UNIVERSITY COLLEGE LONDON

Public Health Applications of Cardiovascular Genomics

by

Michael Vaclav Holmes

A thesis submitted in partial fulfillment for the degree of Doctor of Philosophy

in the

UCL Faculty of Population Health Sciences
Department of Epidemiology and Public Health

January 2014

Declaration of Authorship

I, MICHAEL VACLAV HOLMES, declare that this thesis titled, 'Public Health Applications of Cardiovascular Genomics' and the work presented in it are my own. The work contributing to the thesis involved large-scale collaborations. I can confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

For Chapter 2 (*CYP2C19* Genotype, Clopidogrel and Cardiovascular Disease),

- The project originated from work performed for my Masters thesis in Epidemiology at London School of Hygiene and Tropical Medicine. Since then, I repeated the literature search, updated the original meta-analysis, extended the analyses for new traits and meta-analysed pharmacogenetic subgroup analyses of randomized trials. Thus the analysis pertaining to "effect-modification" that I present in this thesis is completely novel and was not reported in the original Masters thesis. The findings reported in this Chapter are therefore substantially different to the original Masters thesis (which is available on request).

For Chapters 3 to 5 (Secretary Phospholipase A_2 and Cardiovascular Disease: Background),

- Dr. Jackie Cooper (UCL) analysed the dataset for the association of the six tagging single nucleotide polymorphisms (SNPs) in *PLA2G2A* with $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity (under my direction). This analysis led to the selection of the lead SNP for Mendelian randomization analysis

- I worked with Dr. Jackie Cooper (UCL) in synthesizing the Stata analysis script, which was used for analysis of individual participant data (IPD) in the collaborating studies. One figure was adapted from a Stata script originally written by Dr. Daniel Swerdlow (with his kind permission). Dr. Tom Palmer (University of Warwick) kindly provided the Stata do-file to conduct the instrumental variable analysis using summary-level data. All additional Stata scripts for observational, genetic and meta-analyses were written by me.

- I analysed individual participant data in BRHS, BWHHS, CYPRUS, GENDEMIP, EAS, FAST-MI, GRACE-Scotland, IMPROVE and TPT.

- For the following collaborating studies, analysts executed the Stata script remotely for the genetic analysis and I provided support via email: AMC-PAS (Dr. Eric van Iperen); BHF-FHS and GRAPHIC (Dr. Chris Nelson); CCHS (Dr. Christiane Lundegaard Haase), EPIC Netherlands (Dr. Folkert Asselbergs) EPIC Norfolk, GRACE-France, UDACS, Whitehall II (Dr. Jackie Cooper): GENDER (Dr. Jeffrey Verschuren); IHCS (Ms. Kimberly Brunisholz); KAROLA (Dr. Dietrich Rothenbacher); LIFE (Dr. Markus Scholz); MedStar and PennCath (Dr. Mingyao Li); MERLIN and PROVE-IT (Dr. Marc Sabatine); PREVEND (Dr. Pim van der Harst); PROSPER (Dr. Stella Trompet); Rotterdam (Dr. Abbas Dehghan); UCP (Mr. Maarten Leusink). For CURE and PROCARDIS, summary estimates for the main cardiovascular outcomes were provided by Dr Guillaume Pare and Professor Martin Farrell, respectively.

- The analysis of the association between *PLA2G2A* and mRNA in ASAP was conducted by Dr. Lasse Folkerson (Karolinksa Institute) using his own statistical software.

- The literature search and data abstraction for randomized trials of $sPLA_2$ lowering therapies were performed by Dr. Holly Exeter under my supervision.

- The observational analyses were conducted by me for all studies except GRACE-France and MIRACL, for which Miss Salma Kotti and Warren Bao ran the analysis using Stata code that I wrote.

- All meta-analyses of observational studies, genetic studies and randomized trials were conducted by me using Stata scripts that I wrote.

For Chapters 6 to 7 (Alcohol and cardiovascular disease):

- I worked with Dr. Caroline Dale (LSHTM) in synthesizing the Stata scripts used for analyses of individual participant data in each of the collaborating studies.

- Dr. Caroline Dale (LSHTM) wrote the Stata script for the observational analyses
- I wrote the Stata scripts for all meta-analyses of genetic associations with the exception of the Stata do-file for instrumental variable analysis, which was provided by Dr. Tom Palmer (University of Warwick)
- Dr. Chris Finan (UCL) conducted the linkage disequilibrium analysis under my guidance
- Dr. David Prieto (LSHTM) kindly advised on the method to obtain R^2 estimates for the observational analysis in the pooled individual participant dataset
- I conducted genotyping of individuals in Cyprus and TPT datasets using the ABI® TaqMan platform at the UCL Centre for Cardiovascular Genetics under the supervision of, and with the assistance of, Jutta Palmen and Philip Howard.
- I analysed individual participant data (IPD) in ARIC, BRHS, BWHHS, CaPS, CARDIA, CCHS, CFS, CHS, Czech post-MONICA, EAS, ELSA, EPIC-NL, EPIC-Turin, FHS, HAPIEE-CZ, HAPIEE-LT, HAPIEE-PL, HAPIEE-RU, IMPROVE, Inte99, Izhevsk, MESA, MDC, NORDIL, NPHS-II, Whitehall II, WHI, SMART, TPT and ULSAM.
- For the following studies, analysts executed the Stata do-file and/or provided summary estimates under guidance from me and/or Caroline Dale: Copenhagen General Population Study: Debbie Lawlor; EPIC Norfolk: Zheng Ye; EPIC-Potsdam: Dagmar Drogan; DCH: Janne Tolstrup; HPFU/NHS: Shanshan Li; HIMS: Kieran McCaul; InterAct: Stephen Sharp; ISGS-SWISS: ; MRC NSHD: Andrew Wong; MRC 1958BC: Alana Cavadino; NHANES III: Ajay Yesupriya; Portuguese stroke: Astrid Vicente; PREVEND: Pim van deer Hurst; PROCARDIS: Professor Martin Farrell; PROSPER: Stella Trompet; Rotterdam: Abbas Dehghan; UCP: Maarten Leusink.

Signed: _____

Date: _____

UNIVERSITY COLLEGE LONDON

Abstract

UCL Faculty of Population Health Sciences
Department of Epidemiology and Public Health

Doctor of Philosophy

by Michael Vaclav Holmes

Background

Genetic epidemiology is at the interface of translational and basic research and the pace of progress has been unprecedented, with findings representing some of the most robust available in the Scientific literature. However, how we can translate this high-fidelity genomic information into improvements in health of the population? Two distinct translational opportunities include personalized medicine (pharmacogenetics) and using Mendelian randomization to investigate disease aetiology to inform public health policy and develop new therapies.

Objectives

In this PhD thesis, I investigated the evidence base underlying the well-publicized use of the pharmacogenetic biomarker *CYP2C19* genotype to predict the response to clopidogrel, a widely prescribed antiplatelet drug. Second, I used Mendelian randomization to investigate the role of an *endogenous* biomarker, secretory phospholipase A_2-IIA (sPLA$_2$-IIA), thought to be a pro-atherogenic enzyme and a potential drug target for the prevention of cardiovascular disease (CVD). Third, I used Mendelian randomization to investigate the relationship between alcohol, an *exogenous* exposure, and cardiovascular traits and disease events.

Results

CYP2C19 and cardiovascular disease
I identified 32 studies of 42,016 patients reporting 3545 CVD events. Only 6 studies were set within randomized trials ("effect-modification" design) and the remaining 26 reported individuals exposed to clopidogrel ("treatment-only" design). In treatment-only studies, possession of one or more ⋆2-⋆8 *CYP2C19* alleles was associated with

lower cytochrome P450 C19 (CYP2C19) enzyme activity and a higher risk of CVD events (RR 1.18; 95%CI:1.09, 1.28), however, there was strong evidence of small-study bias (Harbord test P=0.001) and, when restricted to large studies (≥200 events), the association of *CYP2C19* ⋆2-⋆8 carrier status with CVD was null (RR 0.97; 95%CI: 0.86, 1.09). In the effect-modification studies, *CYP2C19* genotype did not modify the effect of clopidogrel on CVD end-points. These findings cast doubt on whether information on *CYP2C19* genotype would be helpful to guide selection of the dose of clopidogrel or use of an alternative antiplatelet agent.

The role of secretory phospholipase A_2-IIA ($sPLA_2$-IIA) in CVD
I used Mendelian randomization to make causal inference on the role of $sPLA_2$-IIA in CVD. I identified a single nucleotide polymorphism (SNP) in *PLA2G2A* (rs11573156) that was specific for and had a very strong impact on circulating levels of the $sPLA_2$-IIA isoform. Using data from 36 studies and over 100,000 participants, instrumental variable analysis found no association between $sPLA_2$-IIA with incident, prevalent or recurrent CVD events. These findings suggest $sPLA_2$-IIA is not a valid therapeutic target for CVD prevention, which was in keeping with a phase III randomized clinical trial that was halted for futility in 2012 (during this thesis).

Alcohol and CVD
I used a SNP in *ADH1B* to investigate the relationship between alcohol and coronary heart disease (CHD) in >260,000 participants. The genetic variant (*ADH1B* rs1229984 A-allele) showed very strong association with reduced alcohol consumption when evaluated as volume of alcohol consumed, binge drinking and abstaining from alcohol. The A-allele of rs1229984 showed associations with SBP, CRP, IL-6, BMI and waist circumference that were all directionally concordant with a reduced risk of CHD. Indeed, when the clinical outcome CHD was investigated, individuals carrying the A-allele (who consumed less alcohol than non-carriers) had a reduced risk of CHD at all levels of alcohol consumption. No evidence of a cardioprotective association of alcohol with CHD was identified.

Conclusions My investigation into use of *CYP2C19* genotype as a pharmacogenetic biomarker for clopidogrel response did not identify evidence to support its clinical use and limitations were identified that could apply to other pharmacogenetic tests. Use of Mendelian randomization revealed no evidence to support a causal role of $sPLA_2$-IIA in CVD, which paralleled findings from a phase III randomized clinical trial, and provides support for the use of Mendelian randomization studies more widely to inform drug development. Finally, using the *ADH1B* gene to interrogate the relationship

of alcohol yielded findings that argue against a cardioprotective effect of alcohol consumption. These findings should encourage rethinking of public health advice about the cardiovascular benefits of moderate levels of alcohol consumption.

Acknowledgements

I am incredibly grateful to my supervisors, work colleagues, family and friends who have helped make this thesis possible. I particularly wish to thank:

- my supervisors Juan P Casas, Aroon Hingorani and Mika Kivimaki, who have provided fantastically exciting projects, excellent supervision, intellectual stimulation and unparalleled opportunities to enrich my research skills through development of epidemiological concepts, statistical techniques and orchestrating international collaborations. Opportunities to travel to Philadelphia, Paris and Edinburgh to conduct work related to the projects reported in this thesis greatly enriched my skills and provided invaluable experiences,

- Philippa Talmud, Caroline Dale and Daniel Swerdlow who I have had the pleasure to work with and who have become close work colleagues and friends. I thank them for their invaluable guidance and support through the 4 years of my fellowship,

- Brendan Keating for help with coordinating collaborations, Tom Palmer, Richard Silverwood and Frank Dudbridge for invaluable statistical guidance, Lasse Folkersen for help with mRNA analyses and Steve Humphries for support,

- the UCL/LSHTM Genetic Epidemiology Group members with whom it has been a pleasure to work,

- Chris Finan, Tina Shah and Jorgen Engmann with whom I've had the delight of sharing an open-space office, and in the process enriched my skills for barefoot-running (thanks Chris), baking (thanks Tina) and tea-making (thanks Jorgen)!

- the studies, their contributing scientists and clinicians and the patients/volunteers who contributed their time and efforts to establishing the datasets that enabled the analyses I report in this thesis,

- my family, including my supportive and long-suffering partner Aysha Khanom, my mother Marie, sister Anna and brothers Oliver and Alex (and their respective wonderful families). I thank them wholeheartedly for putting up with me over the years and for accepting and supporting the sacrifices I have made in order to develop my academic career. And finally, my late father, Oliver Holmes, who inspired me to follow in his academic foot-steps. How I cherish the days I spent with him learning neurophysiology in his lab in Glasgow University!

Publications

Original work related to the thesis

Holmes MV*, Dale CE* and Casas JP† (on behalf of the *ADH1B*-CVD collaboration) *ADH1B* genotype, alcohol and cardiovascular disease *Under review at British Medical Journal*

$\star$ joint first authors

† senior author

Holmes MV*, Simon T*, Exeter HJ*, Hingorani AD†, Sabatine MS†, Mallat Z†, Casas JP† and Talmud PJ† (on behalf of the sPLA$_2$ sleuths). Secretory Phospholipase A$_2$-IIA and Cardiovascular Disease: a Mendelian randomization study. *J Am Coll Cardiol.* 2013;62(21):1966-76

$\star$ joint first authors

† joint senior authors

Holmes MV, Perel P, Shah T, Hingorani AD and Casas JP. *CYP2C19* genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA.* 2011;306(24):2704-14

Original work not reported in this thesis

Swerdlow DI, Holmes MV, Kuchenbaecker K, et al. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet.* 2012;379(9822):1214-24.

Holmes MV, Newcombe P, Hubacek JA, et al. Effect modification by population dietary folate on the association between *MTHFR* genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet.* 2011;378(9791):584-94.

Review articles/Editorials

Holmes MV, Casas JP, Hingorani AD. Putting genomics into practice. *BMJ.* 2011;343:d4953. doi: 10.1136/bmj.d4953.

Holmes MV, Harrison S, Talmud PJ, Hingorani AD, Humphries SE. Utility of genetic determinants of lipids and cardiovascular events in assessing risk. *Nat Rev Cardiol.* 2011;8(4):207-21.

Invited Lectures

"Using alcohol genes as instruments: the *ADH1B*-CVD collaboration" (July 12, 2011) *Rank Prize Funds Mini-Symposium on Alcohol and Nutrition*, Lake District, UK

"Translating Genes: Pharmacogenetics and Clinical Trials." (September 25, 2012) *Childrens Hospital of Philadelphia: Genes, Genomics & Pediatric Disease Affinity Group*, Philadelphia, PA

"Ask the Experts: 2C or Not 2C: Should Genotype Influence Antiplatelet Therapy Prescription?" (November 5, 2012) *AHA Scientific Sessions*, Los Angeles, CA

"Beating Heart Disease: Total Eclipse of the Heart" (June 29, 2013) *Medical Research Council (MRC) Centenary Science Fair*, London

"Crash Course in Epidemiology" (August 21, 2013) *International Atherosclerosis Research School*, Prague, Czech Republic

Contents

Declaration of Authorship i

Abstract iv

Acknowledgements vii

Publications viii

List of Figures xviii

List of Tables xxiii

List of Abbreviations xxv

1 Introduction 1

1.1 Cardiovascular Disease: Public Health Importance . . . 1
1.2 Public health approaches to combating CHD . . . 2
1.2.1 Non-pharmacological approaches to CVD prevention . . . 2
1.2.2 Pharmacological approaches to CVD prevention . . . 3
1.3 Trends in CVD - changing incidence . . . 5
1.4 Quest to identify causal risk factors for CVD . . . 6
1.5 Study designs available to investigate role of biological variables . . . 6
1.5.1 Cell lines and animal models . . . 6
1.5.2 Observational studies in Humans . . . 8
1.6 Randomization of exposure . . . 11
1.7 Mendelian Randomisation . . . 13
1.7.1 Gregor Mendel . . . 13
1.7.2 History of Mendelian Randomization: first examples . . . 13
1.7.3 Comparison to Randomized Trial . . . 14
1.7.4 Classification and Application . . . 15
1.7.5 Mendelian triangulation . . . 16
1.7.6 Assumptions . . . 17
1.7.7 Potential Limitations . . . 19

1.8 Using genetic information to guide prescription of medicines (pharmacogenetics) . . . 23
1.8.1 Traditional analysis of "effect modification" . . . 24
1.9 Thesis Themes . . . 25

2 *CYP2C19* Genotype, Clopidogrel and Cardiovascular Disease 26
2.1 Introduction . . . 26
2.1.1 Principles of pharmacogenetics . . . 26
2.1.2 Clopidogrel and Coronary Heart Disease . . . 28
2.1.3 Clopidogrel metabolism . . . 29
2.1.4 GWAs of clopidogrel response . . . 29
2.1.5 Technological advances enabling pharmacogenetic analyses . . . 30
2.2 Aims . . . 30
2.3 Methods . . . 30
2.3.1 Search Strategy . . . 30
2.3.1.1 Systematic review of randomized trials of more vs less clopidogrel . . . 31
2.3.2 Data extraction . . . 31
2.3.3 Grouping of *CYP2C19* for genetic analyses . . . 32
2.3.4 Cardiovascular outcomes . . . 32
2.3.5 Clopidogrel drug metabolite . . . 32
2.3.6 Analyses . . . 32
2.4 Results . . . 33
2.4.1 Study designs, genotyping and reported outcomes . . . 33
2.4.2 Outcomes evaluated . . . 35
2.4.3 Effects of *CYP2C19* genotype on clopidogrel metabolites and platelet reactivity . . . 35
2.4.4 Association of *CYP2C19* genotype and the composite CVD outcome on treatment-only analysis . . . 41
2.4.4.1 Assessment for risk of bias . . . 42
2.4.5 Association of *CYP2C19* genotype and the individual outcomes on treatment-only analysis . . . 44
2.4.6 Investigation of a genetic dose-response relationship . . . 45
2.4.7 Effect-modification analysis . . . 48
2.5 Discussion . . . 50
2.5.1 Problems specific to pharmacogenetic studies . . . 50
2.5.2 Assumptions of the "treatment-only" study design in PGx . . . 51
2.5.3 Scenarios of "treatment-only" studies in pharmacogenetics . . . 53
2.5.4 Implausible effect sizes . . . 55
2.5.5 Separating mechanism-based intended effects from adverse effects . . . 56
2.5.6 Limitations of the star allele nomenclature . . . 58
2.5.7 Problems recognized in other fields . . . 58
2.5.8 Placing these findings into context . . . 58
2.6 Conclusions . . . 60

3 Secretary Phospholipase A_2 and Cardiovascular Disease: Background 62
3.1 Introduction . . . 62
3.1.1 Atherosclerosis as the substrate of Coronary Heart Disease . . . 62

3.1.1.1 Lipid theory of atherosclerosis . . . 62
3.1.1.2 Inflammation theory of atherosclerosis . . . 63
3.1.2 Inflammation and Coronary Heart Disease . . . 64
3.1.3 The interface between lipids and inflammation . . . 65
3.1.4 Phospholipase enzymes . . . 66
3.1.5 Phospholipase A_2 enzymes . . . 67
3.1.6 Secretory phospholipase A_2-IIA . . . 68
3.1.7 Divergent roles of $sPLA_2$-IIA . . . 69
3.1.8 Biological plausibility of $sPLA_2$-IIA in atherosclerosis . . . 71
3.1.9 Experimental association of $sPLA_2$ with CHD in animal studies . . . 71
3.1.10 Observational association of $sPLA_2$ with CVD in humans . . . 72
3.1.11 GWAs studies of CHD . . . 73
3.1.12 Drug development: $sPLA_2$ as a drug target . . . 73
3.1.13 Varespladib . . . 74
3.1.14 Animal studies of varespladib efficacy . . . 75
3.1.15 Safety of varespladib in humans: data from phase II randomized trials . . . 76
3.1.16 Treatment trials of varespladib for CV efficacy: phase III RCTs . . . 76
3.1.17 Genetic variants encoding $sPLA_2$-IIA . . . 77
3.1.18 Use of a *PLA2G2A* genetic variant for Mendelian randomization . . . 77
3.2 Aims . . . 78

4 $sPLA_2$ and Cardiovascular Traits and Events: Observational Analysis 81
4.1 Methods . . . 81
4.1.1 General overview of studies contributing towards the observational associations between $sPLA_2$ and CHD events . . . 81
4.1.2 Measurement of $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity . . . 81
4.1.3 Distribution of $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity . . . 85
4.1.4 Correlation between $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity . . . 85
4.1.5 Association of $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity with cardiovascular traits and potential confounders . . . 86
4.1.6 Association of $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity with cardiovascular events . . . 86
4.1.7 Shape of the association between $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity with cardiovascular traits and clinical events . . . 86
4.1.8 Multivariate analysis to investigate the association of $sPLA_2$ traits and CHD . . . 87
4.2 Results . . . 88
4.2.1 Correlation between $sPLA_2$ mass and $sPLA_2$ enzyme activity . . . 88
4.2.2 Observational Analysis of $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and cardiovascular risk factors . . . 88
4.2.3 Shape of the association between $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and cardiovascular events . . . 92
4.2.4 Observational Analysis of $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and cardiovascular events . . . 92
4.3 Discussion . . . 95
4.3.1 Correlation between $sPLA_2$ traits . . . 95
4.3.2 Association between $sPLA_2$ traits and cardiovascular traits . . . 95

4.3.3 Association between sPLA$_2$-IIA mass and vascular events 96
4.3.4 Association between sPLA$_2$ enzyme activity and vascular events . 97
4.3.5 Independent association between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and vascular events 98
4.4 Conclusions 98

