls in both genders. Cases were selected for inclusion following the standard criteria for CAD and myocardial infarction used in the CARDIoGRAM study. Collections were typed with either the Metabochip array

(60% of samples) or imputed data using HapMap. The data source is publicly available and can be found at http://www.cardiogramplusc4d.org/data-downloads/.

HERMES (Chapter 4-6)

The HERMES (the Heart Failure Molecular Epidemiology for Therapeutic Targets) consortium is a large-scale GWAS of 8,246,881 common and low-frequency (i.e., minor allele frequency > 1%) variants comprising 47,309 cases and 930,014 controls of European ancestry across 26 studies.[205] Genotype data were imputed to either the 1000 Genomes Project (60%), Haplotype Reference Consortium (35%) or study-specific reference panels (5%). Participants from 26 cohorts (with a total of 29 distinct datasets) with either a case-control or population-based study design were included in the meta-analysis. Cases included participants with a clinical diagnosis of heart failure of any aetiology with no inclusion criteria based on left ventricular ejection fraction; controls were participants without HF. The definitions used to adjudicate the heart failure status within each study are detailed elsewhere. All included studies were ethically approved by local institutional review boards and all participants provided written informed consent.

Codes for HF and AF identified from cases in CALIBER (Chapter 3-7)

Following are code lists and corresponding frequencies of all identified cases observed from the data. It should be noted that most patients had multiple codes, so the reported percentages may be added up to more than 100%. Please be noted that the following code lists are used to phenotype outcomes of interest (i.e., HF and AF) throughout my thesis.

Heart failure outcome

HF CPRD Read codes	HF CPRD Procedure-Read codes	HF ICD 10 codes	HF ONS ICD 9 and 10 codes
G58..00: Heart failure (12.72%) G581.00: Left ventricular failure (11.03%) G580.11: Congestive cardiac failure (8.07%) G580.00: Congestive heart failure (5.71%) G5yy900: Left ventricular systolic dysfunction (4.49%) G581.13: Impaired left ventricular function (1.82%) 1O1..00: Heart failure confirmed (1.64%) G58..11: Cardiac failure (1.28%) G5yyA00: Left ventricular diastolic dysfunction (0.93%) G41z.11: Chronic cor pulmonale (0.45%) G58z.00: Heart failure NOS (0.45%) G400.00: Acute cor pulmonale (0.39%) 662W.00: Heart failure annual review (0.38%) G580.12: Right heart failure (0.25%) G580.14: Biventricular failure (0.23%) G581000: Acute left ventricular failure (0.16%) G580000: Acute congestive heart failure (0.15%) G580200: Decompensated cardiac failure (0.13%) G582.00: Acute heart failure (0.10%) G58z.12: Cardiac failure NOS (0.10%) G580.13: Right ventricular failure (0.08%)	585f.00: Echocardiogram shows left ventricular systolic dysfunction (2.59%) 585g.00: Echocardiogram shows left ventricular diastolic dysfunction (0.64%)	I50.0: Congestive heart failure (34.89%) I50.1: Left ventricular failure (30.33%) I50.9: Heart failure, unspecified (13.47%) I11.0: Hypertensive heart disease with (congestive) heart failure (0.63%) I26.0: Pulmonary embolism with mention of acute cor pulmonale (0.31%) I13.2: Hypertensive heart and renal disease with both (congestive) heart failure and renal failure (0.06%) I13.0: Hypertensive heart and renal disease with (congestive) heart failure (0.05%)	I50.0: Congestive heart failure (1.94%) I11.0: Hypertensive heart disease with (congestive) heart failure (1.32%) I50.9: Heart failure, unspecified (0.58%) I50.1: Left ventricular failure (0.38%) I13.0: Hypertensive heart and renal disease with (congestive) heart failure (0.02%) 428.0 Congestive heart failure, unspecified (<0.01%)

HF CPRD Read codes	HF CPRD Procedure-Read codes	HF ICD 10 codes	HF ONS ICD 9 and 10 codes
G580100: Chronic congestive heart failure (0.08%) 662p.00: Heart failure 6 month review (0.07%) 8H2S.00: Admit heart failure emergency (0.05%) 8B29.00: Cardiac failure therapy (0.04%) R2y1000: [D]Cardiorespiratory failure (0.04%) 14A6.00: H/O: heart failure (0.03%) G580300: Compensated cardiac failure (0.03%) G580400: Congestive heart failure due to valvular disease (0.03%) 14AM.00: H/O: Heart failure in last year (0.02%) G554000: Congestive cardiomyopathy (0.02%) G581.11: Asthma - cardiac (0.02%) 9Or0.00: Heart failure review completed (0.01%) G1yz100: Rheumatic left ventricular failure (0.01%) G230.00: Malignant hypertensive heart and renal disease (<0.01%)			