5 Randomized Evidence on sPLA$_2$ and Cardiovascular Disease based on Genetic Studies and Trials 100
5.1 Methods 101
5.1.1 General overview of studies participating in the sPLA$_2$ collaboration 101
5.1.2 Genetic analysis 101
5.1.3 Selection of the genetic instrument and evaluation of its specificity and effect size 102
5.1.3.1 SNP selection 102
5.1.3.2 Specificity of genetic instrument for *PLA2G2A* expression 102
5.1.3.3 Strength of genetic instrument (rs11573156C>G) on sPLA$_2$ 104
5.1.3.4 Association between genetic instrument and putative and established cardiovascular risk factors 104
5.1.4 Cardiovascular outcomes 104
5.1.5 Association between genetic instrument and major vascular events 105
5.1.5.1 Genetic association analysis 105
5.1.5.2 Instrumental variable analysis 105
5.1.6 Systematic review of randomized trials of varespladib 106
5.2 Results 108
5.2.1 Selection and validation of the genetic instrument for sPLA$_2$-IIA . 108
5.2.2 Association of rs11573156 with sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity 108
5.2.3 Systematic review and meta-analysis of the effects of varespladib on circulating sPLA$_2$-IIA, sPLA$_2$ enzyme activity and cardiovascular traits from clinical trials 110
5.2.4 Contextualising the genetic effects on sPLA$_2$-IIA with the sPLA$_2$ inhibitor varespladib 115
5.2.5 Association between rs11573156 with cardiovascular risk factors and carotid atherosclerosis 115
5.2.6 Association between rs11573156 and cardiovascular outcomes in general population studies 115
5.2.7 Association between rs11573156 and recurrent cardiovascular events in patients with acute coronary syndrome 117
5.2.8 Extreme genotype comparison 117
5.2.9 Association between rs11573156 and other cardiovascular events . 117
5.2.10 Instrumental variable analysis of sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and CVD 126
5.2.10.1 General population studies 126
5.2.10.2 Acute Coronary Syndrome studies 127
5.3 Discussion 129
5.3.1 SNP selection 129
5.3.1.1 Specificity of genetic instrument for sPLA$_2$-IIA mass . . 129
5.3.1.2 Strength of genetic instrument on sPLA$_2$-IIA mass 129

5.3.2 Comparison of findings from genetic association analysis with findings from observational analysis for continuous traits . . . 130
5.3.3 Comparison of findings from genetic association analysis with findings from observational analysis for major vascular events . . . 130
5.3.4 Placing these findings into context of the VISTA-16 phase III clinical trial . . . 131
5.3.5 Lack of association of $sPLA_2$ with MVE on observational analysis in ACS cohorts and implications for drugs targeting $sPLA_2$ for CVD prevention . . . 132
5.3.6 Limitations . . . 132
5.3.7 Comparison to other Mendelian randomization studies of $sPLA_2$ . . . 133
5.3.8 Implications for future Mendelian randomization work in $sPLA_2$ traits . . . 133
5.4 Conclusion . . . 134

6 Alcohol and cardiovascular disease 135
6.1 Introduction . . . 135
6.1.1 Alcohol consumption and the global burden of disease . . . 135
6.1.2 Alcohol and coronary heart disease . . . 136
6.1.3 Influence of purported effects of alcohol on CHD on UK governmental guidance . . . 138
6.1.4 Limitations of observational evidence . . . 139
6.1.4.1 The J-shaped curve . . . 140
6.1.5 Experimental and interventional studies . . . 141
6.1.6 Ideal study design . . . 142
6.1.7 Genetic contribution to alcohol consumption . . . 142
6.1.8 Use of Mendelian randomization to investigate alcohol consumption 143
6.1.9 Metabolism of alcohol . . . 144
6.1.10 Alcohol dehydrogenase (ADH1B) . . . 144
6.1.10.1 Association of *ADH1B* rs1229984 with alcohol indices . . . 145
6.1.10.2 Association of *ADH1B* rs1229984 with recognized associations of alcohol . . . 145
6.1.11 Use of *ADH1B* rs1229984 to investigate CHD . . . 146
6.1.12 Consideration of worldwide *ADH1B* genotype frequency . . . 147
6.1.13 Mendelian randomization investigations of alcohol and human health using *ALDH2* genotype . . . 148
6.2 *ADH1B*-CVD project . . . 149
6.2.1 Rationale . . . 149
6.2.2 Aims . . . 149

7 *ADH1B*-Cardiovascular Disease Collaboration: Overview and Observational Associations between Alcohol and Cardiovascular Traits 151
7.1 Introduction . . . 151
7.2 Methods . . . 151
7.2.1 International collaboration . . . 151
7.2.2 Measures of alcohol consumption . . . 152
7.2.3 Data handling . . . 152
7.2.4 Statistical analysis . . . 153

7.2.5 Proportion of variance explained by alcohol . . . 153
7.3 Results . . . 154
7.3.1 Availability of traits and proportion of variance of traits explained by alcohol on observational analysis . . . 155
7.3.2 Observational analysis between alcohol consumption and cardiovascular biomarkers . . . 155
7.4 Discussion . . . 161
7.5 Conclusion . . . 162

8 *ADH1B*-Cardiovascular Disease Collaboration: Genetic and Instrumental Variable Associations 163
8.1 Methods . . . 163
8.1.1 Genetic analysis . . . 163
8.1.2 Meta-analysis of rs1229984 A-allele and cardiometabolic events . . . 164
8.1.3 Subgroup analyses . . . 165
8.1.3.1 Investigation of subgroup analyses using metaregression . . . 166
8.1.4 Sensitivity analysis . . . 166
8.1.5 Investigating potential for confounding by population stratification 166
8.1.6 Instrumental variable analysis . . . 167
8.1.6.1 Linear instrumental variable meta-analysis . . . 167
8.2 Results . . . 169
8.2.0.2 Studies included in the collaboration . . . 169
8.2.1 Allele frequency of *ADH1B* rs1229984 SNP . . . 169
8.2.2 Association of *ADH1B* with alcohol phenotypes . . . 169
8.2.3 Association of *ADH1B* rs1229984 with cardiovascular biomarkers . . . 170
8.2.4 Association of *ADH1B* rs1229984 and lifestyle factors . . . 174
8.2.5 Association of *ADH1B* rs1229984 and coronary heart disease . . . 178
8.2.6 Association between *ADH1B* rs1229984 with stroke (combined subtypes and ischaemic-only) and diabetes . . . 178
8.2.7 Stability of summary estimates to choice of meta-analysis model . . . 180
8.2.8 Stability of summary estimates to adjustment for population structure . . . 180
8.2.9 *In silico* analysis of potential for confounding between *ADH1B* rs1229984 with other SNPs identified from prior GWAs . . . 181
8.2.10 Causal analysis of alcohol on cardiovascular traits and events . . . 183
8.3 Discussion . . . 185
8.3.1 Validation of *ADH1B* rs1229984 as a proxy for alcohol intake . . . 185
8.3.2 Genetic association between rs1229984 and cardiovascular traits . . . 185
8.3.3 Expected effect of alcohol on risk of CHD . . . 186
8.3.4 Potential explanations for the absence of association of *ADH1B* rs1229984 with HDL-C and other traits . . . 186
8.3.5 Association of *ADH1B* with lifestyle factors . . . 187
8.3.6 Instrumental variable analysis . . . 188
8.3.7 Contrast with other Mendelian randomization studies . . . 188
8.3.8 Contrast with existing data and implications for interpretation . . . 188
8.4 Conclusions . . . 189

9 Discussion and Conclusions 190

9.1 Introduction . . . 190
9.2 Reaping the rewards of genomic research . . . 190
9.2.1 Pharmacogenetics . . . 190
9.2.2 Mendelian randomization . . . 192
9.2.3 Mendelian randomization for drug target validation . . . 192
9.3 Mendelian randomization for casual analysis of environmental exposures . 193
9.4 Future aims for Mendelian randomization . . . 195
9.4.1 Multiple SNPs . . . 195
9.4.1.1 Pushing the frontier: multiple SNPs to investigate putative epidemiological interactions . . . 195
9.4.2 Non-linear Mendelian randomization . . . 196
9.5 Conclusions . . . 196

A Supplemental Information for *CYP2C19* and clopidogrel response 198
A.1 Supplementary Methods . . . 198
A.2 Search strategy for identifying studies in the systematic review . . . 198
A.2.1 PubMed Search String . . . 198
A.2.1.1 MeSH Search . . . 198
A.2.1.2 Text Word search . . . 199
A.2.2 EMBASE Search String . . . 199
A.2.2.1 Expanded (exp) headings search . . . 199
A.2.2.2 Text word search . . . 199
A.3 Supplementary Tables . . . 200
A.4 Supplementary Figures . . . 211

B Supplemental Information for $sPLA_2$ and CVD 215
B.1 Definition of myocardial infarction and stroke in the collaborating studies 215
B.1.1 General Population Studies . . . 215
B.1.2 Acute Coronary Syndrome studies . . . 217
B.1.3 Other studies . . . 218
B.2 Supplementary Tables . . . 219
B.3 Supplemental Figures . . . 232
B.4 VISTA-16 Summary from Anthera Pharmaceuticals . . . 234

C Supplemental Information for *ADH1B*, alcohol and cardiovascular disease 240
C.1 Studies included in the *ADH1B* collaboration . . . 240
C.2 Supplemental Tables . . . 256
C.3 Supplemental Figures . . . 285
C.4 Analysis plan . . . 305
5. *ADH1B* invitation letter . . . 305

D Published papers 313
1. *CYP2C19* Genotype, Clopidogrel Metabolism, Platelet Function, and Cardiovascular Events A Systematic Review and Meta-analysis (JAMA) . . . 313

2. Putting genomics into practice (BMJ) 325
3. Effect modification by population dietary folate on the association between *MTHFR* genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials (Lancet) 327
4. Utility of genetic determinants of lipids and cardiovascular events in assessing risk (Nature Reviews Cardiology Review) 338
5. Secretory Phospholipase A2-IIA and Cardiovascular Disease: A Mendelian Randomization Study (JACC) 353

References **364**

List of Figures

1.1 Relationship between LDL-cholesterol and total cholesterol with risk of coronary heart disease. . . . 4
1.2 Trend in mortality from coronary heart disease in high-income countries . 5
1.3 The Epidemiological transition. . . . 5
1.4 Study methods used in Science to investigate biological mechanisms underlying disease processes. . . . 7
1.5 Possible scenarios for an association between an exposure and outcome . . 10
1.6 Comparison of Randomized trial to Mendelian randomization 12
1.7 Gregor Mendel, the "founding-father" of modern genetics 13
1.8 Drug development pipeline 15
1.9 Mendelian randomization concept 18
1.10 Potential confounding of Mendelian randomization studies 21
1.11 Comparison of differential treatment response based on absolute difference or relative difference 24
1.12 Schemata of pharmacogenetics 25

2.1 Contrast between pharmacodynamic and pharmacokinetic. 27
2.2 Characteristics of previous pharmacogenetic studies 28
2.3 PRISMA flow diagram for systematic review of *CYP2C19* and clopidogrel response. 34
2.4 Relationship between *CYP2C19* genotype and active drug metabolite. . . 40
2.5 Relationship between *CYP2C19* genotype and platelet reactivity 41
2.6 Effect of *CYP2C19* genotype on composite cardiovascular outcome in individuals treated with clopidogrel, Treatment-only analysis. 42
2.7 Meta-cumulative plot of *CYP2C19* wild-type ($\star$1) or $\star$17 compared to $\star$2-$\star$8. . . . 43
2.8 Funnel plot and trim-and-fill analysis. 44
2.9 Association between *CYP2C19* genotype (any copy of $\star$2 through $\star$8 vs. $\star$1 or $\star$17) and risk of individual outcomes in the treatment-only analysis. 46
2.10 Meta-analysis of separate effect of one copy or two copies of loss-of-function *CYP2C19* alleles ($\star$2 to $\star$8) compared to normal/increased function alleles ($\star$1 or $\star$17) on risk of CVD in treatment-only analysis. 47
2.11 Analysis of *CYP2C19* genotype on composite cardiovascular end-points in randomized trials where both clopidogrel and placebo arms were genotyped, "Effect modification" analysis. . . . 49
2.12 Potential scenarios and options for analysis when conducting pharmacogenetic studies in drug metabolizing enzymes. . . . 52
2.13 Potential scenarios and implications for interpretation when conducting pharmacogenetic studies using the "treatment-only" design. 54

2.14 Meta-analysis of randomized trials of more-vs-less clopidogrel. 57
2.15 Meta-analysis of Studies Evaluating the Effect of *CYP2C19* ⋆2 to ⋆8 vs ⋆1 or ⋆17 on Cardiovascular Disease (CVD) Events. 60

3.1 Anatomy of an atherosclerotic plaque 63
3.2 Inflammation as a key mediator of atherosclerosis 64
3.3 Synthesis of inflammatory cytokines from arachidonic acid 66
3.4 Catalytic activity of phospholipase enzymes on phospholipids 66
3.5 Comparison of two measures to quantify circulating $sPLA_2$. 69
3.6 Change in $sPLA_2$-IIA mass during acute coronary syndrome 70
3.7 Model of atherogenesis by $sPLA_2$ 72
3.8 Structure of the first-in-class orally-available $sPLA_2$ inhibitor for CVD prevention, varespladib methyl 75
3.9 Map of the *PLA2G2A* gene 77
3.10 Comparison of (A) a randomized trial of varespladib, with (B) random allocation of *PLA2G2A* rs11573156 79

4.1 Distribution of $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity in EPIC-Norfolk 85
4.2 Correlation between $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity 89
4.3 Association of $sPLA_2$-IIA mass or $sPLA_2$ enzyme activity with fatal/non-fatal MI 94

5.1 Association between tagging SNPs and $sPLA_2$ measures 103
5.2 Manhattan plots of the association of SNPs in *PLA2G2A* region and expression of *PLA2G2A*, *PLA2G5*, and, *PLA2G10* mRNA. 109
5.3 PRISMA flow diagram illustrating the search strategy used to identify randomized trials of $sPLA_2$ lowering therapies. 110
5.4 Meta-analysis pooled estimates of the association between *PLA2G2A* rs11573156 with $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity. 111
5.5 Dose-response relationship between varespladib and $sPLA_2$-IIA mass reported in randomized trials. 113
5.6 Meta-analysis pooled estimates of the association between *PLA2G2A* rs11573156 and major vascular events (including individual components) stratified by clinical setting into general population and ACS patients. . 116
5.7 Meta-analysis of the effect of *PLA2G2A* rs11573156 on incident CVD in general population studies 118
5.8 Meta-analysis of the effect of *PLA2G2A* rs11573156 on prevalent CVD in general population studies 119
5.9 Meta-analysis of the effect of *PLA2G2A* rs11573156 on recurrent CVD events in studies of patients with acute coronary syndrome. 120
5.10 Triangulation of the causal association between $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and major vascular events 126
5.11 Observational and instrumental variable estimates per 1 log unit lower $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity with cardiovascular events . 128

6.1 Worldwide alcohol consumption. 136
6.2 Association of alcohol with CHD from 51 observational studies. 138

6.3 Frequency histogram of the sample size of studies included in the meta-analysis of interventional studies of alcohol. . . . 142
6.4 Alcohol metabolism . . . 143
6.5 *ADH1B* gene with exons illustrated as boxes . . . 145
6.6 Implications of the observational association between alcohol and CHD on the interpretation of genetic associations of *ADH1B* with CHD . . . 147
6.7 Contour plot of the allele frequency of *ADH1B* worldwide. . . . 148
6.8 Routes of migration of *ADH1B* genotype. The letters (H1-H7) refer to haplotypes. . . . 148

7.1 Total number of individuals for each trait and the proportion of variance (R^2) of each trait explained by alcohol in the collaborating studies . . . 155
7.2 Observational association between alcohol consumption and other variables (1) . . . 157
7.3 Observational association between alcohol consumption and other variables (2) . . . 158
7.4 Observational association between alcohol consumption and other variables (3) . . . 159
7.5 Observational association between alcohol consumption and other variables (4) . . . 160

8.1 Association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular disease biomarkers showing association on crude analysis, stratified by alcohol intake. . . . 172
8.2 Association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular disease biomarkers **not** showing association on crude analysis, stratified by alcohol intake. . . . 173
8.3 Subgroup and sensitivity analysis of the meta-analysis pooled estimate of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) with HDL-C. . . . 175
8.4 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits and liver enzyme overall and stratified by alcohol intake . . . 177
8.5 Meta-analysis pooled estimate of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and CHD stratified by categories of alcohol intake. . . . 178
8.6 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and stroke and type 2 diabetes overall, and stratified by alcohol intake. . . . 179
8.7 Meta-analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and ischemic stroke . . . 180
8.8 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and binary traits adjusted for principal components analysis . . . 181
8.9 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and continuous traits adjusted for principal components analysis . . . 182
8.10 Triangulation of the causal association between alcohol and cardiovascular traits . . . 183

9.1 Probability of success to market at different stages of drug development pipeline . . . 193
9.2 Stock price of Anthera Pharmaceuticals prior to and after announcement of VISTA-16 termination . . . 194

A.1 Schemata for random allocation of *CYP2C19* alleles akin to the random allocation of an intervention in a RCT . . . 211
A.2 Schemata for metabolism and activation of clopidogrel by *CYP2C19* . . . 211
A.3 Subgroup analysis of association between *CYP2C19* and CVD in "treatment-only" analysis. . . . 212
A.4 Influence of removing one study at a time on the meta-analysis summary estimate. . . . 213
A.5 Potential scenarios for more versus less clopidogrel. . . . 214

B.1 Differential expression of *PLA2G2A* mRNA by rs10732279 genotype . . . 232
B.2 Genotype frequency of *PLA2G2A* rs11573156 in the 34 studies. . . . 233

C.1 *ADH1B* rs1229984 A-allele frequency in the 56 collaborating studies, arranged by geographical region . . . 286
C.2 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and alcohol traits . . . 287
C.3 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and alcohol traits . . . 288
C.4 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and alcohol traits . . . 289
C.5 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 290
C.6 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 291
C.7 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 292
C.8 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 293
C.9 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 294
C.10 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 295
C.11 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits . . . 296
C.12 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits . . . 297
C.13 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits . . . 298
C.14 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits . . . 299
C.15 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits . . . 300
C.16 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits . . . 301

C.17 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiometabolic disorders 302
C.18 Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiometabolic disorders 303
C.19 Directed acyclic graphs to illustrate potential collider bias in Mendelian randomization analysis of alcohol and CVD using the *ADH1B* rs1229984 variant. 304

List of Tables

1.1 Comparison of drug in a randomized trial to a single nucleotide polymorphism (SNP) in Mendelian randomization . . . 14
1.2 Comparison of estimates obtained from observational analysis to instrumental variable analysis . . . 17

2.1 Characteristics of studies included in the systematic review . . . 36

4.1 Characteristics of assays in studies conducted to measure $sPLA_2$ IIA mass and $sPLA_2$ enzyme activity . . . 83
4.2 Association between tertiles of $sPLA_2$-IIA mass and established and emerging cardiovascular risk factors in 3371 individuals in the EPIC-Norfolk nested case control study . . . 90
4.3 Association between tertiles of $sPLA_2$ enzyme activity and traditional cardiovascular risk factors in 3371 individuals in EPIC-Norfolk . . . 91
4.4 Assessment of departure from linearity in the relationship between $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and cardiovascular events, adjusted for age and gender. . . . 92

5.1 Characteristics of the randomized trials of varespladib identified in the systematic review. . . . 114
5.2 Summary associations of *PLA2G2A* rs11573156 per C allele with cardiovascular risk factors and markers of atherosclerosis . . . 121
5.3 Summary effects of *PLA2G2A* rs11573156 per C allele with potential confounders stratified by study setting . . . 122
5.4 Summary effects of varespladib (500mg/day) and *PLA2G2A* rs11573156 on biomarkers reported in randomized trials . . . 123

8.1 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and indices of alcohol consumption. . . . 170
8.2 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) with cardiovascular biomarkers irrespective of alcohol intake. . . . 171
8.3 Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular biomarkers, irrespective of alcohol intake, limited to traits not showing association at a nominal P value equal to 0.05. . . . 176
8.4 Meta-analysis pooled instrumental variable estimates of the effect of a one log unit increase in alcohol volume consumption (units/wk) on selected cardiovascular traits . . . 184

A.1 Characteristics of the studies included in the systematic review: risk of bias 201
A.2 Components of the composite primary cardiovascular outcomes reported by studies contributing to the meta-analysis. . . . 205
A.3 Outcomes reported individually by studies contributing to the meta-analysis. 206
A.4 Genome-wide associations of polymorhpisms in CYP450 enzymes with traits . . . 207
A.5 Details of the studies included in the analysis of *CYP2C19* genotype and platelet response . . . 208
A.6 Association of *CYP2C19* ⋆2-⋆8 vs. ⋆1 or ⋆17 on risk of clinical events in treatment-only analysis using random-effects meta-analysis. . . . 209
A.7 Analysis of *CYP2C19* genotype on composite cardiovascular end-points and major bleeding in randomized trials using random-effects meta-analysis. 210

B.1 Characteristics of the studies included in the $sPLA_2$ collaboration . . . 220
B.2 Associations of *PLA2G2A* gene variants with $sPLA_2$-IIA mass in EPIC-Norfolk and GRACE-France. . . . 223
B.3 Associations of *PLA2G2A* gene variants with $sPLA_2$ enzyme activity in EPIC-Norfolk and GRACE-France. . . . 224
B.4 Genotyping characteristics in the collaborating studies . . . 225
B.5 Data availability for $sPLA_2$ assays, cardiovascular biomarkers and measures of atherosclerosis in the collaborating studies . . . 227
B.6 Contribution of each study to individual outcomes and primary outcome for the Mendelian randomization analysis . . . 229
B.7 Meta-analysis pooled estimates of the per C allele association between *PLA2G2A* rs11573156 and major vascular events (including individual components) stratified by clinical setting using **random** effects modelling. 231

C.1 Design and genotyping characteristics of the studies included in the collaboration . . . 257
C.2 Characteristics of the alcohol questionnaires used in the collaborating studies . . . 260
C.3 Event definitions in the studies included in the collaboration . . . 263
C.4 General and alcohol characteristics of studies included in the collaboration 266
C.5 Number and proportion of outcomes in studies included in the collaboration 272
C.6 Lifestyle characteristics of the studies included in the collaboration . . . 275
C.7 Cardiovascular traits characteristics of the studies included in the collaboration (1) . . . 277
C.8 Cardiovascular traits characteristics of the studies included in the collaboration (2) . . . 280
C.9 Meta-analysis pooled linear and quadratic coefficients of the association between alcohol and traits on observational analysis, adjusted for age and gender . . . 283
C.10 Linkage disequilibrium between rs1229984 and SNPs on chromosome 4 that are associated with cardiometabolic traits from previous GWAs and gene-centric array analyses . . . 284

List of Abbreviations

ACS	**A**cute **C**oronary **S**yndrome
ADH1B	**A**lcohol **Deh**ydrogenase **1B** (class I), beta polypeptide
AMI	**A**cute **M**yocardial **I**nfarction
BMI	**B**ody **M**ass **I**ndex
BP	**B**lood **P**ressure
C-IMT	**C**arotid-**I**ntima **M**edial **T**hickness
CHD	**C**oronary **H**eart **D**isease
CI	**C**onfidence **I**nterval
CK-MB	**C**reatine **k**inase **MB** isoform
CRP	**C**-**r**eactive **P**rotein
CV	**C**ardio**v**ascular
CVD	**C**ardio**v**ascular **D**isease
CYP2C19	**CY**tochrome **P**450 **2C19** hepatic enzyme
CYP450	hepatic **CY**tochrome **P**450 monooxygenases
DBP	**D**iastolic **B**lood **P**ressure
DNA	**D**eoxyribo**n**ucleic **A**cid
FDA	U.S. **F**ood and **D**rug **A**ssociation
GGT	**G**amma-**g**lutamyl **t**ransferase
GWAs	**G**enome **W**ide **A**ssociation **s**tudy
HDL-C	**H**igh **D**ensity **L**ipoprotein-**C**holesterol
IL-6	**I**nterleukin **6**
IPD	**I**ndividual **P**articipant **D**ata
IV	**I**nstrumental **V**ariable
LD	**L**inkage **D**isequilibrium
LDL-C	**L**ow **D**ensity **L**ipoprotein-**C**holesterol

LoF	**L**oss **o**f **F**unction
MI	**M**yocardial **I**nfarction
MR	**M**endelian **R**andomization
mRNA	**m**essenger **R**ibonucleic **A**cid
MVE	**M**ajor **V**ascular **E**vent(s)
NA	**N**ot **A**vailable/applicable
OR	**O**dds **R**isk
PCA	**P**rincipal **C**omponents **A**nalysis
PCI	**P**ercutaneous **C**oronary **I**ntervention
PGx	**P**harmaco**g**enetics
RCT	**R**andomized **C**linical **T**rial
ROS	**R**eactive **O**xygen **S**pecies
RR	**R**elative **R**isk
SBP	**S**ystolic **B**lood **P**ressure
SD	**S**tandard **D**eviation
SNP	**S**ingle **N**ucleotide **P**olymorphism
sPLA$_2$	**s**ecretory **P**hospho**l**ipase **A$_2$**
TG	**T**rig**l**ycerides
T2D	**T**ype **2** **D**iabetes mellitus
WHO	**W**orld **H**ealth **O**rganisation

To my parents, Marie and Oliver Holmes.

Chapter 1

Introduction

1.1 Cardiovascular Disease: Public Health Importance

Cardiovascular disease (CVD, comprising coronary heart disease (CHD), stroke and peripheral vascular disease) is the leading cause of death worldwide.[1] One in 2 men and 1 in 3 women will experience a coronary heart disease (CHD) event during their lifetime,[2] with substantial implications for the population burden of disease, health-care system resources and the economy.[3] Therefore, a reduction in burden of disease from CHD (through prevention of first and recurrent events) is an important research and public health goal.

CHD has genetic[4–6] and environmental[7–9] determinants, with twin studies suggesting that the heritability (the proportion of variance explained by genetic factors) for death from CHD to be approximately 0.57 in men and 0.38 in women.[6] In high-income countries, 96% of all clinical CHD events occur after 50 years of age,[2] with the low event rate in young age arising due to slow progression of subclinical atherosclerosis, which begins from the second decade of life.[10] The slow progression of atherosclerosis, an initially asymptomatic disease, generates problems for inferring causal relationships from observational associations, because it is challenging to disentangle what occurs first: exposure to a putative risk factor, or subclinical atherosclerosis. However, this long preclinical phase provides a window of opportunity to prevent the development of atherosclerosis.

1.2 Public health approaches to combating CHD

The main approaches to CVD prevention involve (i) non-pharmacological interventions and (ii) pharmacological interventions.

1.2.1 Non-pharmacological approaches to CVD prevention

Population-based preventative approaches for CHD take many forms (**Box 1.1**), and typically aim at shifting the population distribution of a causal risk factor. For example, a major non-pharmacological intervention, the Comprehensive Cardiovascular Community Control Programme (CCCP)[11, 12] used a multiple intervention approach (including health education, screening for risk of cardiovascular disease and a hypertension programme) with the aim of reducing multiple risk factors. CCCP was based in multiple countries, however the most widely published study was based in North Finland (North Karelia region).[13] A comprehensive appraisal of the evidence[14] did not support the hypothesis that this intervention had a major impact on cardiovascular risk factors compared to those who did not take part in the programme, the main limitation being ascribed to an individualized approach (vs. population-based). In contrast, a population-wide approach set in Mauritius *was* efficacious with nation-wide substitution to soya bean oil (rich in unsaturated fatty acids) from palm oil (rich in saturated fat), an intervention that reduced total cholesterol.[15]

Box 1.1: Targets for population-based interventions:

- reduce tobacco consumption
- dietary change
  - reduce salt
  - increase fruit and vegetable consumption
  - reduce fat (saturated and trans-fat) intake
- increase physical activity

One area in which progress has been both efficacious and sustainable is the reduction of tobacco smoking, the second leading cause of global disease burden in humans (after hypertension), accounting for 6.3% of global DALYs[a].[16] Since the ground-breaking study by Doll and Hill in 1954,[17] the first prospective study to confirm the elevated association of CVD risk in smokers compared to non-smokers, smoking has become progressively

[a] Disability-Adjusted Life Year (DALY) - one DALY represents one year lost of healthy life due to disease.

less socially acceptable.[18, 19] Population-based interventions through taxation and legislation to ban smoking in public places have been increasingly used to further reduce smoking prevalence. Recent examples include the smoking ban in Scotland[20] and England (Health Act 2006[21]). Implementation of such legislation is supported through observational evidence that suggests a reduction in hospital admissions for CHD following the introduction of smoking bans in UK[22], Italy[23] and USA[24]. More widely, banning smoking in public places has also led to observed improvements in respiratory disease.[25] Of note, the benefits of anti-smoking legislation are likely to become more apparent with longer follow-up.

In addition to the non-pharmacological population health interventions to prevent CVD (such as reduced consumption of dietary salt, fat and increased physical activity, **Box 1.1**), a recent emphasis in UK has been placed on policy making to limit the marketing of unhealthy food to children and improve food labeling.[26] This is in response to a dramatic increase in the prevalence of obesity in children in high-income countries,[27] with a consequential increase in the associated sequelae of obesity - i.e. metabolic diseases and type 2 diabetes.[28, 29] Clearly more action is needed in this arena to reverse the worrying trend in childhood obesity.[30]

1.2.2 Pharmacological approaches to CVD prevention

In contrast to the above approaches, pharmacological intervention involves prescribing drugs to individuals at risk of first (primary prevention) or recurrent (secondary prevention) disease. Drugs classes used for prevention of CVD are listed in **Box 1.2**.

Box 1.2: Major drug classes used for CVD prevention:

- blood pressure lowering
- LDL-C lowering (e.g. HMG-coA reductase inhibitors, statins)
- anti-clotting (anti-platelet drugs such as aspirin)

Treatment trials of blood pressure lowering drugs (such as beta blockers, angiotensin-converting inhibitors, thiazide diuretics and calcium channel blockers) have shown that, whatever the class of drug,[31] reducing blood pressure by 5mmHg is associated with a 20-25% reduction in risk of CHD events.[32] Similarly, data from RCTs of lipid-lowering drugs (predominantly statins) show that a 1 mmol/L reduction in LDL-C results in a 23% reduction in CHD events at 1 year.[33, 34]

One striking feature of the relationship between causal risk factors such as blood pressure or LDL-cholesterol is the nature of the causal effect. Whatever the initial value of

the trait, the relative risk reduction for a given reduction in the risk factor is constant across the distribution, with no threshold (Figure 1.1). This has important relevance to applying interventions (both non-pharmacological and pharmacological) to the population as a whole. First, interventions to reduce these traits will have an effect on modifying disease risk of *all* individuals, irrespective of their absolute levels of their risk factors. Second, most clinical events occur in individuals with average levels of a risk factor. This arises from the normal (or log-normal) distribution of causal traits in the population, and owing to risk factors having a linear (or log-linear) association with CHD risk: therefore, to have greatest impact at reducing *population* risk, the majority of individuals need to reduce their levels of a risk factor, including those with seemingly normal (or low-normal) values of risk factors who might gain little, (the so-called "prevention paradox").[35]

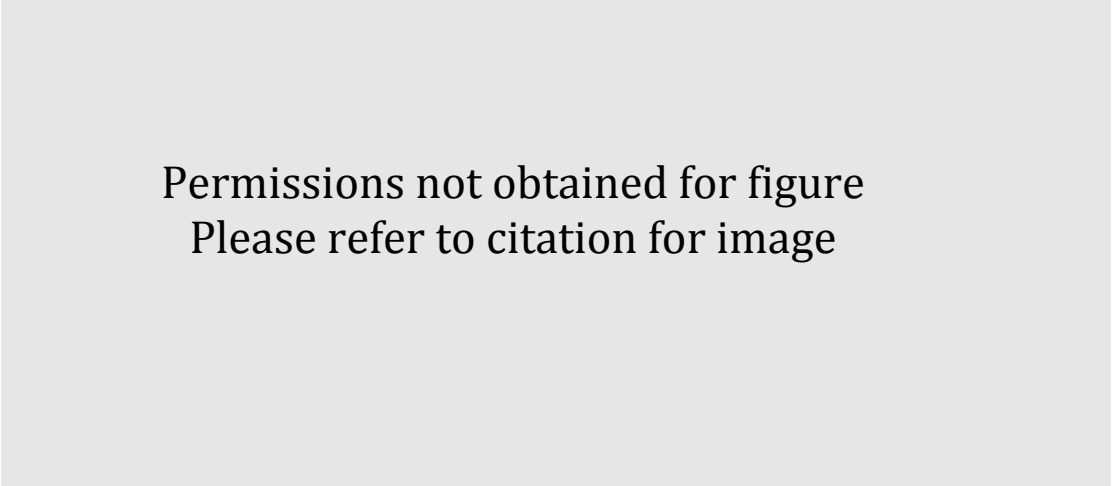


Figure 1.1: Relationship between LDL-cholesterol and total cholesterol with risk of coronary heart disease.
A) Association between LDL-cholesterol reduction from randomized trials of statin therapy and risk of major coronary events.
B) Distribution of total cholesterol in the general population (approximate) showing the observational association between cholesterol level and risk of CHD events.
Abbreviations: CHD, coronary heart disease; TC, total cholesterol. In both (A) and (B), boxes indicate weighted point estimate and vertical lines represent the standard errors)
Figure (A) derived from Baigent *et al*[34]; Figure (B) derived from Jackson *et al*[36]

For pharmacological prevention to be used at a *population* level, certain criteria must be fulfilled. The drug must be:

1. of proven efficacy,
2. safe, so that the risk to benefit ratio is heavily in favour of intervening via pharmacological treatment as part of a mass strategy
3. cost-effective, to encourage population-wide treatment without penalising healthcare resources

1.3 Trends in CVD - changing incidence

Worldwide, CVD mortality has changed remarkably over the past few decades. In high income countries, a fall has been observed (Figure 1.2), whereas in low income countries, CHD mortality has been increasing.

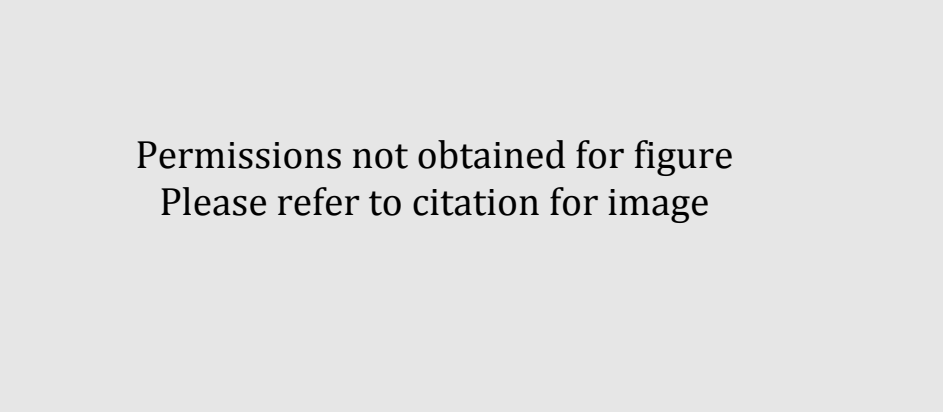


Figure 1.2: Trend in mortality from coronary heart disease in men aged ≤64 in high-income countries
Reproduced from Bandosz *et al*[37].

The major risk factors for CVD are similar across the world, and include smoking, lipid profiles, diabetes, high blood pressure, obesity, diet and physical activity.[38] The difference in temporal trends between high and low income countries may be explained by alterations in the major risk factors. In low and middle income countries, tobacco smoking has increased and diet has become Westernized (i.e. higher in fat and salt). This is in contrast to high income countries, in which tobacco consumption has fallen.[39] These alterations drive in part the epidemiological transition (Figure 1.3)[40]: as low and middle income countries undergo the epidemiological transition, the proportion of deaths from infectious diseases (arising from over-crowding and lack of basic health-care) falls and that from degenerative diseases (such as cardiovascular disease) increases.[41]

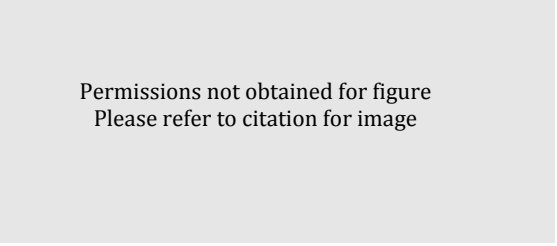


Figure 1.3: The Epidemiological transition.
As life expectancy increases (from improved sanitation, nutritional status, hospital care, etc.), the proportion of deaths arising from infectious disease decreases and is replaced by deaths arising from disease that takes many years to accumulate (such as atherosclerosis).
Reproduced from Omran[40].

Despite the substantial improvement in CVD mortality, vascular disease accounts for 30% of deaths in the UK[b] and despite the use of efficacious treatments, a considerable residual risk of primary and secondary CVD exists.[42]

1.4 Quest to identify causal risk factors for CVD

If we are to further reduce the risk of CVD, identification of novel causal risk factors may provide new targets for therapeutic modification. But, how do we know if a biomarker is causally related to CHD?

Many hundreds of non-genetic risk factors have been identified that associate with risk of CHD on *observational* analysis.[43] However, of this plethora, it is not clear how many represent causal associations. This is important because targeting non-causal risk factors would not yield reductions in CHD and could entail exposing individuals to drug treatment that results in adverse drug reactions.

Thus far, LDL-C and BP are two risk factors that are recognized as important because randomized clinical trials (RCTs) have validated their causal relevance in CHD pathogenesis.[32, 44–46].

1.5 Study designs available to investigate role of biological variables

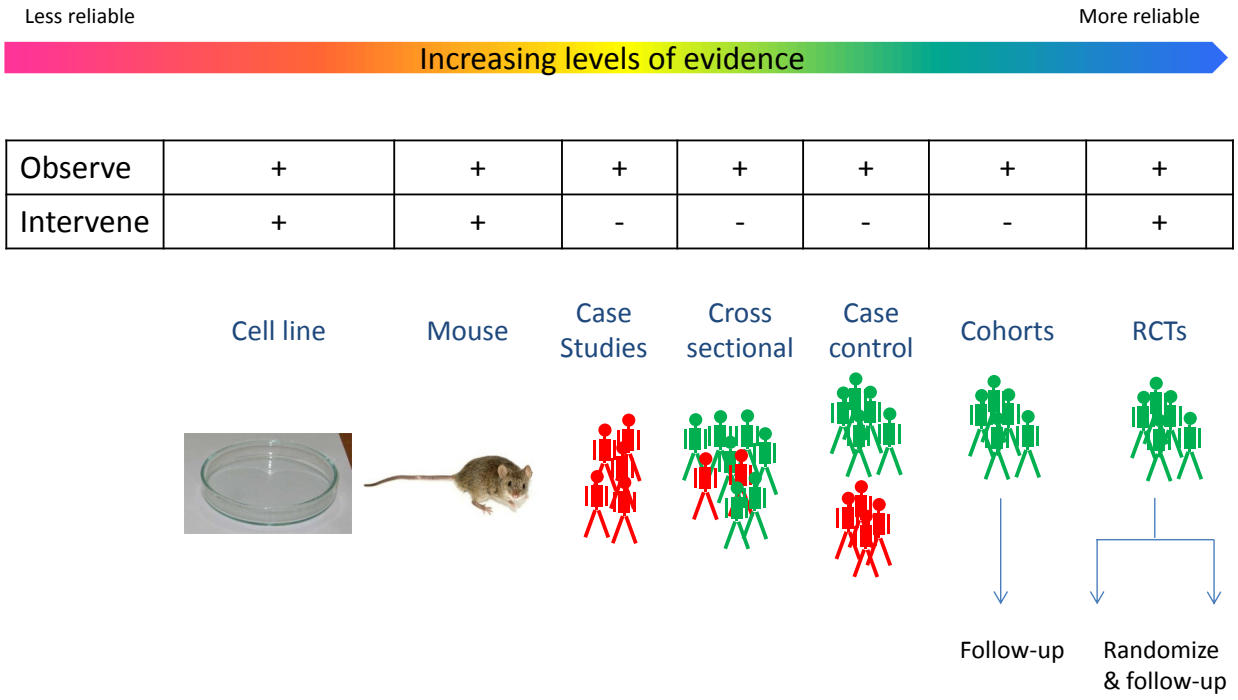
Various study designs exist to investigate whether a relationship exists (and the underlying mechanism) between an exposure of interest and the onset of disease (Figure 1.4).

1.5.1 Cell lines and animal models

The aim of *in vitro* and non-human *in vivo* models of disease is to provide a quick and reproducible means to investigate the role of biological markers in disease pathogenesis.

Animal studies provide a means to investigate whether alteration in a risk factor impacts upon measures of disease. Animal models are particularly effective as they allow the manipulation of biological systems in a way that is not possible in humans.[47] The mouse has provided the main animal model for atherosclerosis, due to: detailed knowledge of genetic information; low costs facilitating larger numbers of animals; fast gestation times; and, a small circulating volume meaning that low amounts of drugs are required.[48]

[b] Data from Office of National Statistics, 2011



Observe	+	+	+	+	+	+	+
Intervene	+	+	-	-	-	-	+

Bias	+++	+++	+++	+++	+++	+	-
Confounding	+++	+++	+++	+++	+++	++	-
Translation	---	---	--	--	-	+	+++
Hypothesis/ Definitive	H	H	H	H	H	H	D

Figure 1.4: Study methods used in Science to investigate biological mechanisms underlying disease processes.
Studies on the left side of the figure are suitable for hypothesis generation, however the pivotal test for casuality arises from randomized clinical trials (RCTs), which if conducted adequately, obviates both measured and unmeasured confounding. Confounding cannot be excluded from any other study design (unless randomization is built-in, e.g. a lab experiment with a randomized intervention).

Footnotes Intervene: in those studies marked with a '+', the study involves an intervention (i.e. controlled exposure); Bias/Confounding: these are the two traditional sources of error in studies, the greater the number '+', the more susceptible the study to these forms of error; Translation: typically only cohorts/randomized trials can directly influence health policy and/or treatment decisions; Hypothesis/Definitive: it can be argued that RCTs represent the only source of definitive evidence on causality.

However, for studying atherosclerosis a challenge arises: mice do not spontaneously develop atherosclerosis in their lifetime.[48] Therefore, to mimic the atherosclerosis process in humans, a genetic knockout mouse that is also fed a high-fat diet for several weeks is required, which results in atheroma deposition.[49]

Although animal models seem appealing in their availability and relatively quick time to investigate (as in theory a mouse can 'model' in weeks what takes decades in humans), several reasons potentially limit their translational benefit. First, a systematic review showed that only 37% of positive findings reported in animal studies were replicated in human randomized trials, and only 10% actually translated into clinical use (thus the attrition rate is 90%, an alarming statistic).[50, 51] A "glass-half-full" perspective would interpret this figure as reassurance that at least some animal models do lead to translational benefits that are highly relevant to humans. But on the other hand, a 63% 'false positive' rate is very high, and means that the majority of findings reported in animal studies will not yield benefit to man. An additional perspective is to consider the biomarkers that have been investigated in animal models but that did *not* show evidence for a role in disease - these could potentially represent false negatives! Second, systematic bias may arise from methodological flaws in animal studies resulting in erroneous conclusions [51]. Such flaws include:

- absence of an *a priori* sample size calculation
- selection bias
- lack of randomization to exposure
- lack of blinding to outcome assessment
- outcome reporting bias
- publication bias

Of note, publication bias is of particular concern as it may over-inflate the scientific focus on a particular biomarker, and has been estimated to account for $\frac{1}{3}$ of the excess efficacy reported in systematic reviews.[52] This selective reporting and publishing of positive studies can misdirect academic focus to investigating non-causal associations.

1.5.2 Observational studies in Humans

Even using the ideal 'animal model' (i.e. humans) to make observations about human disease pathogenesis can be prone to error and identified relationships may not be causal (Box 1.3 and Figure 1.5).