Atrial fibrillation outcome

AF CPRD Read codes	AF CPRD Procedure-Read codes	AF ICD 10 codes	AF OPCS codes
G573000: Atrial fibrillation (22.20%) G573.00: Atrial fibrillation and flutter (8.50%) G573200: Paroxysmal atrial fibrillation (4.16%) G573100: Atrial flutter (1.22%) 14AN.00: H/O: atrial fibrillation (0.49%) 3272.00: ECG: atrial fibrillation (0.49%) 662S.00: Atrial fibrillation monitoring (0.21%)	7L1H000: Direct current cardioversion (2.16%) 7L1H100: External cardioversion NEC (0.74%) 7L1H.11: Cardioversion and stimulation (0.46%) 7934000: Percutaneous transluminal ablation of atrioventricular node (0.17%)	I48: Atrial fibrillation and flutter (83.52%) I48.9: Unspecified atrial fibrillation and atrial flutter (7.23%) I48.0: Paroxysmal atrial fibrillation (0.93%) I48.1: Persistent atrial fibrillation (0.11%) I48.2: Chronic atrial fibrillation (0.08%) I48.3: Typical atrial flutter (0.02%) I48.4: Atypical atrial flutter (0.01%)	X501: Direct current cardioversion (9.51%) K571: Percutaneous transluminal ablation of atrioventricular node (0.69%) K622: Percutaneous transluminal ablation of atrial wall for atrial flutter (0.44%) K621: Percutaneous transluminal ablation of pulmonary vein to left atrium conducting system (0.41%)

AF CPRD Read codes	AF CPRD Procedure-Read codes	AF ICD 10 codes	AF OPCS codes
212R.00: Atrial fibrillation resolved (0.16%) 3273.00: ECG: atrial flutter (0.10%) G573z00: Atrial fibrillation and flutter NOS (0.07%) 14AR.00: History of atrial flutter (0.05%) 9Os..00: Atrial fibrillation monitoring administration (0.04%) 6A9..00: Atrial fibrillation annual review (0.02%) G573500: Persistent atrial fibrillation (0.01%) G573400: Permanent atrial fibrillation (0.01%) 9hF1.00: Excepted from atrial fibrillation qual indic: Inform dissent (0.01%) 9Os0.00: Atrial fibrillation monitoring first letter (0.01%) G573300: Non-rheumatic atrial fibrillation (<0.01%) 9hF..00: Exception reporting: atrial fibrillation quality indicators (<0.01%) 9Os1.00: Atrial fibrillation monitoring second letter (<0.01%)	793M100: Percutaneous transluminal ablation of atrial wall for atrial flutter (0.08%) 7934200: Transluminal radiofrequency ablation heart conducting system NEC (0.06%) 793M300: Percutaneous transluminal ablation conduct sys heart for atrial flutter NEC (0.04%) 7934500: Percutaneous transluminal ablation of atrial wall (0.04%) 7934800: Percutaneous transluminal ablation of atrial wall NEC (0.03%) 793M200: Percutaneous transluminal internal cardioversion NEC (0.03%) 7930000: Open ablation of atrioventricular node (0.03%) 793M000: Percutaneous transluminal ablation pulmonary vein to left atrium conduct system (0.03%) 793M.00: Therapeutic transluminal operations on heart (0.02%) 7L1H200: Internal electrode cardioversion (0.02%) 7L1H300: Electrical sinus rhythm conversion (0.01%) 7L1H.13: Defibrillation (0.01%) 7L1H800: Chemical cardioversion (0.01%) 7L1H.12: Direct current cardiac shock (0.01%) 7936A00: Implant intravenous pacemaker for atrial fibrillation (0.01%) 7L1H111: External electrode cardioversion (<0.01%) 7L1H400: Electrical operative cardiac stimulation (<0.01%) 793Mz00: Therapeutic transluminal operations on heart NOS (<0.01%) 7936900: Implantation of intravenous atrial overdrive pacemaker (<0.01%)		K575: Percutaneous transluminal ablation of atrial wall NEC (0.36%) X502: External cardioversion NEC (0.30%) K623: Percutaneous transluminal ablation of conducting system of heart for atrial flutter NEC (0.15%) X504: External ventricular defibrillation (0.13%) K624: Percutaneous transluminal internal cardioversion NEC (0.05%) K521: Open ablation of atrioventricular node (0.05%)

Codes for subtypes of HF and AF identified from cases in CALIBER (Chapter 3-7)

Following are the code lists used to identify subtype of HF and AF throughout my thesis.

Subtypes of heart failure

HF CPRD Read codes	HF CPRD Procedure-Read codes	HF ICD 10 codes
1 Heart failure with left ventricular systolic dysfunction	585f.00: Echocardiogram shows left ventricular systolic dysfunction (2.59%) 11284	
2 Heart failure with left ventricular diastolic dysfunction	585g.00: Echocardiogram shows left ventricular diastolic dysfunction (0.64%) 11351	
3 Heart failure due to chronic respiratory disease G41z.11: Chronic cor pulmonale (0.45%) 5695 G400.00: Acute cor pulmonale (0.39%) 8464 G581.11: Asthma - cardiac (0.02%) 23481		I26.0: Pulmonary embolism with mention of acute cor pulmonale (0.31%)
4 Heart failure due to hypertension G230.00: Malignant hypertensive heart and renal disease (<0.01%) 67232		I11.0: Hypertensive heart disease with (congestive) heart failure (0.63%) I13.2: Hypertensive heart and renal disease with both (congestive) heart failure and renal failure (0.06%) I13.0: Hypertensive heart and renal disease with (congestive) heart failure (0.05%)
5 Heart failure due to valvular disease G580400: Congestive heart failure due to valvular disease (0.03%) 194870 G1yz100: Rheumatic left ventricular failure (0.01%) 22262		