Box 1.3: Examples of observational evidence that have failed to translate to true causal associations:

- **Hormone replacement therapy (HRT) and CVD**: observational evidence suggested that HRT was cardioprotective[53], however results of a large-scale RCT (Women's Health Initiative)[54] showed an increase in CHD risk associated with HRT use. This discrepancy is likely due to confounding of HRT use by favourable socioeconomic factors (e.g. diet, exercise) in the observational analysis.
  One striking feature is that the confounding was of such magnitude as to bias the underlying (true) harmful effect of HRT and make it directionally opposite to create an apparent cardioprotective effect. This serves to illustrate just how misleading results from observational evidence can be.
- **vitamin E and CVD**: Large-scale prospective observational evidence supported a protective role of vitamin E in CHD[55, 56], with dose-response and temporality criteria fulfilled.[a] However, RCTs have failed to reproduce this finding[58] and a meta-analysis of high-dose (≥400 IU/day) vitamin E suggests that contrary to being beneficial, high-dose vitamin E may *increase* risk of all-cause moratlity[59]

[a] A dose-response relationship and temporality (exposure measured prior to outcome) are arguably two Bradford-Hill[57] criteria that are most important in supporting a causal association between an exposure and outcome when interpreting observational evidence

In simplistic terms, error can be grouped into 4 principle domains:

1. **chance**: a chance finding is the scenario where a study identified an association between an exposure and outcome that is not true (a false-positive, or type 1 error). The primary cause of chance findings is small sample sizes, resulting in a randomly erroneous estimate. Such false positives do not represent the 'truth' and are typically not replicated on analysis of larger data-sets (unless there is publication bias, which can distort the field as a whole[60, 61]).
2. **bias**: in this scenario, there is deviation from the true association; bias can be divided into two main forms:
   (a) **selection bias**: this type of bias typically affects case-control studies in which the controls are not representative of the population from which the cases arose; on measuring an association between disease status and exposure, selection bias can yield misleading findings. Selection bias can also affect cohort studies if follow-up is different between exposed and non-exposed groups.
   (b) **measurement error**: error in the measurement of an exposure or outcome can be either non-differential or differential
      i. **non-differential**: in this example, there is no relationship between the error in exposure measurement and the outcome (or vice versa). If the

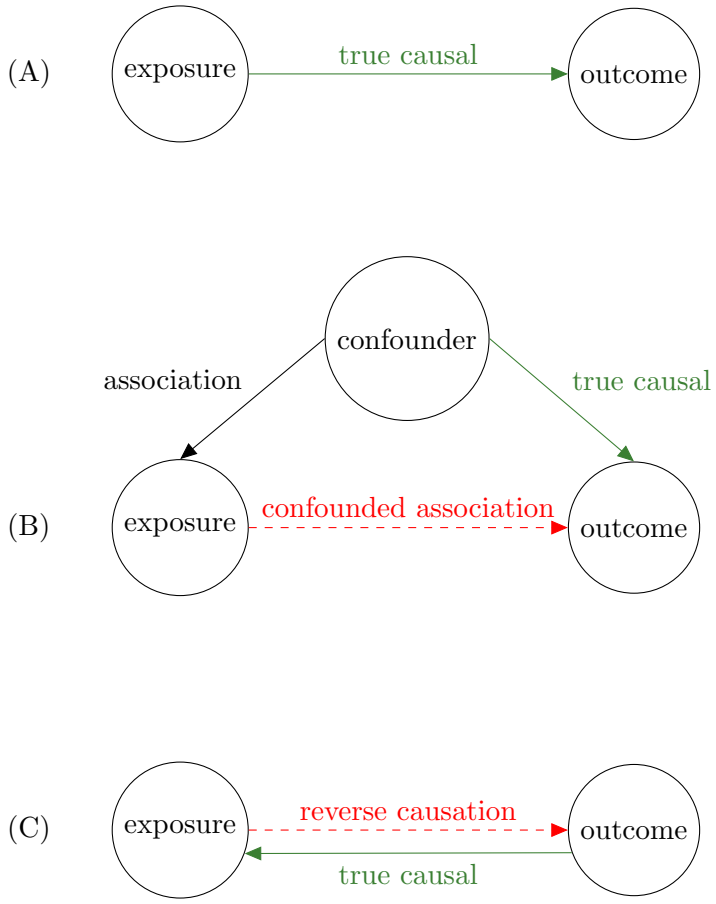


Figure 1.5: Possible scenarios for an association between an exposure and outcome
(A) the observed association between an exposure and outcome is causal. If the exposure is modifiable (e.g. LDL-cholesterol), then an intervention to modify the exposure (e.g. treatment with HMG-CoA reductase inhibitors, more commonly known as statins) will alter the risk of disease (e.g. CHD)
(B) the association between the exposure of interest arises due to a relationship with a confounding factor. This creates a confounded association (red line) between the exposure and outcome. An example of this is an association between yellow teeth and lung cancer, which is confounded by smoking status. In this example, an intervention to reduce the yellow colouring of teeth is unlikely to alter risk of lung cancer (whereas reducing smoking would)
(C) the *outcome* results in altered levels of what is thought to be the 'exposure'. The "temporality" criterion of Bradford Hill[57] attempts to minimise this source of confounding, however in the case of CHD, subclinical disease may alter biomarkers many years prior to the manifestation of clinical CHD. An example of this is the relationship between C-reactive protein (CRP) and CHD. An intervention to change CRP is unlikely to alter risk of CHD, as evidence to date suggests that the association is entirely driven by reverse causality.[62]

error is in the exposure, this usually leads to regression dilution bias in which the effect estimate is biased towards the null.[63]

ii. **differential**: this type of bias is systematic and means that the measurement of an exposure is systematically different according to the outcome, or vice-versa. An example is **recall bias**, which is particularly common in case control studies where exposure is determined retrospectively and knowledge of a persons's disease status can influence how exposure is

remembered.[c] Differential measurement bias can result in either attenuation of the association towards or away from the null, depending on the scenario.

3 **confounding**: this is *the main limitation* of observational studies (Figure 1.5B). Despite measurement of what may be considered 'known' confounders and statistical adjustment in multivariate models, residual confounding can arise from: (i) measurement error of the confounder; (ii) known but unmeasured confounders, and/or; (iii) unknown confounders. It is perhaps unknown confounding that is the most frustrating component - e.g. even in the most richly phenotyped cohorts, confounders are likely to exist that are not known, and which, despite the best efforts of the scientific investigators, result in error. Even if all potential confounders are measured and adjusted for in a multivariate analysis[d], conditioning on a potential confounder can *induce new* relationships between exposures and other confounders by opening 'back-door' pathways.[64] Thus, even with careful selection and measurement of co-variates in analysis, it is impossible to say with certainty that all confounding has been eliminated, and therefore on observational (non-genetic) analysis, an association cannot be stated to be causal.

4 **reverse causality**: in this scenario, disease status (especially subclinical disease) results in changes in levels of the biomarker of interest (Figure 1.5C). On identifying an association between the biomarker and risk of disease, the biomarker may erroneously be interpreted as causing disease whereas the reverse is true. This can affect both case control studies (as cases are collected after disease onset) and prospective cohorts (where the disease process begins years prior to the clinical manifestations, as in CHD).

1.6 Randomization of exposure

R.A. Fisher famously stated "randomization relieves the experimenter from the anxiety of considering and estimating the magnitude of the innumerable causes by which his data may be disturbed."[65]

[c] One example may be a case-control study of autism, with the investigators interested in vaccination history. Parents with children with autism are likely to be aware of the media attention on the apparent (yet false) association of the measles, mumps and rubella (MMR) vaccine with autism. This could subtly alter how the parents of children with autism recall their child's vaccine information, potentially creating an apparent association between vaccination history and risk of autism

[d] It is worthy to note that the selection of confounders is a subjective process and what one scientist may consider a confounder, another may not. This is partly why studies that conduct similar analyses may adjust for different traits labelled as 'confounders'

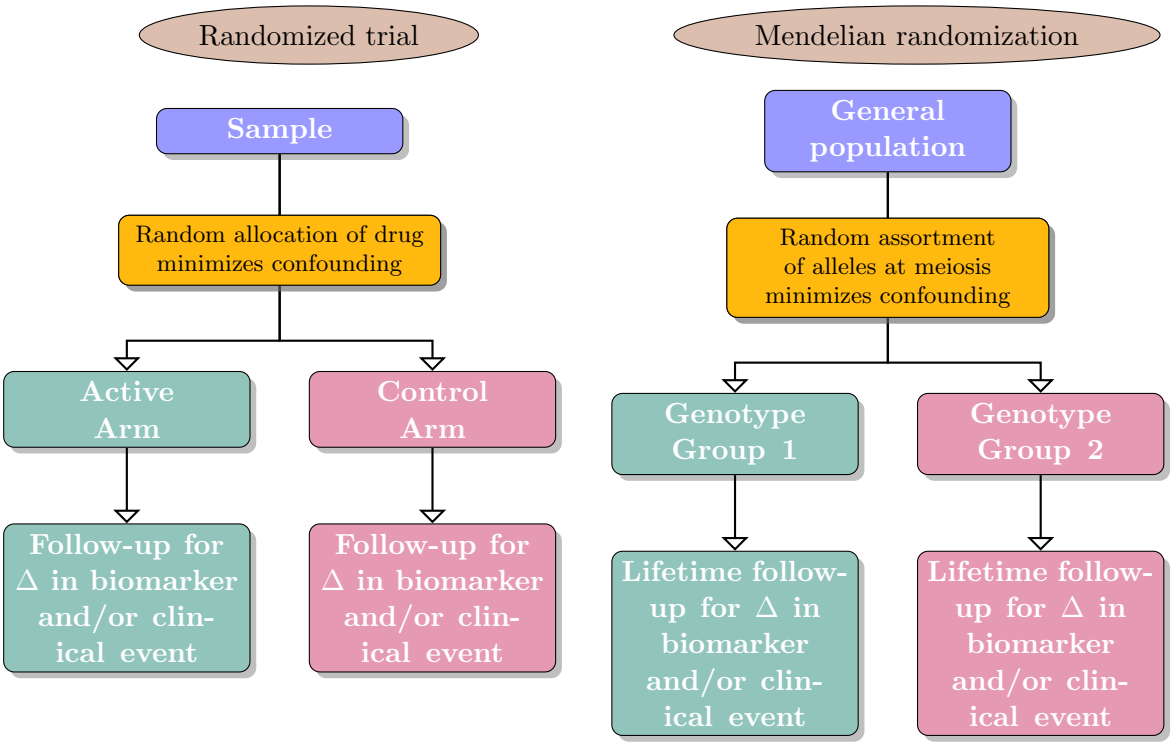


Figure 1.6: Comparison of a Randomized trial to Mendelian randomization. In both study designs, the random allocation of exposures permits the investigation of associations between the exposure and outcome that is free from confounding, and from which causal associations can be inferred. Δ refers to change

The gold standard of evidence on causal relationships arises when both known and unknown confounders are equally balanced between exposed and unexposed groups. This can only occur when the exposure is randomly allocated to individuals and there are 2 scenarios where this arises (Figure 1.6):

1. **RCT**: the gold-standard of study design. Participants enrolled into the clinical trial are randomly allocated a treatment intervention. When a trial is adequately conducted[e] and suitably powered, it is this randomization that eliminates confounding

2. **Mendelian randomization**: so-called "Nature's randomized trials".[67] Alleles are randomly allocated from parents to offspring. Thus, whether an individual receives an allele at a locus is independent of confounders in the parents. At the population level, this means that individuals grouped according to their genotype status at a particular locus should be similar (with respect to confounders) to individuals in the alternate genotype group (as depicted in Figure 1.6).

[e] Not all RCTs are created equal and trial characteristics such as adequacy of allocation concealment and blinding can greatly influence the robustness of trial findings.[66]

1.7 Mendelian Randomisation

1.7.1 Gregor Mendel

Mendelian randomization has flourished within the past decade as a tool to differentiate causal from non-causal relationships between exposures and outcomes. The cornerstone of this approach was the discovery by the Moravian scientist in 1865, Gregor Mendel (Figure 1.7), that inheritance of one trait is independent of inheritance of another trait (so-called 'Mendel's second law'). Although Mendel wasn't aware that the unit of inheritance was the "gene", the law which he established arises from the independent assortment of alleles, which occurs in the meiotic phase of gametogenesis.[67] The random cross-linking between the paternal and maternal chromosomes means that gene variants are inherited independently of one another, resulting in a genome that is randomly allocated - akin to the random allocation of a drug in a clinical trial (Figure 1.6).[f]

Figure 1.7: Gregor Mendel, the "founding-father" of modern genetics[68]

1.7.2 History of Mendelian Randomization: first examples

The concept of using genes to investigate deconfounded associations between exposures and disease risk originally arose from an idea suggested by Katan in 1986.[69] At the time, the scientific community was uncertain about whether a reduction in cholesterol would increase the risk of cancer. This was because observational studies had shown that low serum cholesterol was associated with increased risk of cancer (especially colon cancer).[70] Despite observational studies measuring cholesterol up to 18 years prior to the cancer diagnosis,[71] the concern remained that the observational association between cholesterol and cancer could arise from confounding by another variable or due to reverse causation (as in Figure 1.5 B and C). Katan suggested using gene polymorphisms in *APOE* to investigate the de-confounded association between cholesterol and cancer,[69] and in doing so, opened up the Scientific community to the potential use

[f] It is important to note that this randomization process applies only to variation in germline DNA and not somatic mutations or mitochondrial DNA.

of gene variants as instruments to test casual relationships. Katan's ideas were further developed by Davey-Smith and Ebrahim to extend the use of Mendelian randomisation to environmental exposures.[72, 73] Such was the importance of Katan's ideas that a special series of articles in the International Journal of Epidemiology was dedicated to this.[74–79]

1.7.3 Comparison to Randomized Trial

Although the basic principle is that Mendelian randomization shares many features of a RCT, distinct differences between the two approaches exist, summarized in Table 1.1. Drug treatments tend to have a large effect on the pharmacodynamic target (in order to yield a maximal treatment response), whereas a single SNP tends to have a much weaker association. This means that the sample size for a Mendelian randomization analysis may be many-fold greater than for a RCT. Despite this, the cost of a Mendelian randomization project is far less than a RCT as a trial involves recruiting patients and heavy costs for monitoring adverse drug reactions. In contrast, Mendelian randomization studies can be conducted in general population cohorts who have already been recruited and followed up for incident events. Furthermore, a trial of a drug means exposing individuals to drugs that can have life-threatening adverse drug reactions, whereas measurement of a SNP involves no intervention.

Given the high-risk profile of drug development, principally due to attrition (Figure 1.8), the advantageous properties (cost and safety) of Mendelian randomization analyses mean that their conduct would be well-placed prior to embarking upon clinical trials in the drug development pipeline. These concepts are further developed during this thesis.

Table 1.1: Comparison of drug in a randomized trial to a single nucleotide polymorphism (SNP) in Mendelian randomization

Domain	Feature	Drug treatment	Randomized allocation of genotype
Design	Strength of exposure	Typically high	Tends to be small
	Confounding	Absent	Absent⋆
	Factorial design possible	Yes	Potential
Practicality	Cost (£)	Millions	Thousands
	Duration	Years	Months-years
	Sample size requirement	Usually large	Very large
Drug development: application	Place in drug development pipeline	Late	Early
	Potential harm of intervention	Possibly high	None
	Risk of negative finding to pharma	High	Low

Footnote. ⋆ assuming absence of linkage disequilibrium and population stratification, discussed later in this Chapter

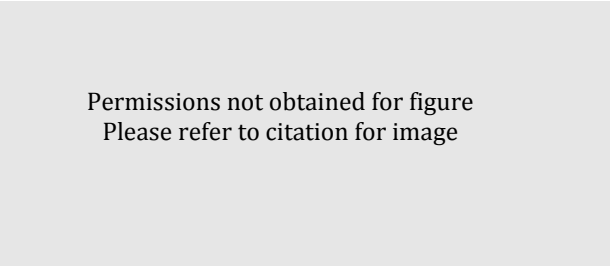


Figure 1.8: Drug development pipeline.
The drug development process is very high risk. After initially screening thousands of compounds, only a handful is taken forward to investigate in human studies. If these fail, then the cost can be devastating to both the individual drug companies that have invested millions to billions of dollars but also to development of new therapies in general. Reproduced from Willmann et al.[80]

1.7.4 Classification and Application

Mendelian randomization can be broadly categorised into two main types, depending on the exposure (intermediate phenotype) under investigation:

endogenous trait: these are traits that have originated from within the human body (e.g. C-reactive protein)

- cis: in this example , the intermediate phenotype is a known cognate protein, an example of which is secretory phospholipase A_2-IIA (as discussed in chapter 3). This is the prototype example of a "simple well-defined phenotype" referred to by Katan,[74] that is ideal for 'classical' Mendelian randomization. Because the protein can usually be measured specifically, and a known gene encodes the protein, in this scenario it is often straight forward to select a SNP that is specific for the protein exposure of interest (by selecting a SNP in the gene that specifically encodes the protein) and take it forward for instrumental variable analysis.
- trans: contrary to the `cis`-form, in `trans` MR studies, the intermediate phenotype is endogenous and not a protein (such as a circulating lipid fraction, e.g. HDL-C, or systolic blood pressure). This type of biomarker is more challenging to investigate using Mendelian randomization as it makes certain assumptions. For example, use of a single SNP for Mendelian randomization in this setting relies on the assumption that the SNP does not have effects on other traits that can result in confounding of the instrumental variable estimates.[81] An example of using a single SNP to make causal inference on an endogenous non-protein trait is the investigation of homocysteine and risk of stroke. Using the *MTHFR* variant, I investigated the role of homocysteine in stroke and found that in populations with low levels of folic acid,

the *MTHFR* variant (which was involved in homocysteine metabolism) had a large effect on homocysteine levels and was associated with risk of stroke.[81]

exogenous trait: in contrast to endogenous traits, exogenous traits are exposures that originate outside of the human body. Use of Mendelian randomization analyses for exogenous traits are subject to the same limitations and assumptions as those for `trans`-Mendelian randomization, namely specificity of the SNP for the exogenous trait.[81] An example of using a single SNP to make causal inference on an environmental exposure (alcohol) is reported in Chapters 6 to 8.

Recent developments have enabled the use of combining several SNPs together into an allele score.[82] This has advantages for Mendelian randomization when applied to both endogenous cis/trans and exogenous traits. Use of multiple SNPs will increase the proportion of variance of the intermediate phenotype, meaning that there will be greater statistical power. Furthermore, it enables the investigation of non-protein traits (not encoded for by any single gene), with the bonus that any associations of individual SNPs in the allele score with traits *other* than the index phenotype should be non-systematic and therefore diluted by the combined effects of the multiple SNPs in the allele score.

Another means to categorise Mendelian randomization is based on the *application*:

drug target validation - in this scenario (a special type of `cis` Mendelian Randomisation), Mendelian randomization is used to validate a potential drug target using a SNP that encodes a drug target receptor. Information from these studies informs on whether drug targets should be pursued in further studies (ideally randomized trials). A recent example is use of a SNP in the *IL6R* gene,[83] which indicates that targeting the IL-6 receptor may represent a novel means to reduce risk of CHD. This study also served to validate the role of inflammation (acting via the IL-6 receptor) in CHD aetiopathogenesis.

validation of risk factors - in this scenario, Mendelian randomization is used to investigate endogenous or environmental exposures that are amenable to public health intervention.

1.7.5 Mendelian triangulation

One key component of Mendelian randomization is the instrumental variable analysis, also known as "Mendelian triangulation," which refers to the statistical technique in

which data on the associations between the genetic instrument (SNP) with the intermediate phenotype and the outcome are used to generate an un-biased estimate between the intermediate phenotype and the outcome. The investigator can then compare the estimate derived from the crude observational analysis to that derived from the instrumental variable analysis to investigate whether there is a discrepancy. Absence of a discrepancy would suggest that the biomarker is causally related to disease. Presence of a discrepancy could be explained by confounding in the observational analysis. Four potential scenarios comparing the observational and instrumental variable estimates are outlined in Table 1.2.

Table 1.2: Comparison of estimates obtained from observational analysis to instrumental variable analysis

Scenario	Observational estimate	Instrumental variable estimate	Potential explanation(s)
1	Associated	Null	Observational estimate confounded or lack of power in IV estimate
2	Associated	Associated	Biomarker is likely to be causal
3	Associated +	Associated ++	Causal (IV estimate of greater magnitude owing to lifetime exposure to genotype)
4	Null	Associated	Negative confounding⋆

Footnotes: IV: instrumental variable, derived for example from a Mendelian randomization analysis. ⋆ this is an unlikely scenario given that if the observational association is null, it is unlikely that the biomarker would be prioritized for a Mendelian randomization analysis!

1.7.6 Assumptions

There are three basic assumptions in the use of genetic variants as instrumental variables:[84]

1. the SNP (Z in Figure 1.9) must associate with the intermediate phenotype (X in Figure 1.9)
2. the SNP must not have independent associations with the outcome of interest via alternative pathways (the dotted red line between Z and Y in Figure 1.9)
3. the SNP must not associate with any confounders (the dotted red line between U and Z in Figure 1.9)

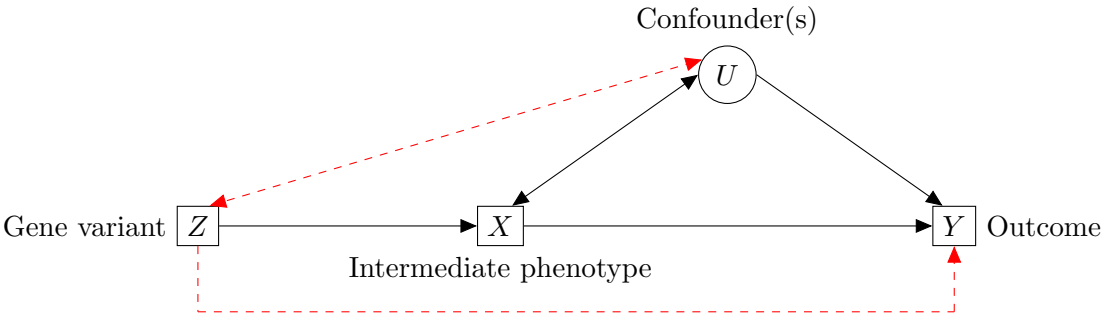


Figure 1.9: Mendelian randomization concept.
The investigator is primarily interested in the causal relationship between the intermediate phenotype (X in figure above) and the outcome (Y). Observational studies that report this association are limited by potential confounding from known and unknown confounders (U). Use of an instrument (in this case a gene variant, Z) that associates with the intermediate phenotype but *not* with the confounders (owing to Mendel's second law) can be used to "triangulate" the true (deconfounded) association between X and Y. Mendelian randomization makes three assumptions:
(i) The red dotted line between Z and U refers to the assumption that there is no association between the gene variant with known and unknown confounders; (ii) The red dotted line between Z and Y refers to the assumption that there is no association between Z and Y that is independent of X; (iii) The instrument (Z) must associate with the intermediate phenotype (X)

1.7.7 Potential Limitations

Limitations to Mendelian randomization exist, but with large sample sizes and appropriate choice of the genetic instrument, they can be largely overcome. Limitations include:

Small effect size One of the greatest criticisms thrown at Mendelian randomization is that the genetic instruments typically have a small effect size on the intermediate phenotype, explaining only a small proportion of variance (R^2) in the exposure. However, this is not universally the case, e.g. the SNP used in Chapter 3 explained a large proportion of the variance of circulating sPLA$_2$-IIA mass.
It is worthy to note that drugs used in conventional clinical trials tend also to only explain a small proportion of variance on the pharmacodynamic target. E.g. the proportion of variance of LDL-C explained by statins is only 8% (data from Whitehall-II cohort[85]) Yet meaningful conclusions on drug efficacy can come from trials with a few thousand individuals.

Sample size Due to the small effects of individual SNPs on the intermediate phenotype, Mendelian randomization studies rely on large sample sizes. Thus, even if the SNP explains only a small proportion of variance of the intermediate phenotype, as in the example of C-reactive protein where the proportion of variance is small (6%),[86] by amassing studies to have suitable power,[62] even a variant with a relatively weak effect can be used to make meaningful causal inference. Thus when conducting a Mendelian randomization study, both the impact of the genetic variant on the intermediate phenotype and the sample size need to be taken into consideration in order to determine whether there is sufficient power to make meaningful causal inference.

Confounding There are three ways in which Mendelian randomization studies can be confounded.[87] These are:

1. Linkage disequilibrium (LD): in this case, the genetic instrument is associated with another SNP which has a true "causal" association with the outcome of interest (Figure 1.10A). Thus, the instrumental SNP must be carefully selected such that LD with other SNPs (typically those in adjacent genes) is not an issue. This can be investigated using 'in-house' data to generate LD plots, but also various external tools exist including the SNP Annotation and Proxy Search (Broad Institute)[88] and an online tool provides researchers with information on long-range LD GLIDERS.[89][g]

[g]Of note, this does not test if the SNP in the gene is responsible for the alteration in the encoded protein - this comes e.g. from investigating the association of SNPs in the region with for example mRNA expression of the gene (as I present in Chapter 5 for secretory phospholipase A$_2$).

2. Population stratification: genotype frequency often differs by population subgroups. It is well established that so-called ancestry associates with various disease frequencies including cardiovascular disease.[90] Thus if individuals of differing ancestry are used in the same analysis, ancestry may introduce confounding between the gene and outcome (Figure 1.10B). In a GWAs, confounding by population stratification can be assessed by means of a Chi-squared quantile-quantile(Q-Q) plot, where a striking or early deviation from the 45 degrees line suggests genomic inflation, as assessed by the lambda statistic.[91]

   In order to minimize potential for population stratification, researchers commonly limit genetic analyses to individuals of a single ancestry (e.g. European, African, Asian). However, in this scenario, confounding may still arise by 'residual' population stratification, and it may be necessary to statistically adjust analyses by principal component traits.[h][91, 94]

3. Pleiotropy: in this scenario, a SNP may be non-specific for the trait under investigation. This can result in an association between the gene and outcome arising from the association of the SNP with an intermediate phenotype separate to the one that you are investigating (Figure 1.10C). This can introduce confounding into the instrumental variable analysis.

   An example is *APOE* genotype, which has effects on multiple traits including C-reactive protein, LDL-C and HDL-C. If one were to use *APOE* to investigate the effect of CRP on CHD, a positive association between the genotype and outcome may be incorrectly ascribed to a causal effect of CRP on CHD. However, this association would arise from the pleiotropic effects of the *APOE* gene with the other "true" causal intermediate traits (such as LDL-C). It ought to be noted that even if pleiotropy is identified, it may be possible to statistically adjust for traits in order to try and mitigate confounding by association of the SNP with another trait.

   Some SNPs used for Mendelian randomization may be pleiotropic by the nature of their effect on the phenotype - e.g. SNPs used for trans-MR are often non-specific for the trait under investigation. For example, if a SNP is involved in metabolism of the exposure of interest, the SNP would associate with both the biomarker of interest and its metabolites. Thus identification of pleiotropy in the scenario of a trans-acting SNP may be permissable.

[h] Principal component traits are derived from SNPs in the dataset using EIGENSTRAT[92] and catch the main axes of genetic variation in the study participants.[93]

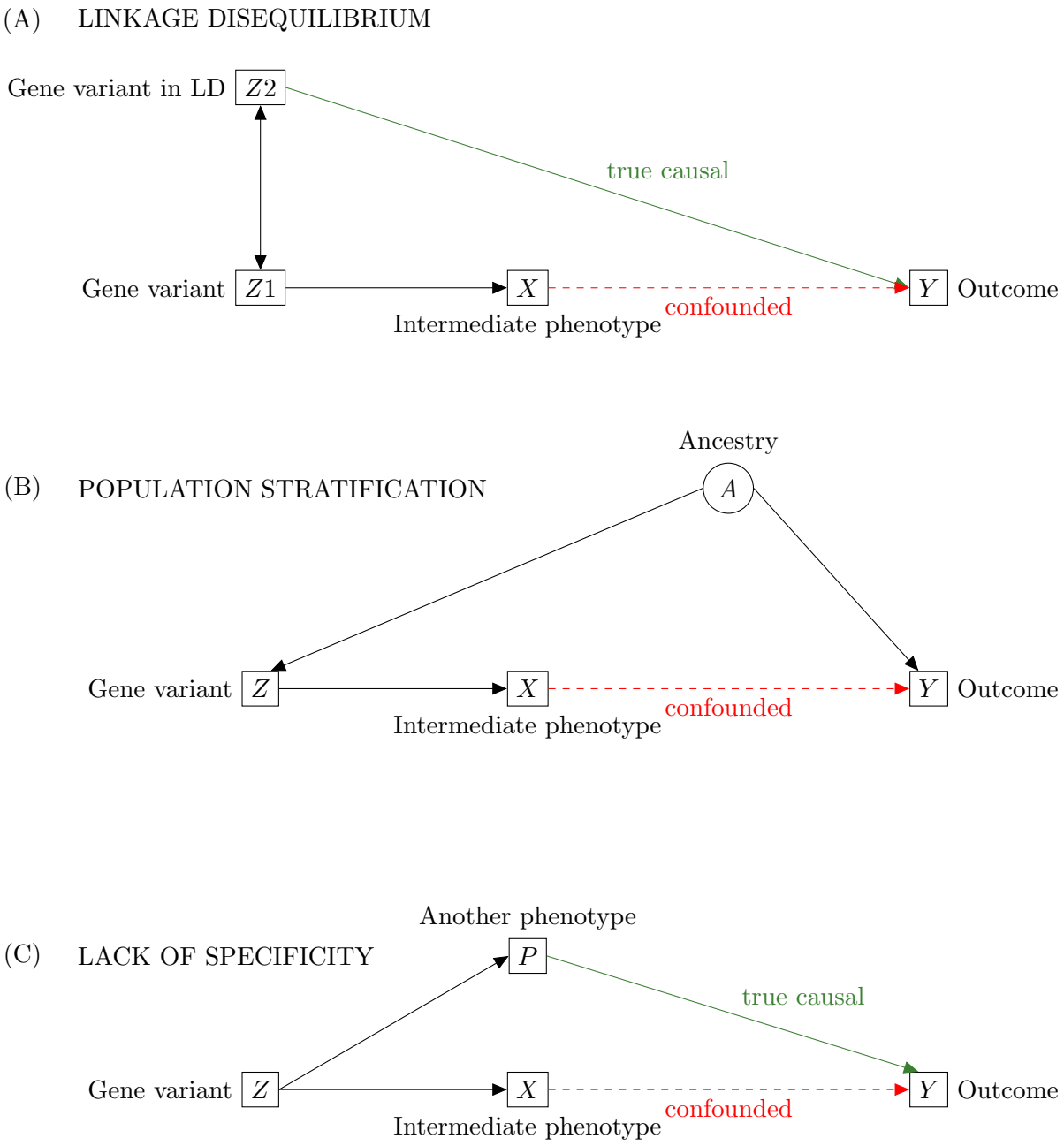


Figure 1.10: Potential confounding of Mendelian randomization studies.
(A) In this scenario, the original SNP (Z1) chosen as an instrument for the intermediate phenotype (X) is in LD with SNP Z2. Z1 is not associated with the outcome via the intermediate phenotype. However, an association between Z1 and Z2 through LD induces an association between Z1 and the outcome. This may then be erroneously interpreted as a causal association between X and Y.
(B) In this scenario, genotype frequency and disease risk are both associated with ancestry, which can confound the association between genotype and disease risk.
(C) In this scenario, the gene has associations with several intermediate phenotypes (X and P). If the sum causal effect is thought to be solely mediated via X, the magnitude of the association may be confounded.

Canalisation This oft-proclaimed limitation[95] suggests that developmental adaptations can mitigate the disease predisposition arising from deleterious genetic variants. In practise, few if any biological examples of canalisation in humans have

been convincingly demonstrated.[96] An alternative take on canalisation is human intervention to mitigate the effects of genetic variants - e.g. genetic predisposition to increased LDL-C may make an individual more likely to be prescribed lipid-lowering drugs (such as statins). This form of canalisation could mitigate the genetic effect.

These limitations are all, by and large, addressable if Mendelian randomization studies are adequately powered, and with careful selection of SNPs for use as genetic variants in instrumental variable analysis.

1.8 Using genetic information to guide prescription of medicines (pharmacogenetics)

A separate opportunity to translate genomic information is its potential use to group individuals into expected therapeutic response to a drug, so-called "personalized medicine".

As described above, owing to the inherent properties of design, randomized trials are the optimal method to judge the effect of an intervention on a health outcome. Results from randomized trials will yield an *average* treatment effect for all individuals. However, it is possible that *individual* characteristics (e.g. presence of comorbidities or levels of a trait of interest) may modify the individual response to a drug. This individual response may result in reduced efficacy and/or an increased risk of adverse effects, which collectively make a substantial contribution to the burden of ill-health.[97] If such treatment-modifying individual-level characteristics can be identified, this information can be used to used to target treatments to those predicted to respond more favourably to treatment: the basis of stratified or 'personalized' medicine.

The aims of stratified medicine are to:

1. reduce costs of drug prescribing, by limiting prescribing to selected individuals that are predicted to benefit from treatment (rather than to all individuals)
2. prevent adverse drug reactions, by preventing exposure of the drug to individuals with a characteristic that associates with adverse drug reactions
3. maximize therapeutic benefit, by limiting prescribing to individuals with markers that associate with beneficial outcomes

Stratified medicines can be categorized into two ways[98]: (Figure 1.11):

Absolute In this scenario, the treatment response in terms of relative risk is the same across subgroups, however the *absolute* risk difference differs by strata.
For example, primary prevention of CVD using statins is stratified using risk scores such as Framingham coronary risk score (FRS).[99] Guidance from National Institute for Health and Clinical Excellence (NICE) in England and Wales,[100] the European Society of Cardiology[101] and the American Heart Association[102] recommend statins in individuals estimated to have a 10-year risk of CVD in excess of an agreed threshold value, the precise value of which varies from guideline to guideline. The *relative* benefit of statins on risk of CVD is consistent according to

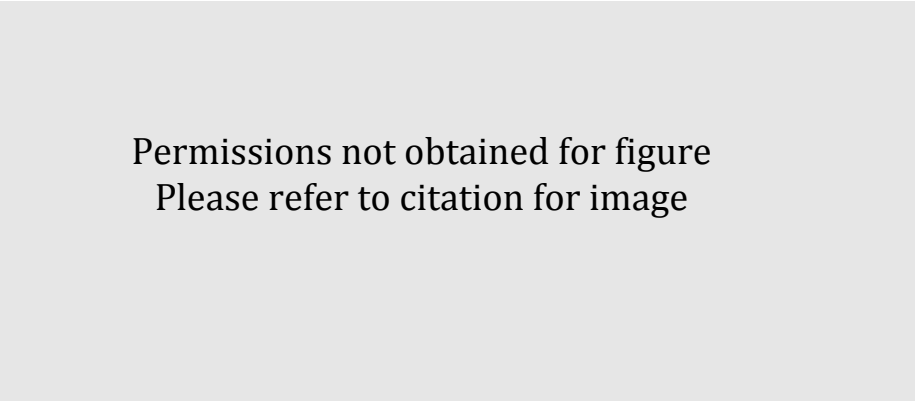


Figure 1.11: Comparison of differential treatment response based on absolute difference or relative difference In (A) the relative treatment response of the drug is constant across strata, however the absolute difference differs. In (B), the relative treatment response differs according to strata.
Reproduced from Hingorani et al.[98]

values of FRS. However, the *absolute* difference is greater in those with a higher FRS (as in Figure 1.11(a)). Identification of differences in absolute risk means that expensive drugs or drugs with high risks of side effects can be targeted to individuals that will derive most absolute benefit.

Relative This is the more conventional form of interaction, whereby a difference in the relative treatment effect occurs when stratifying by a subgroup (as in Figure 1.11(b)).

An example of this would be use of a genetic variable that encodes a drug target receptor, for example Epidermal growth factor receptor (EGFR) status and treatment response to the EGFR-inhibitor, gefitinib in patients with lung cancer for which evidence suggests patients experience a more beneficial treatment response to gefitinib when EGFR is highly expressed.[103, 104]

1.8.1 Traditional analysis of "effect modification"

The traditional analytical method to evaluate effect modification of individual characteristics on drug response (i.e. to identify differences in relative treatment effect) is sub-group analysis, through interaction testing. However, reliable detection of a differential effect among particular subgroups of patients requires larger-sample sizes (i.e. power is often limited to conduct subgroup analyses within conventional trials). Therefore it is not entirely surprising that most claims of sub-group effects have not been replicated.[105]

Use of genomic information for subgroup analyses (Figure 1.12) is discussed in further detail in Chapter 2, *CYP2C19* Genotype, Clopidogrel and Cardiovascular Disease).

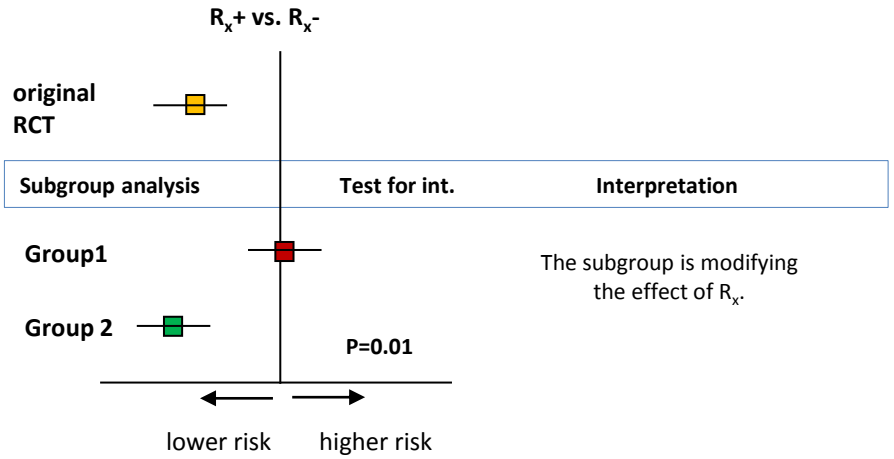


Figure 1.12: Schemata of pharmacogenetics As with any patient characteristic, genomic information can be used to classify individuals into groups. If this classification can be shown to modify treatment response (as in this figure, where individuals with genotype status in Group 2 receive a large risk reduction in response to treatment, whereas Group 1 seem to experience no benefit), then genomic information could be used to guide prescribing - this is a form of "personalized" medicine called "pharmacogenetics".

This type of subgroup analysis is desirable as it forms a means of 'factorial design' since both drug and SNP are randomly allocated. This is in contrast to traditional (phenotypic) subgroup analyses, which, can re-introduce confounding yielding misleading findings.[106, 107]

1.9 Thesis Themes

During the work for this PhD, I will use three different approaches to illustrate the contrasting applications of genomics to further the understanding of cardiovascular disease, with the ultimate aim of making improvements in public health.

Pharmacogenetics: in chapter 2, I will examine the evidence base regarding the clinical use of *CYP2C19* genotype to guide antiplatelet treatment.

Mendelian randomization for drug target validation: in chapters 3 to 5, I will investigate the role of secretory phospholipase A_2-IIA (sPLA$_2$-IIA) in CHD. sPLA$_2$-IIA is a pro-inflammatory enzyme that is thought to play important roles in the pathogenesis of CVD. I will use Mendelian randomization to provide a deconfounded association between sPLA$_2$-IIA and CVD risk.

Mendelian randomization for investigation of an environmental exposure: in chapters 6 to 8, I will use variation in a gene encoding an enzyme involved in the main metabolic pathway of alcohol, to investigate the evidence-base for a cardioprotective role of alcohol consumption.

Chapter 2

CYP2C19 Genotype, Clopidogrel and Cardiovascular Disease

2.1 Introduction

2.1.1 Principles of pharmacogenetics

For more than 50 years,[108] genetic variation has received recognition as a potential modifier of treatment response (pharmacogenetics). Gene polymorphisms can influence drug response by encoding enzymes involved in drug handling (absorption, distribution, metabolism and excretion; a "pharmacokinetic" action) and through encoding proteins that are drug targets (e.g. cell receptors; a "pharmacodynamic" action) (see Figure 2.1).

With the completion of the human genome project[109] and advances in genomic sequencing technologies, facilities that allow rapid and cheap genotyping are increasingly available. A recent surge in the number of published pharmacogenetic studies has occurred,[110] which, together with numerous commentary articles, has fuelled expectation that pharmacogenetics will help to deliver a genomic means to personalize drug prescribing (i.e. personalized or stratified medicine).[111–114]

To investigate why, despite 20 years of active research in this field, the output from the field of pharmacogenetics seemed to lag that of progress made in gene-disease association studies, I previously performed a systematic review and field synopsis,[110] that identified several features that might account for the slow translation from bench to bedside. These included:

- an excess of small studies (median sample size 93; Figure 2.2 (A))

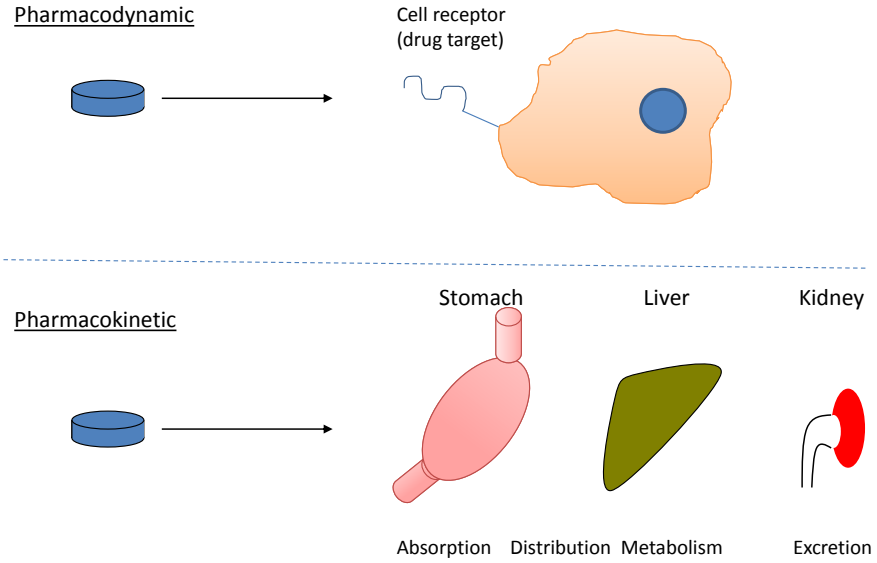


Figure 2.1: Contrast between pharmacodynamic and pharmacokinetic.
The term 'pharmacodynamic' refers to the action of the drug on the intended drug target (in the figure above, a cellular receptor), whereas 'pharmacokinetic' refers to the process by which enzymes in the body modify the drug (ADME: absorption, distribution, metabolism, excretion)

- a large proportion of studies (75%) reporting nominally significant ($P \leq 0.05$) results, suggesting possible reporting/publication bias (Figure 2.2 (B))
- a high proportion of reviews/commentaries to original articles (25:1) which may "stoke the flames" of expectation
- a lack of focus (so-called "mile-wide inch-deep" phenomenon[115]) on gene-drug combinations with many genes for which only single studies had been performed, meaning that evidence for replication was missing for many signals, but also hampering efforts to pool data for meta-analyses

Despite these issues, several high-profile pharmacogenetic tests have received attention for their potential clinical utility. Notable early successes in genes used to predict adverse response to HIV medications[116, 117] have become established in routine clinical care. Furthermore, a GWAs enabled the discovery of loci associated with statin-related myopathy[118]. However, these successes are the exception rather than the rule and have predominantly focused on *adverse reactions* to drugs: most pharmacogenetic tests of *intended effects* have yet to translate into clinical use.[119–121]

One of the most highly anticipated pharmacogenetic tests to predict intended drug effects was the use of *CYP2C19* genotype as a marker of identifying treatment response to the second most widely-prescribed drug in the world, clopidogrel.[122]

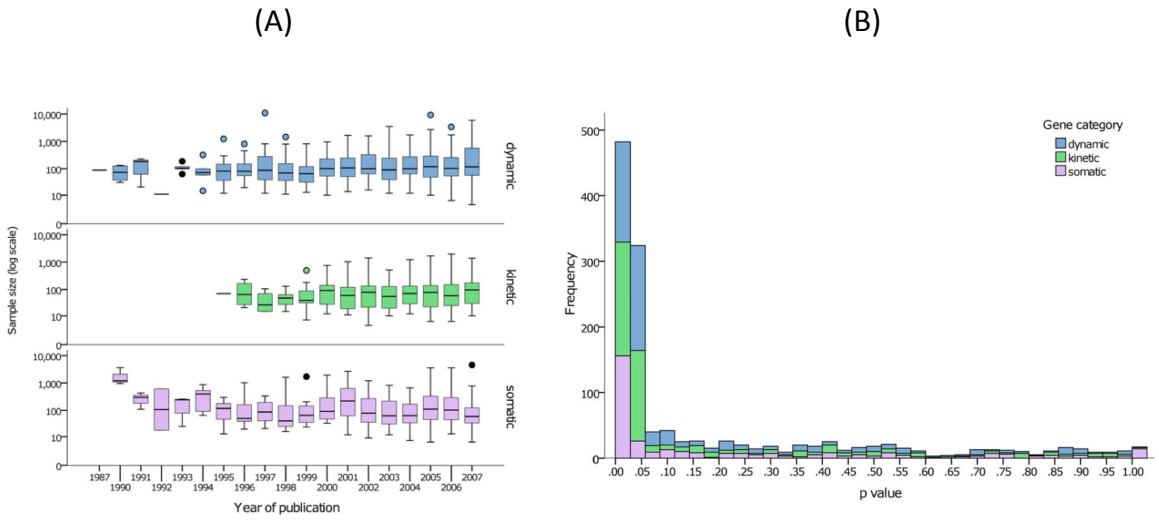


Figure 2.2: Characteristics of previous pharmacogenetic studies.
Panel (A) shows the median sample size for studies conducted between 1987-2007 (inclusive), panel (B) shows the P-values reported in full-text of 161 articles. For both, genes are classified according to whether they are the drug target (pharmacodynamic, shaded blue), involved in drug metabolism (pharmacokinetic, shaded green) or somatic variants (shaded purple).
Reproduced from Holmes et al[110]

2.1.2 Clopidogrel and Coronary Heart Disease

Anti-platelet agents such as aspirin and clopidogrel have proven highly effective in the treatment or prevention of a range of atherothrombotic disorders, including ACS, coronary stent thrombosis and stroke.[123, 124] A recent overview of randomized placebo-controlled trials including 7,384 cardiovascular events in 79,613 individuals with acute or chronic coronary heart disease or with multiple CHD risk factors indicated clopidogrel treatment reduced the odds of cardiovascular events (odds ratio [OR]: 0.88; 95%CI: 0.83, 0.93), but at the expense of a mechanism-based increase in odds of major bleeding (OR 1.28; 95%CI: 1.13, 1.45).[125] Clopidogrel was approved by the US Food and Drug Administration (FDA) in 1997 and is currently used by more than 40 million patients worldwide, being the second most widely prescribed drug after atorvastatin.[122] Therefore, any change in its prescription guidelines is likely to have important repercussions.