Subtypes of atrial fibrillation

AF CPRD Read codes	AF ICD 10 codes
1 Paroxysmal atrial fibrillation	
G573200: Paroxysmal atrial fibrillation (4.16%)	I48.0: Paroxysmal atrial fibrillation (0.93%)
2 Persistent or permanent atrial fibrillation	
G573500: Persistent atrial fibrillation (0.01%) G573400: Permanent atrial fibrillation (0.01%)	I48.1: Persistent atrial fibrillation (0.11%)
3 Atrial flutter	
G573100: Atrial flutter (1.22%) 3273.00: ECG: atrial flutter (0.10%)	I48.3: Typical atrial flutter (0.02%) I48.4: Atypical atrial flutter (0.01%)

Statistical analysis and multiple imputations (Chapter 4-6)

EHR Cohort study

All imputations were done in Stata software, version 13 (StataCorp LP., College Station, USA). We verified the proportional hazards assumption of Cox models by plotting the Schoenfeld residuals and (-log(-log(survival)) vs log(time) plots for continuous and categorical variables, respectively. Risk factor data appeared to be missing at random after the adjustment for major confounders (e.g. age, sex, diabetes, BMI and blood pressure). Hence, multiple imputations were implemented by the MICE (Multiple Imputation by Chained Equation) method using the "mi" algorithm in Stata, to replace missing values in risk factors and other variables. For non-linear continuous variable, we logarithmically transformed them before imputation. Imputation models were estimated separately for each of the outcomes and included:

1) All the baseline covariates used in the main analysis (age, smoking, systolic blood pressure, LDL-cholesterol, index of multiple deprivation, diabetes status, HDL-cholesterol, Triglyceride [logarithmic transformation], and C-reactive protein [logarithmic transformation]);

2) Baseline average measurements of covariates not considered in the main analysis (diastolic blood pressure, alcohol intake, ethnicity, physical activity, serum albumin, and estimated Glomerular Filtration Rate [eGFR]);

3) Medications (statins, other lipid-lowering drugs, and blood pressure-lowering medications);

4) The Nelson-Aalen hazard, follow-up time and the event status for each of the three endpoints (i.e, HF, AF, and MI) analysed.

Twenty multiply-imputed datasets were generated for each outcome, and coefficients were combined using Rubin's rules. Trace plots of summaries of imputed values were visualised and imputation diagnostics checked by considering values of total imputation variance, relative variance increase (RVI), fraction on missing information (FMI), and relative efficiency (RE) to

ensure the good performance of multiple imputation processes and a sufficient number of imputations.

Statin use was marked for participants who had received at least two separate prescriptions of a form of statin drugs within a window of three months. If this window had an overlap with the period of 6 months before or after the baseline date for an individual, it would be considered “statin use at baseline”. For the adjustment of treatment effects, the use of statins at baseline or at any time after that (but before the first CVD presentation) was considered.

For a single exposure affecting one fifth of the CPRD population (estimation of 1,262,280 from a total population of 3,637,715 individuals, based on our work with the data) we were powered at the two-sided alpha of 0.95 and the beta of 0.2 level to detect heterogeneous relative effects across three endpoints (i.e., HF, AF, and MI), which range evenly from 0.96 to 1.05 at the extremes, assuming that the minimum incidence rate of any endpoints during the follow-up was 0.27% (equivalent to 270 events per 100,000 person-years, based on our work with the data).

All models were visualised to assess the validity of the proportional hazards assumption. Sensitivity analyses were carried out to examine the robustness of the results. The potential effect modifiers and mediators are as follows: i) comparison between using average and single TG values; ii) comparison between men and women; iii) comparison amongst three age groups (i.e., 18-54, 55-74, and ≥75 years old); iv) comparison amongst different intercurrent MI, HF, AF, and any CVDs statuses; v) comparison amongst those with and without hypertension at baseline; vi) comparison amongst those with and without comorbidity (i.e., having been previously diagnosed with cancer, kidney disease, and COPD) at baseline; vii) restricting analysis only in events occurring after the first four years of baseline TG measurements; viii) restricting analysis only in people without diabetes, or not using statins at baseline.

Analysis of meta-regression

The main results were derived from the univariable meta-regression model. MI was used to ensure the validity of this approach. We also performed multivariable meta-regression by adjusting for the following variables (all were trial-level data):

i) Types of study: categorical variable (i.e., primary prevention, secondary prevention, and mixed),
ii) Types of intervention: categorical variable (i.e., statins, PCSK-9 inhibitors, CETP inhibitors, fibrates, and others),
iii) Average age of the studied population: continuous variable,
iv) Baseline LDL-C in all participants: binary variables (≥ 2.59 mmol/L or < 2.59 mmol/L)[67], and
v) Mean follow-up period: continuous variable.