Because cardiovascular disease is common[1, 2] and anti-platelet drugs are the second most widely prescribed drugs worldwide,[122] they are also an important cause of admissions for gastrointestinal haemorrhage and other bleeding complications.[97] The risk of gastrointestinal bleeding lies between 1.3-12% within 30 days of commencing dual anti-platelet therapy.[123, 124] Therefore, to maximize benefit and minimize harm, clinicians currently target patients at highest risk of thrombotic events and the lowest risk of bleeding, based on routine clinical assessments. However, there is emerging concern

that individuals may differ both in their response to the anti-thrombotic effects, and their susceptibility to the haemorrhagic complications of anti-platelet therapy.[126–132]

It is suggested that the average 12% relative risk reduction in major CV events ascribed to clopidogrel (as well as the 28% increased risk of major-bleeding) could differ between subgroups based on presence of *CYP2C19* gene variants.[120] Since 2008, several genetic studies aiming to evaluate this have been conducted, and by 2010, using the available evidence at the time, the FDA issued a boxed warning[133], used to highlight potentially fatal, life-threatening or disabling adverse effects and recommended consideration of *CYP2C19* genotype prior to prescribing clopidogrel, warned of reduced effectiveness in patients who are poor metabolizers, and advised clinicians of the availability of a test to identify genetic differences in *CYP2C19* function.[133] One year after the FDA warning, a more cautious announcement was made by the American Heart Association/American College of Cardiologists, arguing insufficient evidence and advising physicians to adhere to current (non-genetic) guidelines.[134] Amid this uncertainty, FDA-cleared tests for *CYP2C19* genotype[135] were offered direct to consumers with sales escalating.[136]

2.1.3 Clopidogrel metabolism

Clopidogrel is a pro-drug, converted to its active form through metabolism by the hepatic cytochrome P450 (CYP450) enzyme system including CYP450 2C19 (CYP2C19) (Figure A.2). Genetic variants in *CYP2C19* have been associated in vitro with differences in CYP2C19 enzyme activity, as indicated by small mechanistic studies with carriers of some *CYP2C19* alleles (e.g. $\star$2) showing reduced, and carriers of other alleles (e.g. $\star$17) having increased activity.[137–139] Individuals have therefore been classified as either slow or fast metabolizers based on *CYP2C19* genotype, which is assumed to modify the levels of active clopidogrel metabolites, platelet reactivity and thus the mechanism-based benefits (reduction in CVD events) and harms (increased bleeding) arising from clopidogrel treatment (see Figure A.1 for further explanation).

2.1.4 GWAs of clopidogrel response

To date, only one GWAs of clopidogrel response has been conducted. In the Amish PAPI study, Shuldiner et al[140] administered clopidogrel to 429 healthy Amish adults and measured response to clopidogrel through adenosine diphosphate-stimulated platelet aggregation. In GWAs analysis, 13 SNPs on chromosome 10q24 (in strong LD with each other) were identified to associate with platelet aggregation. Of these, rs1277823 showed strongest association at P=1.5 x 10^{-13}. Of note, this SNP was in LD (at R^2=0.87) with

the SNP that characterizes *CYP2C19**2 (rs4244285).

No GWAs has been conducted to investigate clinical outcomes such as CVD or bleeding events in relation to clopidogrel treatment.

2.1.5 Technological advances enabling pharmacogenetic analyses

Point of care testing (POCT) is now available and enables a rapid means to determine an individual's *CYP2C19* genotype.[141] The availability of this and other FDA-approved 'devices'[135] means that it is now possible to perform the genetic test in the clinic to rapidly assign an individual to presence/absence of reduced-function *CYP2C19* alleles. However, what is the evidence base for use of *CYP2C19* genotype in informing choice of antiplatelet drug?

2.2 Aims

To ascertain the totality of research evidence, I conducted a systematic review and critically appraised existing evidence underpinning *CYP2C19* loci and clopidogrel response.

2.3 Methods

2.3.1 Search Strategy

I followed guidance from the Human Genome Epidemiology Network (HuGEnet) on gene disease association studies[142] and the reporting of systematic reviews from PRISMA.[143] I searched PubMed and EMBASE from inception to 25th October 2011. The search terms were adapted from a previous HuGENet article[144] and comprised: (i) drug name (including the generic name, clopidogrel, and trade names e.g. Plavix, clopilet etc.); (ii) metabolic enzymes (including *CYP2C19*), and; (iii) gene (please see Appendix A for details of the full search strings) to identify studies describing patients treated with clopidogrel and reporting treatment response (platelet response or clinical outcomes) in relation to *CYP2C19* genotype (Ensembl Gene ID:ENSG00000165841). Articles with abstracts containing the keywords clopidogrel and *CYP2C19* that reported new data were eligible for inclusion (i.e. editorials and reviews were omitted). I additionally interrogated bibliographies of included articles, identified previous meta-analyses and narrative reviews and searched articles listed in the Pharmacogenomics Knowledge Base

(http://www.pharmgkb.org, accessed October 25th 2011), Medicines and Healthcare products Regulatory Agency (MHRA, UK Department of Health) and FDA. Articles were not restricted to a language.

2.3.1.1 Systematic review of randomized trials of more vs less clopidogrel

In addition to the main search, I also identified randomized trials that compared more vs. less clopidogrel. This was in order to able to contextualize the effect of *CYP2C19* on CVD risk.

I searched PubMed from inception to July 2011 using the search criterion "clopidogrel" and restricted the search to randomized clinical trials in humans using the PubMed built-in filter. The retrieved abstracts were filtered for more versus less clopidogrel randomized trials. I examined the outcomes CVD and major bleeding. Analysis was limited to the most widely-reported clopidogrel doses (600mg versus 300mg loading dose). I noted the number of events per treatment arm and synthesized the relative risk. I used fixed-effect (Mantel-Haenszel) meta analysis to pool summary estimates in Stata v11.2.

2.3.2 Data extraction

I extracted information on 2 separate occasions and checked for consistency of data to minimize error. Information extracted included: study design; duration of follow-up; proportion of eligible individuals included in the pharmacogenetic analysis; recruitment criteria e.g. acute coronary syndrome (ACS), emergency or elective percutaneous intervention or stable CHD; and the proportion of individuals receiving concomitant therapy with proton pump-inhibitors or aspirin. In the case where it was not clear what outcomes were reported, I contacted the corresponding authors. Any remaining uncertainties were resolved by consensus with my supervisors.

To evaluate risk of bias, I recorded how studies ascertained clinical outcomes, whether studies reported if investigators were blinded to clinical status when ascertaining *CYP2C19* genotype, genotype indices (Hardy Weinberg equilibrium and call rate) and the source of funding.

2.3.3 Grouping of *CYP2C19* for genetic analyses

The main analysis compared individuals with one or more copies of any *CYP2C19* genetic variant associated with reduced enzyme function (i.e. ⋆2, ⋆3, ⋆4, ⋆5, ⋆6, ⋆7, ⋆8) with individuals categorized as having none of these alleles (⋆1/⋆1) or having one or more ⋆17 gain-of-function alleles (the reference group). I separately conducted analyses of the effect of one loss-of-function or two loss-of-function alleles with the same reference group (to investigate evidence of a genetic dose-response relationship).

2.3.4 Cardiovascular outcomes

The main cardiovascular outcome for the analysis was a composite including any or all of the following: all-cause mortality, fatal/non-fatal CHD, fatal/non-fatal stroke, stent thrombosis, target vessel revascularization and hospitalization for ACS. Studies that exclusively reported stent thrombosis (and no other outcome) were excluded from the primary analysis, but were included in the analysis for stent thrombosis. Additionally I conducted separate analyses for the following component end-points: fatal and non-fatal MI and stroke, stent thrombosis and all-cause mortality. In studies that only reported hazard ratios, if provided I used the hazard proportion to estimate the number of events per genotype group, otherwise I substituted the relative risk for the summary hazards ratio. In one study that did not provide numerical values of effect estimates, I derived the point estimates and 95%CI from the published forest plots.[145]

2.3.5 Clopidogrel drug metabolite

For analysis of the association of *CYP2C19* genotype and clopidogrel metabolites, I used data from the largest study by Mega *et al*[146]. For studies of *CYP2C19* genotype and platelet function, I noted the number of individuals and the mean (or median) and standard deviation (or interquartile range) for the relevant platelet function measure in different genotype categories.

2.3.6 Analyses

For binary outcomes, I used relative risk (RR) as a measure of effect. I performed meta-analyses using fixed and random effects modeling and quantified between-study heterogeneity using the I^2 statistic[147] and Cochrane's Q statistic. I investigated sources of heterogeneity using meta-regression and used the method described by Altman and Bland[148] to test for interaction of summary estimates. I restricted the analysis of

platelet reactivity to studies with more than 500 participants and calculated standardized mean differences using fixed-effect (inverse variance) models.

I assessed small study bias through: (i) comparison of effect estimates in studies stratified by number of cardiovascular events (1-99,100-199,≥200); (ii) the Harbord test;[149] (iii) visual inspection of funnel plots; and (iv) trim and fill analysis.[150] To evaluate the change in effect estimates with the addition of new evidence, I synthesized cumulative meta-analysis plots.

I used $P<0.05$ to suggest evidence against the null hypothesis of no association and all statistical tests were 2-sided. I used Stata version 11.2 for all statistical analyses.

2.4 Results

2.4.1 Study designs, genotyping and reported outcomes

I identified 32 studies[138, 140, 145, 146, 151–177] including 3,545 CVD events in 42,016 participants (Figure 2.3), with a weighted mean age of 63, of which 29% were female (Table 2.1).

Only 6 out of 32 studies were nested within a RCT: in five of these, *CYP2C19* genotyping was conducted in participants from both the clopidogrel and comparator arms of a randomized trial, and of these, for four (referred to as "effect modification" studies) the comparator was placebo (CURE, ACTIVE-A[164], CHARISMA[151] and CLARITY-TIMI[145]), and in one (PLATO)[173] the comparator was prasugrel. In the pharmacogenetic substudy of the TRITON-TIMI trial[146] only the clopidogrel arm was genotyped.

The remaining 26 studies were either prospective cohorts (24) or case-control (2) studies limited to individuals receiving clopidogrel (referred to as "treatment-only" studies).[138, 140, 145, 152–163, 165–172, 174–177] The majority of studies (21 of 32) included patients with ACS at the time of recruitment and 8 included only patients with stable CHD, mainly recruited at the time of placement of coronary stents (CHD status was not reported in the remaining 3 studies). Of the 28 star alleles reported to date in *CYP2C19*,[178] only 13 (the loss of function *2 to *10 and *12 to 14 inclusive, as well as the gain-of-function *17) have been evaluated in the pharmacogenetic studies of clopidogrel. The ⋆2 allele was typed by 31 of 32 studies (97%); both ⋆3 and ⋆17 alleles each by 12 studies (38%), and the remaining ten ⋆ alleles were genotyped in 5 or fewer studies. Only 6 of the 28 SNPs that uniquely identify the known *CYP2C19* ⋆ alleles were listed in the Human HapMap (builds 21, 22 and 3(2)).[179] Only two studies reported detailed

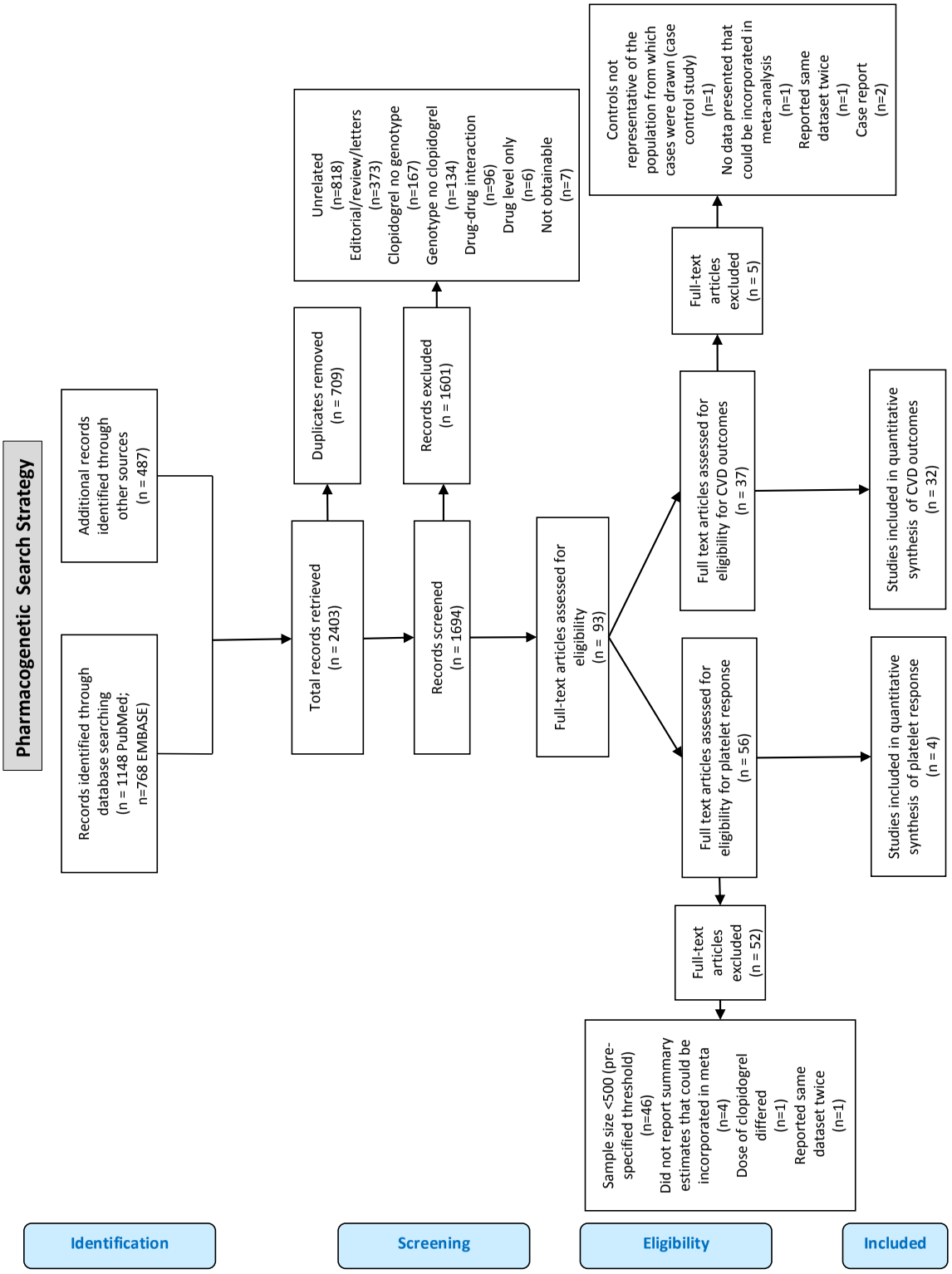


Figure 2.3: PRISMA flow diagram for systematic review of *CYP2C19* and clopidogrel response.

linkage disequilibrium between *CYP2C19* SNPs[140, 159] and one study imputed an un-typed SNP.[145]

The genotyping and reporting of the nine other genotyped alleles varied considerable among studies (Table 2.1). Over half of studies (15 of 25 that reported sources) were funded directly or via study investigators by the manufacturer of a new, competitor anti-platelet agent, prasugrel (Table A.1).[180]

Other study characteristics including risk of bias are reported in Table A.1.

2.4.2 Outcomes evaluated

Twenty-six of 32 studies (81%) evaluated a composite outcome, but the individual components varied substantially between studies and included both hard clinical end-points (e.g. ST-segment elevation MI) and softer end-points (such as hospitalization for ACS or target vessel revascularization). A considerable proportion of disease outcomes were ascertained but were not reported either individually or as part of the combined end point (Tables A.2 and A.3).

2.4.3 Effects of *CYP2C19* genotype on clopidogrel metabolites and platelet reactivity

At a dose of 75mg of clopidogrel (the usual maintenance dose) individuals with the *CYP2C19* alleles ⋆2 , ⋆3, ⋆4, ⋆5, ⋆6, ⋆7 or ⋆8 associated with lower enzyme activity, had a 0.14 unit reduction in geometric mean active metabolite concentration ($AUC_{0\text{-}t}$; $\mu\times$hour) compared with individuals carrying ⋆1 or ⋆17 alleles (Figure 2.4). A similar reduction in exposure to the active clopidogrel metabolite was observed for the same genotype comparison at the 600mg clopidogrel dose (0.13 unit reduction in geometric mean active metabolite concentration; $AUC_{0\text{-}t}$; $\mu\times$hour). Of note, at both doses of clopidogrel, the difference in the active clopidogrel metabolite concentration between the two *CYP2C19* genotype categories (⋆2-⋆8 vs. ⋆1 or ⋆17) was smaller than the difference between the overall concentration difference between treated and untreated individuals, regardless of the *CYP2C19* genotype (Figure 2.4).
In four studies using the treatment only approach (4,341 individuals; Table A.5)[167, 172, 181, 182] reporting platelet reactivity following 600mg clopidogrel, a genotype dosage-dependent response was observed with number of ⋆2 alleles and increased platelet aggregation (i.e. reduced platelet inhibition) when compared to subjects with ⋆1/⋆1 genotype (Figure 2.5).