To take into account the potential interaction in meta-regression results, we carried out subgroup analyses, including

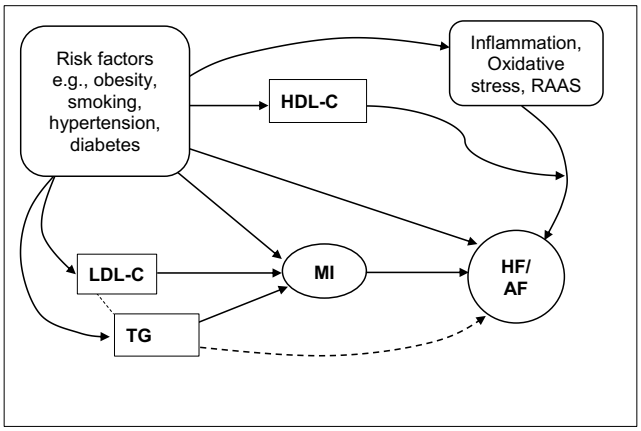
i) Sensitivity analysis: pooled results from the univariable model compared with results from the multivariable model,
ii) Sensitivity analysis: all trials included compared with only major trials (i.e., those with at least 1,000 participants with > 1 year of follow-up).
iii) Subgroup analysis according to types of lipid-lowering agents, and
iv) Subgroup analysis according to types of prevention setting (i.e., primary-, secondary-, or mixed prevention).
v) Subgroup analysis according to types of comparison (i.e., head-to-head comparison or placebo comparison)

Any studies with missing data for any particular analysis were excluded from a specific analysis. A funnel plot in which the SE of the log RR was plotted against the RR was constructed to examine the potential publication bias, and the asymmetry of the plot was examined visually. Statistical heterogeneity was assessed using the I^2 statistic with the cut-off $I^2 > 75\%$ or $P > 0.10$ considered statistically nonsignificant. For the summary treatment effect (meta-

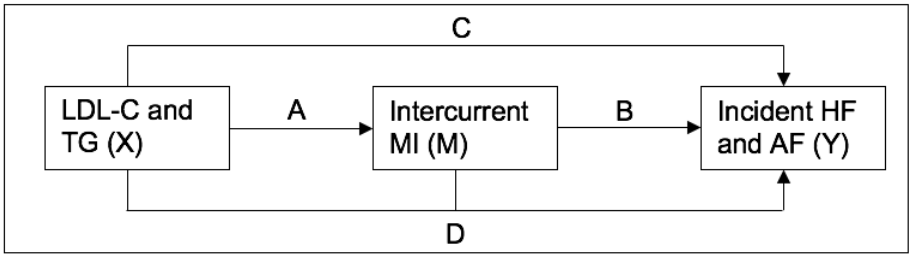
regression) estimate, a two-tailed P value of less than 0.05 was considered statistically significant.

Mediation analysis

In this section, I perform a simple mediation analysis using the four-step approach suggested by Baron and Kenny.[298] This approach, I will do regression analysis (Cox-model) at each path separately and compare coefficients at each step. According to a causal diagram below (taken from Figure 1-1, page), MI (intercurrent MI, to be more specific) is considered as a potential mediator of the association between lipids (i.e., LDL-C and TG) and outcome of interest (i.e., HF and AF).



From the above diagram, I can draw a simple pathway linked among lipids, MI, and outcome as follows:



There are four regression models for each step, including

Path A: Regress M on X => $M = x_0 + xX$

Path B: Regress Y on M => $Y = m_0 + mM$

Path C: Regress Y on X => $Y = x_0 + xX$

Path D: Regress Y on X condition on M (i.e., direct effect) => $Y = b_0 + x_1X + m_1M$

The purpose of path a, b, and c is to establish that zero-order relationships among the variables (e.g., variable X, M, and Y) exist. If one or more of these relationships are nonsignificant, we can conclude that mediation is unlikely. In the path d, some forms of mediation can be supported providing that the effect of variable X (e.g., LDL-C and TG) remains significant after controlling for M (e.g., intercurrent MI). If the coefficient of variable X (x_1) is no longer significant when M is controlled, the finding supports full mediation, whereas if it is attenuated, the finding supports partial mediation. If the coefficient is still the same (x and x_1) after controlling for the potential mediator, it is unlikely to be mediator. In addition, I do further subgroup analysis of intercurrent MI (i.e., with and without intercurrent MI) as an effect modifier (interaction) of the association between lipids and outcome in order to comprehensively investigate the role of MI in the association.

Following is the results of four-step approach to test mediation in my thesis.

1. **LDL-C and HF** (total n = 1,142,472, intercurrent MI cases = 14,168, incident HF cases = 25,751)

Path	Tested association	Unit of measurement	Fully adjusted HR (95%CI), P-value
A	LDL-C and intercurrent MI	Per 1 SD decreased LDL-C	0.90 (0.88, 0.92), < 0.001
B	Intercurrent MI and incident HF	Having MI, compared to not having MI	2.50 (2.39, 2.62), < 0.001
C	LDL-C and incident HF	Per 1 SD decreased LDL-C	1.09 (1.08, 1.11), < 0.001
D	LDL-C and incident HF adjusted for intercurrent MI	Per 1 SD decreased LDL-C	1.10 (1.09, 1.12), < 0.001
Subgroup analysis			
Without intercurrent MI		Per 1 SD decreased LDL-C	1.10 (1.09, 1.12)*
With intercurrent MI		Per 1 SD decreased LDL-C	1.03 (0.98, 1.08)*

*P-value for heterogeneity = 0.009

3. **LDL-C & AF** (total n = 1,142,472, intercurrent MI cases = 14,243, incident AF cases = 45,690)

Step	Tested association	Unit of measurement	Fully adjusted HR (95%CI), P-value
1	LDL-C and intercurrent MI	Per 1 SD decreased LDL-C	0.88 (0.87, 0.90), < 0.001
2	Intercurrent MI and incident AF	Having MI, compared to not having MI	0.97 (0.92, 1.02), 0.19
3	LDL-C and incident AF	Per 1 SD decreased LDL-C	1.11 (1.10, 1.12), < 0.001
4	LDL-C and incident AF adjusted for intercurrent MI	Per 1 SD decreased LDL-C	1.11 (1.10, 1.12), < 0.001
Subgroup analysis			
Without intercurrent MI		Per 1 SD decreased LDL-C	1.12 (1.10, 1.13)*
With intercurrent MI		Per 1 SD decreased LDL-C	1.09 (1.01, 1.19)*

*P-value for heterogeneity = 0.52

4. **TG & HF** (total n =1,261,973, intercurrent MI cases = 16,879, incident HF cases = 30,486)

Step	Tested association	Unit of measurement	Fully adjusted HR (95%CI), P-value
1	TG and intercurrent MI	Per 1 SD decreased TG	1.12 (1.08, 1.16), < 0.001
2	Intercurrent MI and incident HF	Having MI, compared to not having MI	2.45 (2.35, 2.55), < 0.001
3	TG and incident HF	Per 1 SD decreased TG	1.11 (1.08, 1.15), < 0.001
4	TG and incident HF adjusted for intercurrent MI	Per 1 SD decreased TG	1.12 (1.09, 1.15), < 0.001
Subgroup analysis			
Without intercurrent MI		Per 1 SD decreased TG	1.11 (1.08, 1.14)*
With intercurrent MI		Per 1 SD decreased TG	1.05 (0.95, 1.15)*

*P-value for heterogeneity = 0.26

6. **TG & AF** (total n = 1,261,973, intercurrent MI cases = 16,946, incident AF cases = 45,690)

Step	Tested association	Unit of measurement	Fully adjusted HR (95%CI), P-value
1	TG and intercurrent MI	Per 1 SD decreased TG	1.09 (1.06, 1.13), < 0.001
2	Intercurrent MI and incident AF	Having MI, compared to not having MI	0.98 (0.94, 1.03), 0.41
3	TG and incident AF	Per 1 SD decreased TG	1.31 (1.28, 1.33), < 0.001
4	TG and incident AF adjusted for intercurrent MI	Per 1 SD decreased TG	1.31 (1.28, 1.33), < 0.001
Subgroup analysis			
Without intercurrent MI		Per 1 SD decreased TG	1.31 (1.28, 1.34)*
With intercurrent MI		Per 1 SD decreased TG	1.09 (0.93, 1.27)*

*P-value for heterogeneity = 0.012

From all above information, it is found that the association between LDL-C and incident HF and AF is less likely to be mediated through intercurrent MI. This is partly supported by the subtype analysis in which the association between LDL-C and HF still remains when I restricted the analysis to only HF cases due to chronic respiratory disease (Figure , page). Therefore, LDL-C should be associated with HF through other pathways rather than MI or there might be residual confounders that artifact the findings.

However, MI might play a role as an effect modifier of the association between LDL-C and HF since among patients without intercurrent MI, the strength of the association is significantly stronger than that of those with intercurrent MI (HR 1.10 [95%CI 1.09 to 1.12] VS 1.03 [95% CI 0.98 to 1.08], p-value for heterogeneity = 0.009).

Regarding the association of TG with HF and AF, it can be observed that the risk of HF and AF is unlikely to be mediated through intercurrent MI. However, MI might be an interaction between the association between TG and incident AF. Hazards ratio (HR) per 1 SD decrease in TG levels among patients without intercurrent MI was 1.31 (95%CI 1.28 to 1.34), whereas that among individuals with intercurrent MI was 1.09 (95%CI 0.93 to 1.27), and p-value for heterogeneity was 0.012.

Static VS Dynamic population design

There are three reasons to support that dynamic population study design (i.e., the analysis that accounts for the change in an exposure over time due to dynamic movement of population in and out the study) is not necessary in my work as follows:

1) Although my study period is between 1 Jan 1997 and 30 Jun 2016 (around 19 years), cohorts of my study had a relatively short follow-up period of around 5 years (median) only. Whereas, the median measurement of lipids in my cohorts is 2-3 times. This means that, in general, patients had their lipid measurements once in every 1.7-2.5 years, which is not very often.
2) The analysis of the pattern of change in blood lipids in individual patients using growth curve model allowing for random intercept and random slope have shown that per each visit, patients' lipid levels did not clinically significantly change. As per each visit:
   - LDL-C levels decrease by 0.133 (95%CI 0.132 to 0.134) mmol/L.
   - HDL-C levels increase by 0.003 (95%CI 0.002 to 0.003) mmol/L.
   - TG levels decrease by 0.034 (95%CI 0.033 to 0.035) mmol/L.
3) Our previous analysis using a dynamic population study design (i.e., using a complementary log-log model) in different outcomes, such as type 2 diabetes, cataract, and COPD, always show the similar results compared to those from a static population study design (i.e., using typical Cox-model). Moreover, the static design yielded more conservative results (bias toward null) than the dynamic one (Figure S1), and our results showed significant results. Therefore, using the dynamic design is not necessary in my work.