Table 2.1: Characteristics of studies included in the systematic review

First Author / Study Name (Year of Publication)	Outcome(s) reported	ACS at study entry (%)	Women, %	Age, Mean, (SD), y	Participants (n, % of total sample size)	Follow-up, Months (range)	*CYP2C19* ⋆ alleles geno-typed
Anderson *et al* [152]/ IHCS (2009)	CVD-c, MI, death	NR	27	63 (NR)	1250 (100)	12 (NR-24)	2
Bouman *et al* [153]/ MAPCAT (2011)	ST	50.9	21	61.3 (7.7)	112 (1.5)	18 (NR)	2, 17, (3-8)
Campo *et al* [154]/ NA (2011)	CVD-c, death, ST, bleeding	61	23	66 (13)	300 (59.2)	12 (NR)	2,17
Collet *et al* [155]/ AFIJI (2009)	CVD-c, MI. ST, death	100	7.7	40.1 (5.1)	259 (68.5)	12 (NR-96)	2 (3-6)
Giusti *et al* [156]/ RECLOSE (2009)	CVD-c, ST, death	65.67	25.4	69 (11)	772 (96)	6 (NR)	2
Harmsze *et al* [159]/ NA (2010)	ST	24.6	20.5	62.1 (9.4)	596 (NA)	NA	2, 3
Harmsze *et al* [157]/ POPular (2011)	CVD-c	0	24	63.2 (10.2)	725 (68)	12 (NR)	2, 17
Jeong *et al* [158]/ACCEL (2011)	CVD-c	100	30.9	63.0 (12.4)	266 (48)	>12 (NR)	2, 3, 17
Komarov *et al* [160]/NA (2011)	CVD-c	0	NR	59.4 (NR)	399 (NR)	18 (NR)	2
Malek *et al* [138]/ NA (2008)	MI, ST	100	30	60 (11.4)	105 (100)	12 (NR)	2
Malek *et al* [161]/ NA (2010)	Death	100	33	60(11)	261 (94.6)	48 (NR)	2

Continued on next page

Table 2.1 – *Continued from previous page*

First Author / Study Name (Year of Publication)	**Outcome(s) reported**	**ACS at study entry (%)**	**Women, %**	**Age, Mean, (SD), y**	**Participants (n, % of total sample size)**	**Follow-up, Months (range)**	***CYP2C19* ⋆ alleles genotyped**
Oh *et al* [162]/ Sky Registry (2011)	CVD-c, MI, death, ST	20.6	34.3	60.8 (9.8)	2146 (64.8)	12 (NR)	2
Ono *et al* [163]/NA (2011)	CVD-c	0	24.3	68.8 (9.8)	202 (100)	≤ 12 (NR)	2, 3
Sawada *et al* [165]/ NA (2010)	CVD-c, MI, death, ST	9	15	69.6 (9.9)	100 (27.3)	8 (3)	2
Shuldiner *et al* [140]/ Sinai Hospital of Baltimore Study (2009)	CVD-c	0	40.1	64.3 (11.2)	227 (100)	12 (NR)	2
Sibbing *et al* [168]/ ISAR-REACT, ISAR-REACT 2, ISAR-SMART 2, ISAR-SWEET (2009)	CVD-c, death, MI, stroke , ST	34	22	66.5 (10.2)	2485 (46.2)	1 (0.8, 1.2)	2
Sibbing *et al* [166]/ NA (2010)	CVD-c, death, MI, ST, bleeding	11	22.6	67.4 (10.7)	1524 (94.8)	1 (NR)	17
Sibbing *et al* [167]/NA (2011)	ST	NR	22.4	67.5 (10.4)	1566 (95)	NA	2
Simon *et al* [169]/ FAST-MI (2009)	CVD-c	100	29.4	66.2 (12.2)	2208 (60.2)	12 (NR)	2-5,17
Tello-Montoliu *et al* [170]/NA (2011)	CVD-c	100	NR	NR	471 (95.5)	6 (NR)	2, 17
Tiroch *et al* [171]/ NA (2010)	CVD-c, death, MI, ST, stroke	100	25	64.8 (12.7)	928 (100)	12 (NR)	2, 17

Continued on next page

Table 2.1 – *Continued from previous page*

First Author / Study Name (Year of Publication)	**Outcome(s) reported**	**ACS at study entry (%)**	**Women, %**	**Age, Mean, (SD), y**	**Participants (n, % of total sample size)**	**Follow-up, Months (range)**	***CYP2C19* ⋆ alleles genotyped**
Trenk *et al* [172]/ EXCELSIOR (2008)	CVD-c	0	21.8	66.4 (9.1)	797 (99.4)	12 (NR)	2
Worrall *et al* [174]/ NA (2009)	CVD-c	100	NR	NR	104 (40.2)	12 (NR)	2
Yamomoto *et al* [175] /NA (2011)	MI, death, stroke	0	33	68.6 (10.0)	123 (100)	12 (NR)	2,3
Yan *et al* [176]/ NA (2011)	CVD-c	100	17.3	65.2 (10.7)	497 (100)	20 (NR)	2
Yuan *et al* [177]/ NA (2011)	CVD-c	NR	NR	NR	267 (NR)	12 (NR)	2
Mega *et al* [146]/ TRITON-TIMI 38 (2009)	CVD-c, MI, stroke, ST, bleeding	100	29.3	60.1 (11.1)	1477 (10.9)	NR (6, 15)	2-10 (7, 12-14)
Wallentin *et al* [173]/ PLATO (2010)	CVD-c, bleeding, ST	100	31	62.5 (11.0)	10285 (55.2)	NR (NR, 12)	2-8, 17
Bhatt *et al* [151]/ CHARISMA (2009)	CVD-c, bleeding	0	29.7	64 (9)	4862 (31.2)	28 (NR)	2, 3, 17
Mega *et al* [145]/ CLARITY-TIMI 28 (2008)	CVD-c	100	19.7	57.5 (10.3)	465 (13.3)	1 (NR)	2
Pare *et al* [164]/ ACTIVE-A (2010)	CVD-c, bleeding	0	45.4	71.0 (9.9)	1156 (15.3)	43.2 (NR)	2, 3, 17
Pare *et al* [164]/ CURE (2010)	CVD-c, bleeding	100	41	63.8 (11.0)	5059 (40.3)	NR (3, 12)	2, 3, 17

Abbreviations: ACS, acute coronary syndrome, comprising ST-segment elevation myocardial infarction, nonST-segment elevation myocardial infarction, and unstable angina; CVD-c, cardiovascular disease composite; MI, myocardial infarction; NA, not applicable; NR, not reported; PCI, percutaneous coronary intervention; PGx,

pharmacogenetic; STTH, stent thrombosis.

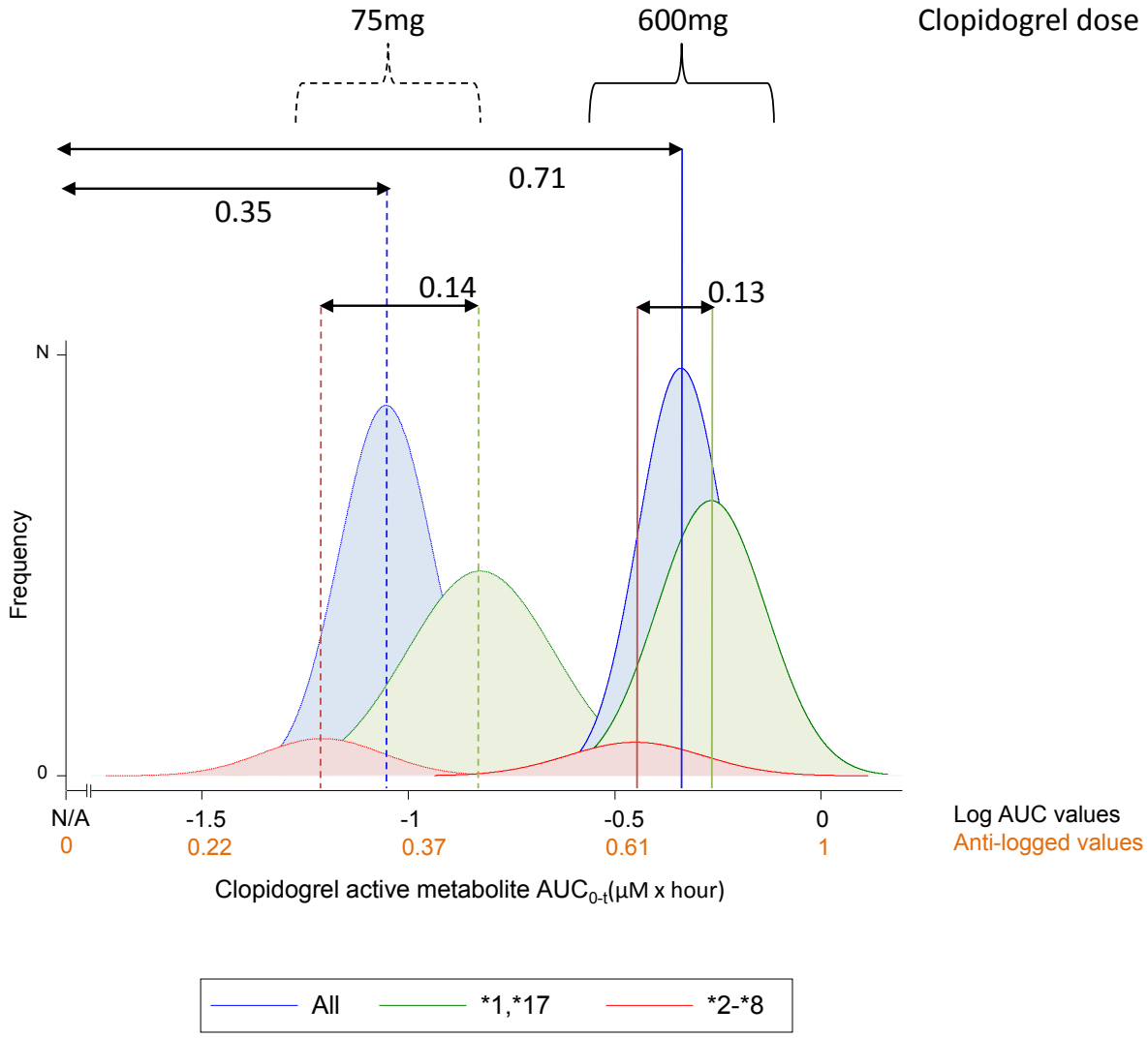


Figure 2.4: Relationship between *CYP2C19* genotype and active drug metabolite. The expected mean active clopidogrel metabolite concentration in a Caucasian population for all individuals treated at 75mg and 600mg, and for individuals with loss-of-function and normal/increased function *CYP2C19* alleles.

The mean active clopidogrel metabolite concentration regardless of genotype $AUC_{0\text{-}t} = 0.35$ μ×hr; difference in clopidogrel active metabolite concentration between *2-*6 and *1 or *17 $AUC_{0\text{-}t} = 0.14$ μ×hr. The central tendency and measure of dispersion are obtained from Mega *et al*[146]: *CYP2C19**1 or *17 summary estimates were pooled from ultra and extensive metabolizer groups and *2-*6 from intermediate and poor metabolizer groups. The height of the plots are proportional to the allele frequency of *2 (the most common loss-of-function * allele; rs4244285 MAF=0.13, Caucasians, dbSNP); i.e. as *1 is more common (87%) than *2 (13%), the height of the plot for the *1 or *17 group is higher than that for *2, reflecting the number within the population that will harbor this genotype.

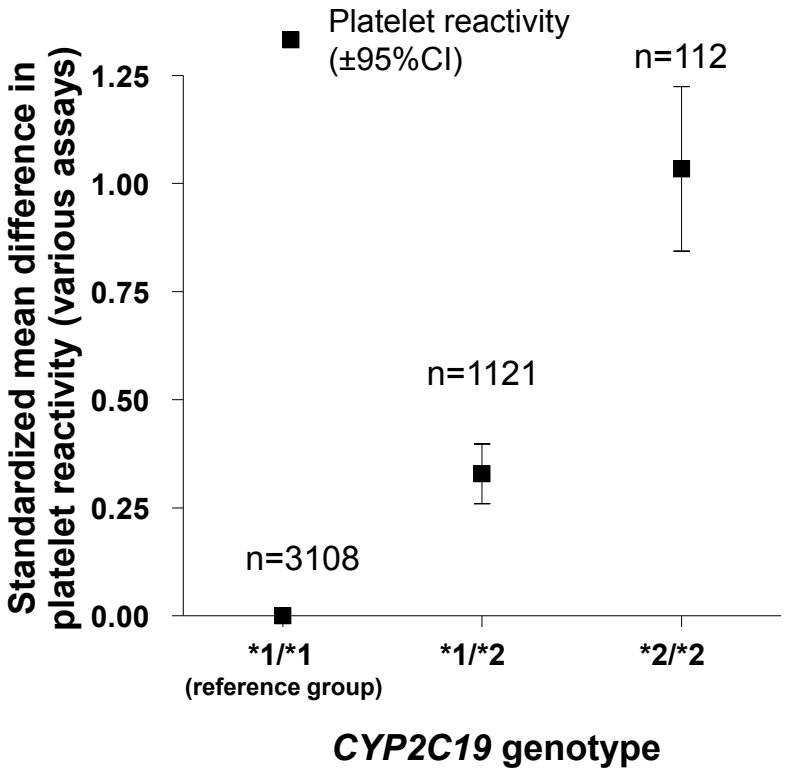


Figure 2.5: Relationship between *CYP2C19* genotype and platelet reactivity.
Meta-analysis of four treatment-only studies[167, 172, 181, 182](Table A.5) including 4,341 individuals reporting *CYP2C19* genotype and platelet reactivity following a 600mg loading dose of clopidogrel.

2.4.4 Association of *CYP2C19* genotype and the composite CVD outcome on treatment-only analysis

A pooled analysis of 22 studies using the treatment-only approach[138, 140, 146, 152, 154–158, 160–163, 165, 168–172, 174, 175, 177](i.e. all study participants were exposed to clopidogrel with no comparison arm), supplemented by a treatment-only analysis using data from the clopidogrel-treatment arm of 4 randomized trials[151, 164, 173] with a total 2,465 clinical events amongst 26,251 individuals, indicated that individuals with any copy of *CYP2C19*⋆2 to ⋆8 had an increased relative risk (RR) of CVD events (RR 1.18; 95%CI: 1.09, 1.28; I^2= 60%; 95%CI: 38%, 75%) using fixed-effects and RR 1.34 (95%CI: 1.15, 1.56) using random-effects models when compared to individuals with ⋆1 or ⋆17 alleles (Figure 2.6).

Meta-cumulative analysis, with the genotype group reversed to make the comparison similar to a more vs. less intensive dose of active clopidogrel metabolite, showed that at the time of US Food and Drug Administration approval, individuals with ⋆1 or ⋆17 had a RR of CVD events of 0.82 (95%CI: 0.72, 0.93) for fixed-effect and RR 0.72 (95%CI: 0.57, 0.92) for random-effects modeling compared to individuals with ⋆2 through ⋆8,

with some attenuation of the summary estimate with addition of new studies (most recent estimate: RR 0.86; 95%CI: 0.79, 0.94 for fixed-effect Figure 2.7, and RR 0.76; 95%CI: 0.65, 0.89 for random-effects modeling).

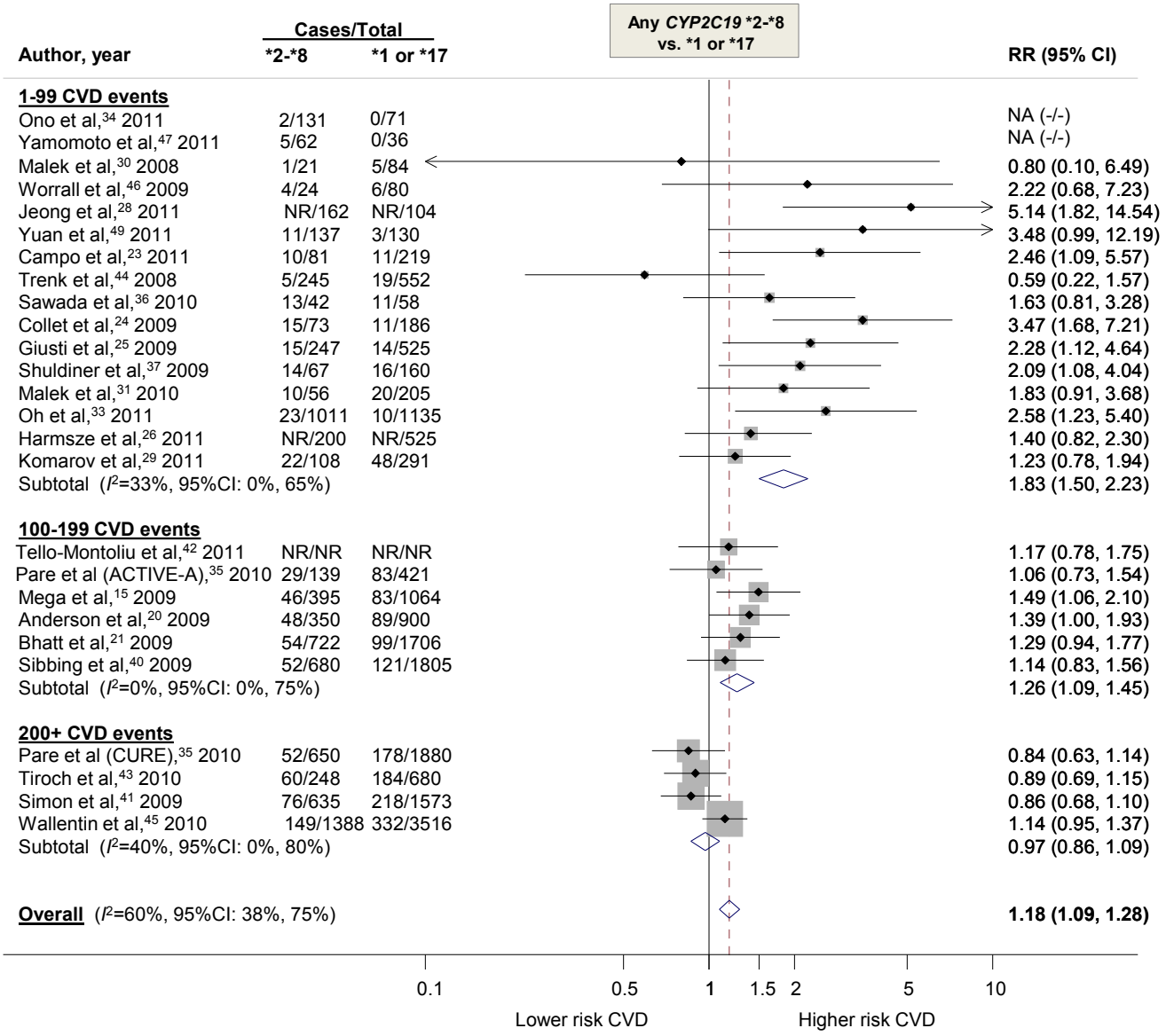


Figure 2.6: Effect of *CYP2C19* genotype on composite cardiovascular outcome in individuals treated with clopidogrel, Treatment-only analysis.

Meta-analysis of risk of composite cardiovascular outcome comparing any copy of *CYP2C19**2 through *8 to wild-type (*1) or *17 (reference), stratified according to the number of events (1-99, 100-199, $\geq$ 200) per study. Plot shows fixed-effects meta analysis model.

2.4.4.1 Assessment for risk of bias

When I stratified studies according to the number of events, a clear trend towards the null was observed in larger studies (Figure 2.6), providing strong evidence of small study bias (P=3.2×10^{-7} on χ^2 test for heterogeneity). The Harbord test for small-study bias

was positive (P=0.001) and the funnel plot was asymmetric (Figure 2.8). When I quantified the potential impact of small study bias using the trim and fill analysis, after addition of 6 hypothetical missing studies, the summary RR decreased from 1.18 to 1.08 (95%CI: 0.997, 1.178). In the four largest studies reporting ≥200 CVD events (58% of all reported events) the summary RR was 0.97 (95%CI: 0.86, 1.09).

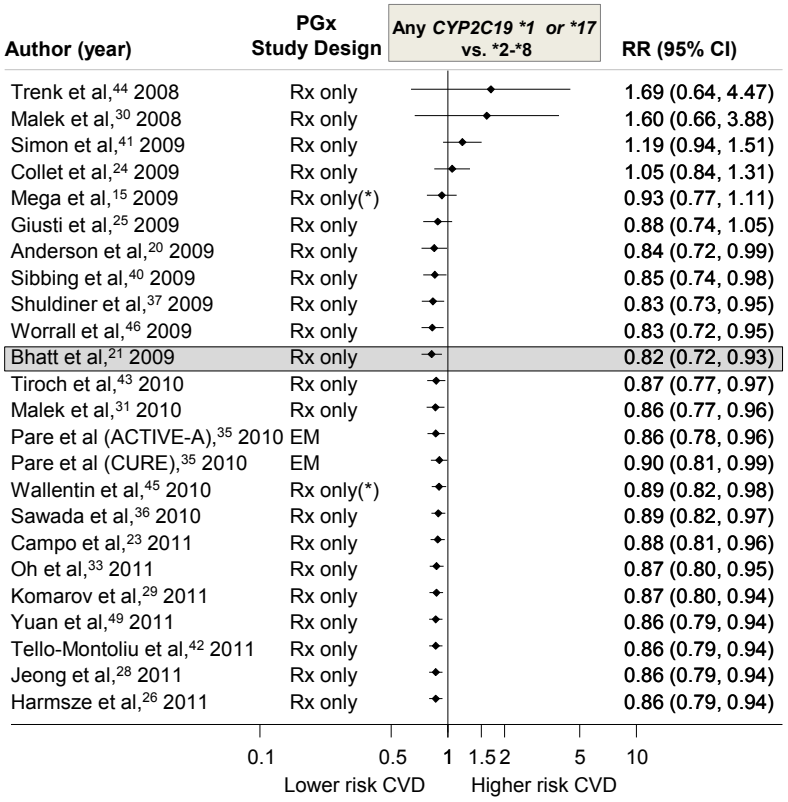


Figure 2.7: Meta-cumulative plot of *CYP2C19* wild-type (⋆1) or ⋆17 compared to ⋆2-⋆8, to make the relative risk directionally consistent with a more-versus-less clopidogrel trial. The shaded box shows the level of evidence at the time of FDA boxed warning[133] and attenuation of the summary estimate with subsequent studies.
EM: effect modification; Rx: treatment; ⋆ although set in an RCT, effect modification analysis was not permitted. Plot shows fixed-effects meta analysis model.

Subgroup analyses by the main study characteristics did not yield evidence that the summary estimate differed according to type of CHD at baseline (ACS or stable CHD), source of funding (Pharma or Academia), whether or not patients received concomitant proton pump inhibitors (some of which, such as omeprazole, are thought to inhibit the CYP2C19 enzyme[183]) or whether the study investigators were blinded to the outcome when ascertaining genotype (Figure A.3).