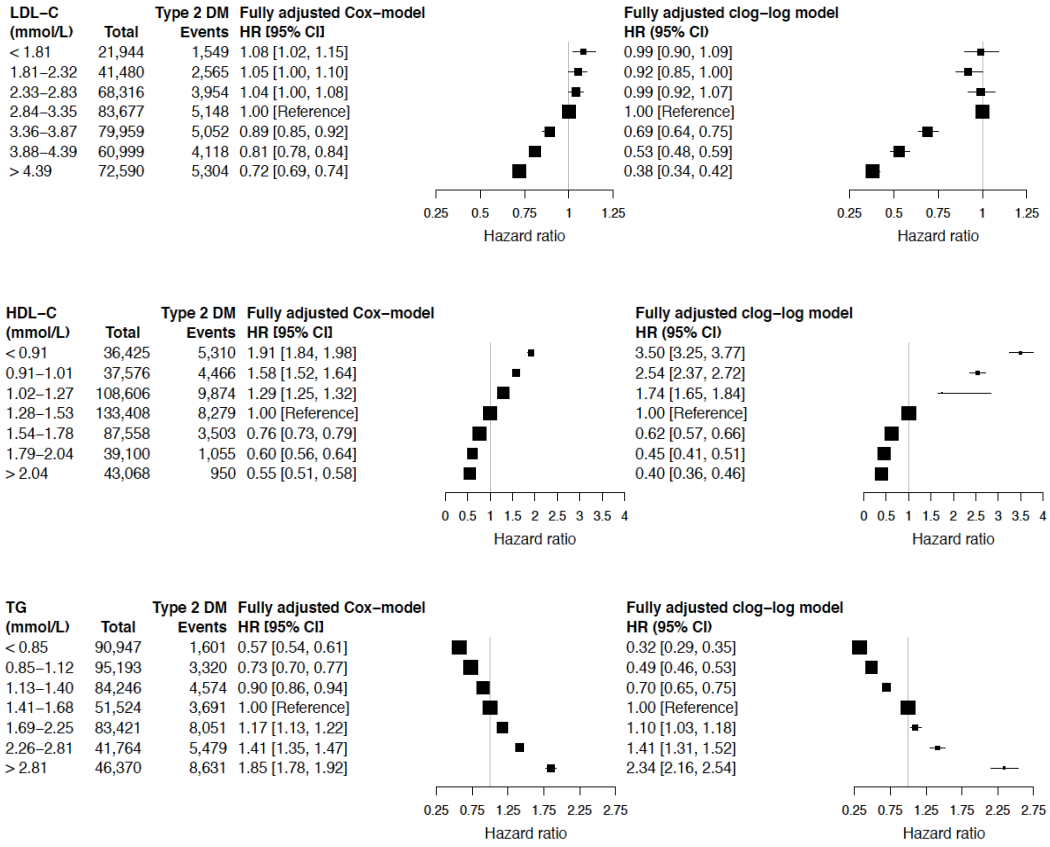


Figure S1 Comparisons of the results between a static population study design (Cox-model) and a dynamic population study design (clog-log model). The association between blood lipids and incidence of type 2 diabetes.

The effect of averaging LDL-C over a year and medication effect on the observational findings

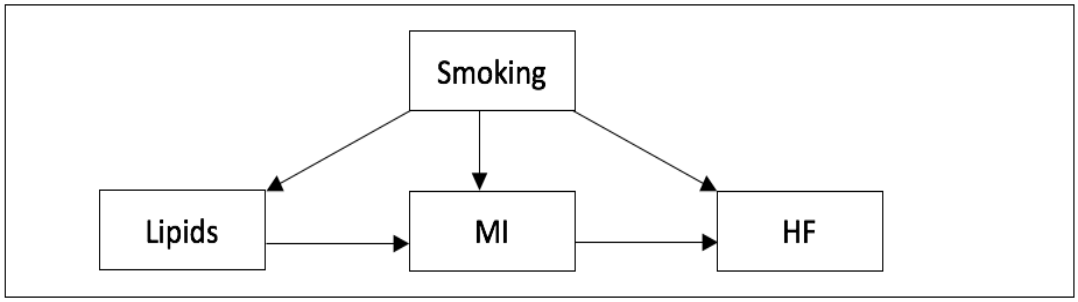
According to sensitivity analysis (see chapter 4 Figure 4-2, page 190, chapter 5 Figure S5-5 to S5-7, page 267 to 272, and chapter 6 Figure S6-4, page 330), average lipid levels over a year gave the similar results to the use of single values at baseline. As being explained in 4.1 that cohorts of my study had a relatively short follow-up period of around 5 years (median). Whereas, the median measurement of lipids in studied population is 2-3 times. Therefore, patients had their lipid measurements once in every 1.7-2.5 years, which is not very often, and a yearly average value should be close to a single value.

Concerning the medication effect, especially from statins use at baseline, I found that in multivariable adjustment model, the use of statins at baseline is significantly associated with the increased risk of both HF and AF. For example, patients who used statins at baseline lipid measurement is associated with the increased risk of HF by 54%, compared with individuals who did not use statins (HR 1.54 [95%CI 1.50-1.59]). However, this seems to be a result of reverse causation or confounders rather than the true causal effect because patients who were prescribed with statins must have underlying cardiometabolic risk factors, such as dyslipidaemia or diabetes, that increase the risk of HF.

However, patients who used statins at baseline were not excluded from the analysis. Instead, I performed the subgroup analysis of not using statins at baseline and compared the results with the main findings, which can be found from chapter 4 Figure 4-2 (page 190), chapter 5 Figure S5-5 to S5-7 (page 267 to 272), and chapter 6 Figure S6-4 (page 330).

The potential effect of collider bias on the observational findings

collider bias is the cause to concern in my work. This is because according to the causal diagram (Figure 1-1, page 45), risk factors are not collider (i.e., a variable in a path with both arrows pointing towards the variable since it is a common effect), which is logically sensible. Smoking, for example, is associated with lipid levels (e.g., increase LDL-C but decrease HDL-C levels) and can increase the risk of HF and AF. It is unlikely to be in the opposite direction that lipid levels, HF, or AF cause patients to smoke. Therefore, adjusting for smoking is necessary in order to close all paths in the causal diagram. On the other hand, intercurrent MI might be considered as collider since it shares a common effect with lipids and smoking. Therefore, we should not adjust for intercurrent MI otherwise the path can be opened and introduce collider bias. Below shows the simple direct acyclic graph (DAG) and each path as being described.



1. Lipids <----- Smoking -----> HF: This path should be closed by conditioning on smoking.
2. Lipids ------> MI ------> HF: This path should keep open as it is a causal effect of our interest.
3. Lipids <------ Smoking ------> MI ------> HF: This path should be closed by conditioning on smoking.
4. Lipids ------> MI <------- Smoking -------> HF: This path is already closed since it contains collider (MI). Therefore, this is no need for further conditioning. However, conditioning on MI will open this path and can introduce collider bias.

Mendelian randomisation

General concept of one- and two-sample MR

Mendelian randomisation (MR) is the use of genetic variatns in non-experimental data to make causal inferences about the effect of an exposure on an outcome.[299] It is a form of instrumental variable (IV) analysis that has been described as nature's or your god's randomised controlled trial (RCT) due to the random allocation nature of genetic variants at conception.[241] Random allocation properties of genetic instrument and the fact that genes always precede the outcome of interest make the use of genetic instrument can avoid the classical problem of residual confounders and reverse causation found in observational study.[44]

There are three fundamental conditions for a genetic variant to satisfy to be an IV, including i) the genetic variant is associated with the exposure, ii) the genetic variant is not associated with any confounder of the exposure-outcome association, and iii) the genetic variant does not affect the outcome, except possibly via its association with the exposure.[299]

One-sample MR is the use of individual-level genetic data from only one genome-wide association study (GWAS). Therefore, we can obtain the effect of the instrumental variable-risk factor association (ratio denominator) and instrumental variable-outcome association (ratio numerator) from the same sample of participants using either the ratio of coefficients method or two-stage least squares regression.[241]

Two-sample MR is further developed method that allows researchers to use just the summary genetic data (e.g., beta-coefficient and standard error from a regression model) from two different GWAS consortia. The assumptions of two-sample MR are similar to those of one-sample MR as being described earlier. Indeed, there are some advantages of using two different sets of participants. In particular 'winners' curse', which can underestimate true causal effects in one-sample MR, is unlikely to happen in two-sample MR. Moreover, unlike the impact of weak instrument bias in one-sample MR, which biases effects towards the confounded multivariable regression results, in two-sample MR, weak instrument bias is towards the null. The main advantage of using

summary data from GWAS consortia in two-sample MR is the increased statistical power, particularly in relation to testing effects on binary disease outcomes.[241]

However, there are some limitations of two-sample MR due to nature of summary-level data, which is often publicly available. First, it is not possible to check whether the genetic instruments are associated with observed confounders (i.e., assumption ii). Second, it is unlikely to be able to do subgroup analysis and effect moderation in two-sample MR without individual participant data. Third, we have to accept the potential bias from adjustment made in different GWAS consortia. Last, it is more complicated to conduct the test of non-linear effect, compared to using individual-level genetic data in one-sample MR.[241]

Causal inference and sensitivity analysis of 2-sample MR

In contrast to one-sample MR, various approaches have been developed to make a causal inference from two-sample MR. For single variant analysis, Wald ratio is mostly used. For multiple variants analysis, we can apply fixed/ random effect meta-analysis of Wald ratios, inverse variant weighted (IVW) linear regression, maximum likelihood method, MR-Egger, weighted median (WM) MR, or multivariate MR (MVMR) depending on the assumptions that can be less strictive and the nature of genetic instrument used.[300]

IVW-MR is the standard approach in which estimate of the causal effect combines the ratio estimates of multiple variants using the approximate variance just derived. It is a weighted average of slope estimates with no intercept term implying that there should not have an evidence of pleiotropic effect (i.e., assumption iii) and all genetic instrument must be valid (i.e, InSIDE assumption, see the next section). However, it is likely that genetic instrument can affect the outcome through other pathways rather than exposure of interest. Therefore, MR-Egger is a useful method to check and adjust for pleiotropy (if present) since it allows for the intercept term not to be zero[301] (i.e., in the linear regression plot between genes-exposure association on the X-axis and genes-outcome association on the Y-axis, the intercept not equal to zero means that if there is no association between genes and exposure (genes-exposure association is zero) but the association between genes and

outcome still exists (genes-outcome association is not zero), then genes affect the outcome through other pathways. However, MR-Egger is more sensitive to valid instruments that IVW method and can give a bias estimate if the InSIDE assumption is violated.

Weighted median (WM) MR is the method based on the idea that if the first set of genetic instruments (e.g., 50% of all included SNPs) are invalid IV and the second set are valid IV, then median ratio estimate can give an unbias results.[300] In addition, for most of genes that regulate more than one trait, such as genes that regulate LDL-C also affect HDL-C and TG, there is a method that controls for the effect of the genetic instrument on other traits the closely related to our exposure of interest called multivariate MR (MVMR).[300] The idea of MVMR is derived from the situation that suppose genes (SNPs) are associated with only known confounders, direct adjustment for that confounders could introduce a collider bias, and adjustment for genetically predicted confounders can avoid this bias. MVMR is useful for disentangling the effect of closely bound exposures, such as lipid traits. Moreover, the method still yields the robust and unbias results even in the situation that the InSIDE assumption is violated. A table below is a summary of various causal inference approach used in my thesis.

Approach	**Give an unbias estimates if violating**	
	Pleiotropic assumption	**InSIDE (valid IV) assumption**
IVW	No	No
MR-Egger	Yes	No
WMMR	No	Yes
MVMR	Can be Yes/No (depend on what has been adjusted for)*	Yes

*For example, if there is a biological pathway that links between genes that regulate LDL-C and HF through HDL-C, then adjusting for genetically predicted HDL-C can give an unbias estimates. However, if the pleiotropy occurs in other pathways but is not accounted for, then MRMR will give a bias estimation.

Assessment for pleiotropic effect

In my thesis, the test for horizontal pleiotropy has been conducted using 2 methods: Cochrane's Q test, and MR-Egger test. The idea of MR-Egger has been described earlier (please see the subsection "*Causal inference and*

sensitivity analysis of 2-sample MR", page). A significant Q test implies horizontal pleiotropy and significant Egger intercept further implied a directional pleiotropy. If:

- Q test shows significant p-value of < 0.05 but Egger intercept shows no significance (P > 0.05, 95%CI includes zero), it can be interpreted as existing balanced pleiotropy, which IVW can provide an unbiased estimate as long as the InSIDE assumption is met.
- Q test > 0.05 -> no pleiotropy (assume an appropriate weight order used in IVW-MR).
- Q test < 0.05 and significant Egger intercept -> existing directional pleiotropy, then MR Egger might be more appropriate than IVW-MR.

The InSIDE assumption

Due to the fact that the underlying MR models assumed a linear dose response, the instrumental variable effect estimates must be independent of the exposure effect in MR analysis (i.e., the Instrument Strength Independent of Direct Effect [InSIDE] rule). The null hypothesis that the instrumental variable effect (derived from the ratio of outcome to exposure) estimates for the SNPs in an instrument were independent of the exposure (lipid) effect estimates for the same SNPs for all outcomes of interest was tested and shown as correlation coefficients. The significant correlation coefficients between IV effect and exposure effect suggested the violation of the InSIDE assumption. This approach of checking InSIDE assumption has been successfully implemented in previous work by White *et al*.[62] In all scenarios, the InSIDE assumption was satisfied (Table S 4-5, Table S 5-9, and Table S 6-7).

Power calculation in two-sample MR

For power calculation, we followed the method explained by Brion *et al* (http://cnsgenomics.com/shiny/mRnd/) and Hermani *et al*.[223] By using the total number of individuals, proportion of cases, estimates R^2 and the odds ratio observed from the results, we could calculate the power of statistics to detect

the difference from at least, or more extreme, the magnitude (odds ratio) we observed at a two-sided alpha value of 5% (Table S 4-4, Table S 5-8, and Table S 6-6).

For example, we would like to calculate the power at a two-sided alpha value of 5% from two-sample MR from which there were 1,000 cases and 100,000 controls from the outcome consortium (UK Biobank in my thesis). We found that the overall R^2 between SNPs and exposure was 0.066 and the overall strength of the association (e.g., odds ratio) was 0.70. Statistical power can be calculated as shown in the following R-script, which has been shown that we have 82% of power to detect at least the given difference.

```
ncases<- c(1000) #number of cases in the outcome consortium
ncon<-c(100000) #number of controls in the outcome consortium
r2  = c(0.066) #r2 for SNPs-exposure, which was calculated from:
#mutate(T = (beta.exposure^2)/(se.exposure^2)) %>%
#mutate(r2 = 1-exp(-T/samplesize.exposure))
#When:
#beta.exposure = beta coefficient of each SNP in an exposure consortium
#se.exposure = standard error of each SNP in an exposure consortium
#samplesize.exposure = sample size of each SNP in an exposure consortium

ratio = ncases/ncon
sig = 0.05
b1 = c(log(1/0.70))
#b1 = log odds ratio of the outcome
#please noted that b1 must be flipped to always be > 1
n = ncases+ncon

#power is then calculated by the following formulae:

pnorm(sqrt(n*r2*(ratio/(1+ratio))*(1/(1+ratio)))*b1-qnorm(1-sig/2))
```

```
## [1] 0.8220736
```