When each study was omitted, one at a time, in the analysis between any reduced function *CYP2C19* allele and risk of the composite CVD outcome, I did not find evidence

that any one study overly-influenced the analysis. (Figure A.4)

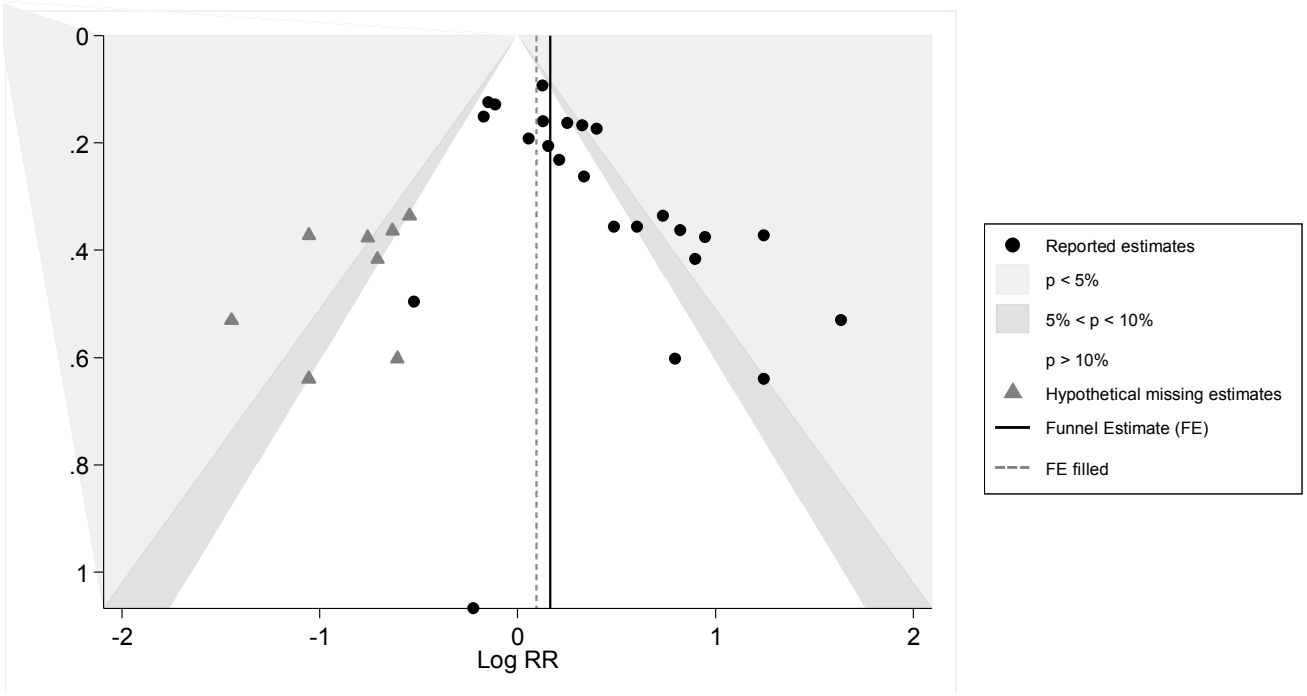


Figure 2.8: Funnel plot and trim-and-fill analysis. Trim and fill contour-enhanced funnel plot of the pharmacogenetic studies comparing *CYP2C19* ⋆2 through ⋆8 versus ⋆1 or ⋆17 and risk of CVD events in treatment-only analysis.
Footnote: Circles represent summary estimates from the 24 pharmacogenetic studies contributing towards the treatment-only analysis in Figure 2.6 with the summary estimate shown by the solid vertical line. Triangles represent 8 hypothetical missing studies generated by the Trim and Fill analysis with the dashed vertical line illustrating the summary estimate which includes the hypothetical studies.

2.4.5 Association of *CYP2C19* genotype and the individual outcomes on treatment-only analysis

Examining the individual outcomes on treatment-only analysis identified that individuals with any copy of *CYP2C19*⋆2 to ⋆8 had a higher risk of fatal/nonfatal MI (RR 1.37; 1.13, 1.65), nonfatal MI (RR 1.48; 95%CI: 1.05, 2.07) and higher risk of stent thrombosis (RR 1.75; 95%CI 1.50, 2.03). However, for stent thrombosis, as with the composite CVD outcome, a trend towards the null with larger sample sizes was also observed (Figure 2.9).

In contrast, individuals carrying any copy of *CYP2C19*⋆2 to ⋆8 alleles had a lower risk of all-cause bleeding when compared to individuals carrying the ⋆1 or ⋆17 allele (RR: 0.85; 95%CI 0.76, 0.95; Figure 2.9). Values were similar under a random-effects meta-analysis model (Table A.6).

2.4.6 Investigation of a genetic dose-response relationship

Eleven of 32 studies involving 10,291 individuals provided data that allowed me to obtain the effect of one loss-of-function *CYP2C19* allele (>238 CVD events) or two loss-of-function *CYP2C19* alleles (>37 CVD events) compared to the *CYP2C19*⋆1 or ⋆17 reference group. The RR of CVD events among carriers of one loss-of-function allele was 1.77 (95%CI: 1.27, 2.47) for fixed-effect and 2.01 (95%CI: 1.21, 3.34) for random-effects modeling in studies with <100 cases. In studies with ≥ 100 events, the values for the same exposure were RR 0.94 (95%CI: 0.80, 1.10) for fixed effect and RR 0.95 (95%CI 0.78, 1.15) for random-effects modeling.

By comparison, the RR of CVD events among carriers of two loss-of-function alleles was 3.75 (95%CI: 2.40, 5.86) for fixed effect and 3.76 (95%CI 2.34, 6.06) for random-effects modeling in studies with <100 cases and 1.52 (95%CI: 1.04, 2.21) for fixed effect and 1.45 (95%CI 0.82, 2.56) for random-effects modeling in studies with ≥ 100 cases (Figure 2.10).

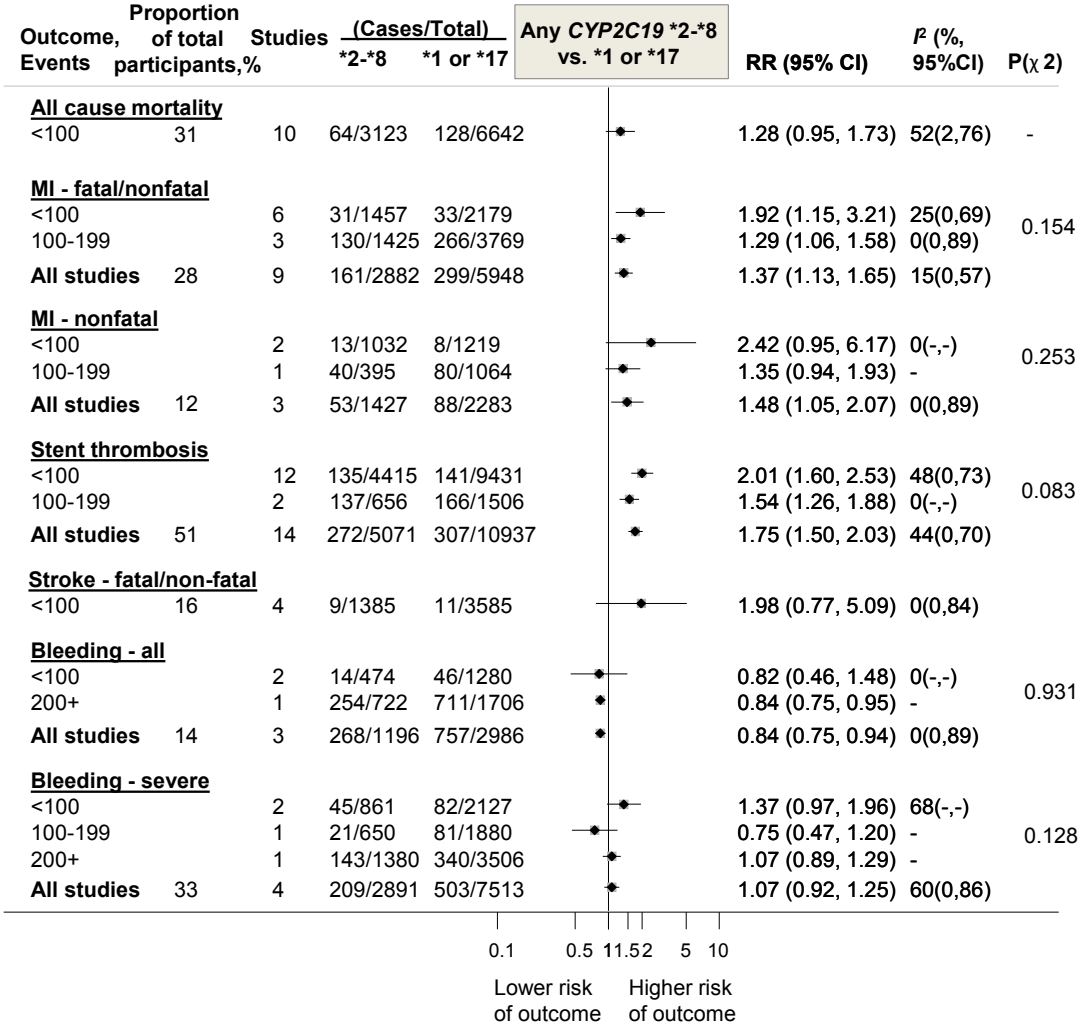
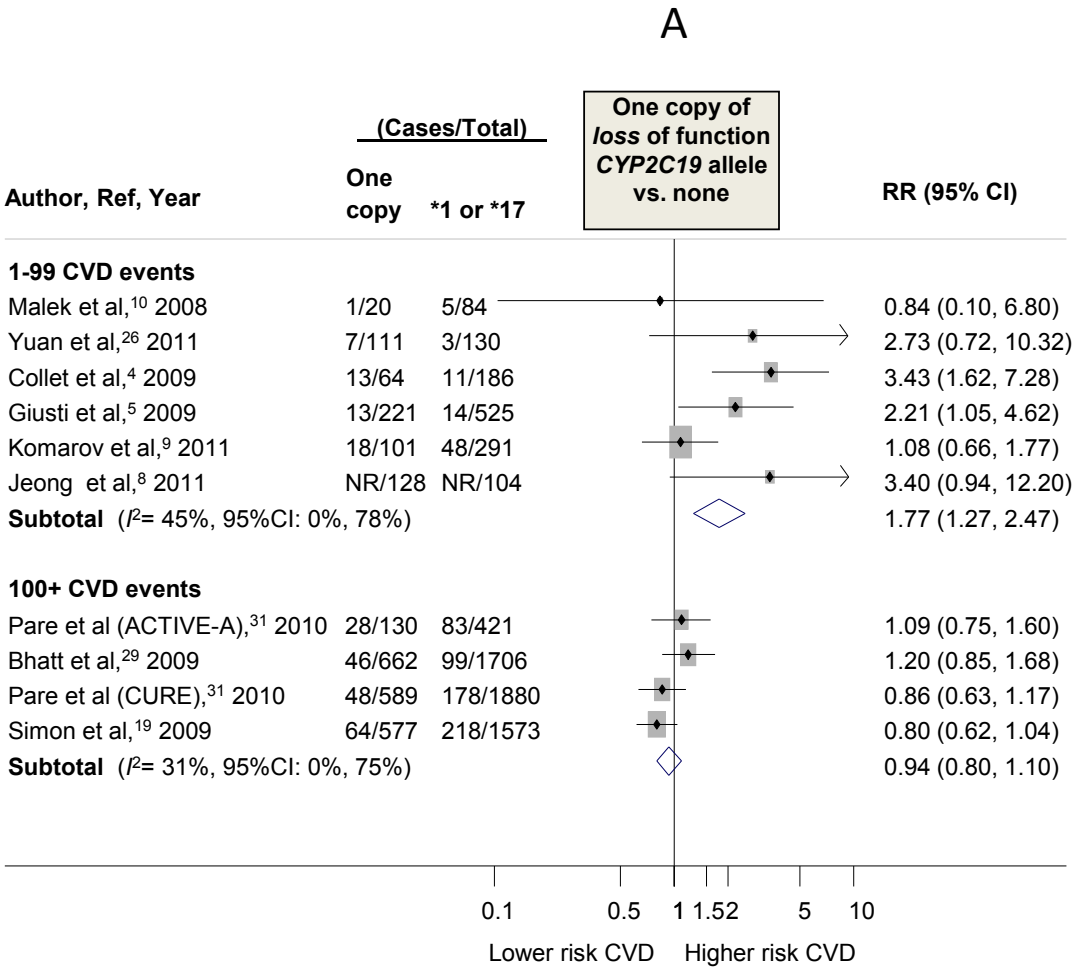


Figure 2.9: Association between *CYP2C19* genotype (any copy of ⋆2 through ⋆8 vs. ⋆1 or ⋆17) and risk of individual outcomes in the treatment-only analysis. Each outcome is stratified by number of events per study.

Footnote: Proportion of total participants calculated by dividing the number of individuals contributing to each individual outcome by the total number of individuals contributing towards the treatment-only analysis (n=31,076). Fixed-effects meta-analysis model.



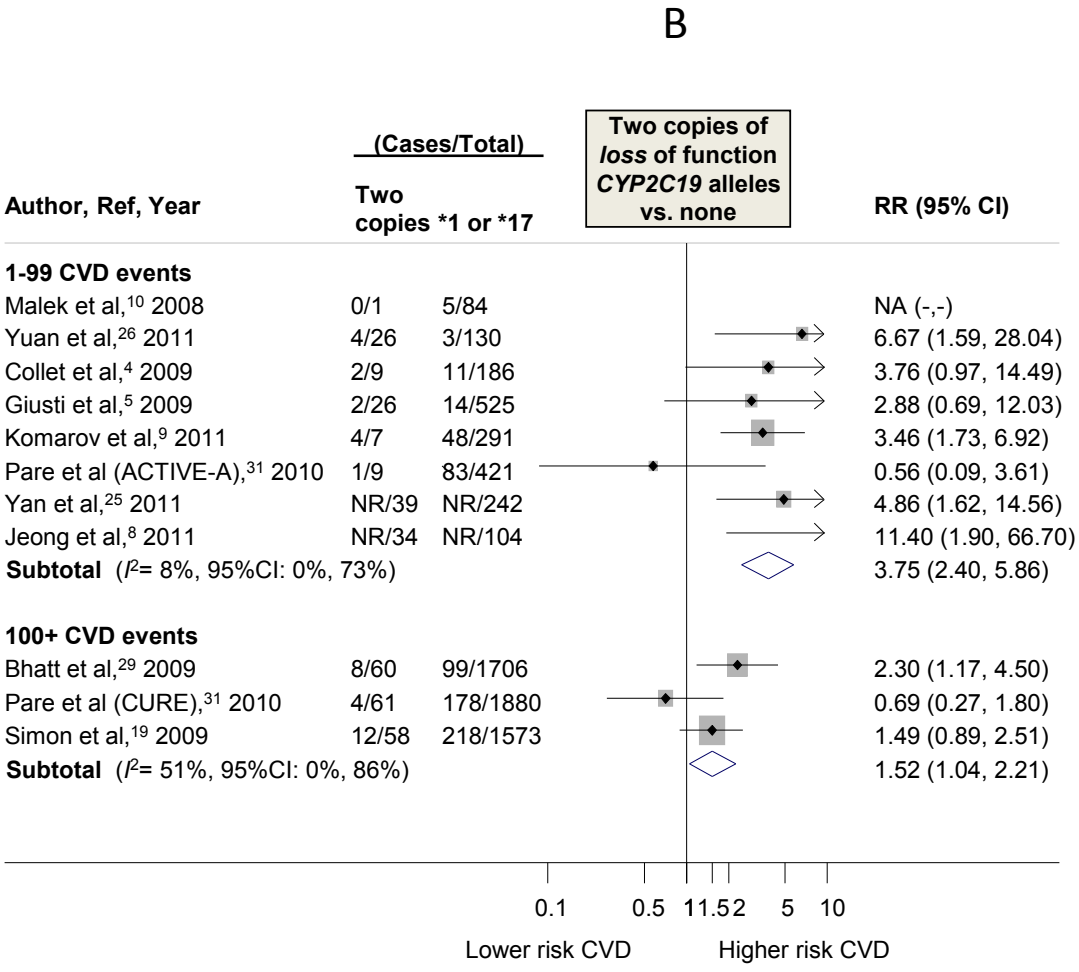


Figure 2.10: Meta-analysis of separate effect of one copy or two copies of loss-of-function *CYP2C19* alleles ($\star$2 to $\star$8) compared to normal/increased function alleles ($\star$1 or $\star$17) on risk of CVD in treatment-only analysis. Panel A shows effect of one copy loss-of-function allele compared to none; Panel B illustrates effect of two copies of loss-of-function alleles, compared to none.

Footnote: Fixed-effect meta-analysis model.

2.4.7 Effect-modification analysis

Genetic studies embedded within randomized trials permitted the evaluation of effect modification of a genetic variant on treatment response by means of an interaction test. When I conducted an analysis of effect modification using data from 4 RCTs in which the comparator was placebo (with more than 1,097 major cardiovascular events in 11,477 subjects), the effect of clopidogrel on major CVD events was not modified by *CYP2C19* categories (P for interaction 0.37) (Figure 2.11A). I also found no evidence for a treatment by genotype interaction for major bleeding (P for interaction 0.07; Figure 2.11B). Values were similar under a random-effects meta-analysis model (Table A.7).

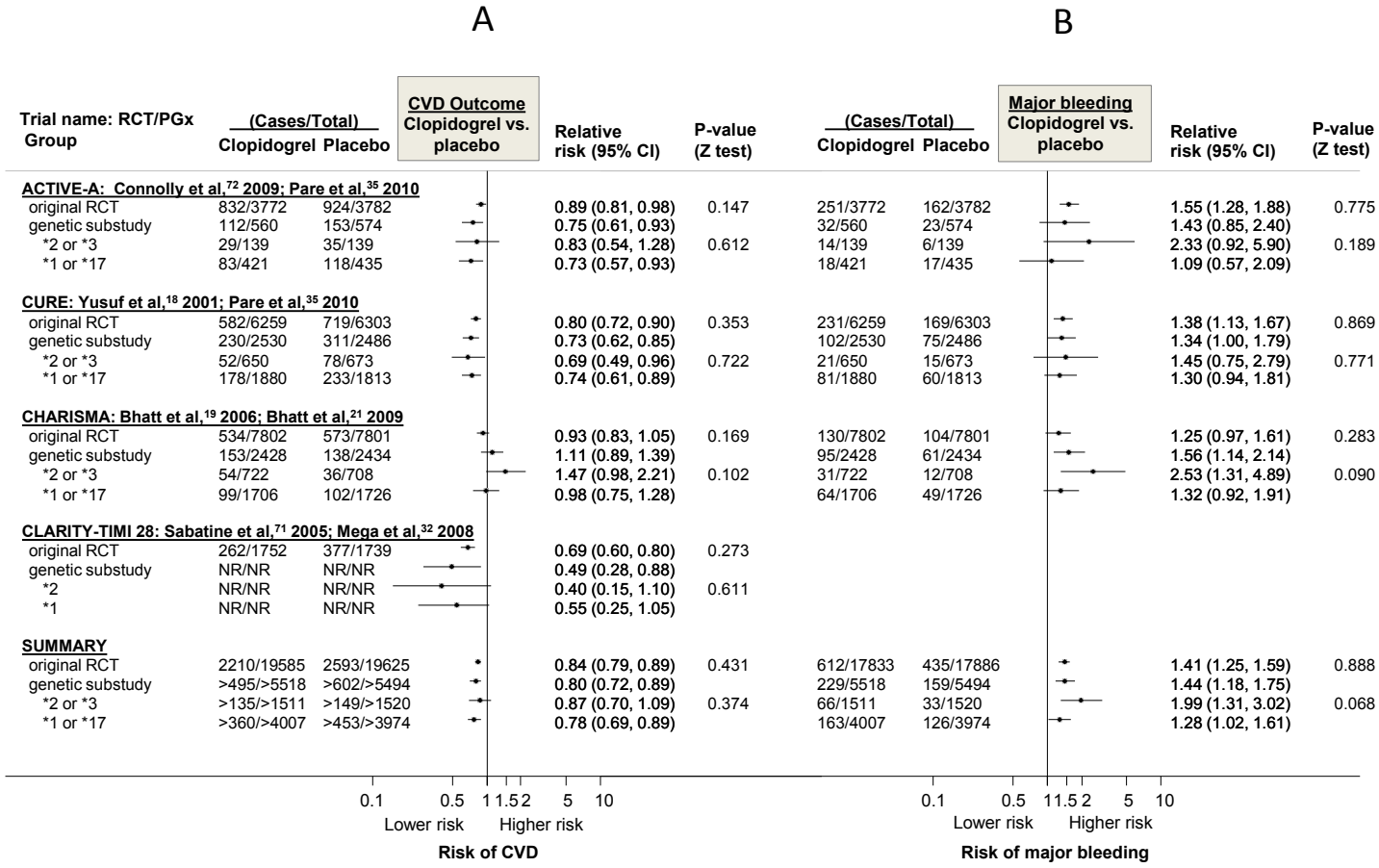


Figure 2.11: Analysis of *CYP2C19* genotype on composite cardiovascular end-points in randomized trials where both clopidogrel and placebo arms were genotyped, "Effect modification" analysis.

Meta-analysis of risk of (A) composite cardiovascular outcome and (B) major bleeding event, comparing clopidogrel to placebo, stratified by: (i) findings from original RCTs; (ii) genetic substudy, and; *CYP2C19** allele status into (iii) any copy of *2 or *3 (iv) *1 or *17. The P value reflects the Z-test for interaction between subgroups, comparing (i) original RCT and genetic substudy, which assesses the representativeness of the genetic substudy to the original cohort; (ii) *2 or *3 compared to *1 or *17, which tests for effect modification of the effect of clopidogrel vs. placebo by *CYP2C19* genotype. "Summary" represents values pooled by fixed-effects meta-analysis

2.5 Discussion

A total of 32 studies, including 42,016 individuals and 3,545 cardiovascular events evaluated the potential modification of clopidogrel response by *CYP2C19* genotype. These studies encompassed two distinct groups: those in which participants were all treated with clopidogrel ("treatment only") and studies set within a randomized trial ("effect modification" studies).

Evidence derived from "treatment-only" studies indicated that subjects classified as poor metabolizers (any ⋆2 to ⋆8 *CYP2C19* allele) when compared to normal/fast metabolizers (⋆1 or carriers of the ⋆17 allele) had, on average, lower levels of the active clopidogrel metabolites (Figure 2.4), less platelet inhibition (Figure 2.5), lower risk of bleeding (RR= 0.84; 95%CI: 0.75, 0.94; Figure 2.9), and a higher risk of CVD events (RR= 1.18; 95%CI 1.09, 1.28; Figure 2.6). However, there was evidence of small study and outcome reporting bias which greatly undermined the validity of the results derived from these studies. This is supported by the pooled analysis of 4 randomized trials that evaluated the potential effect modification of *CYP2C19* on clopidogrel response and found no evidence for effect modification on cardiovascular end-points or bleeding (both P-values for interaction test >0.05; Figure 2.11). Until further evidence is obtained from high-quality studies (ideally within randomized trials) that overturns existing evidence, the use of *CYP2C19* genotype to guide prescription of clopidogrel should be discouraged. The studies of *CYP2C19* genotype and clopidogrel response are likely to exemplify several general issues in pharmacogenetic studies serving to highlight important, yet rectifiable limitations in this field, in particular relating to studies of genetic variants involved in drug metabolism, such as the CYP-enzymes, which were among the most common pharmacogenetic studies in my previous field synopsis.[110]

2.5.1 Problems specific to pharmacogenetic studies

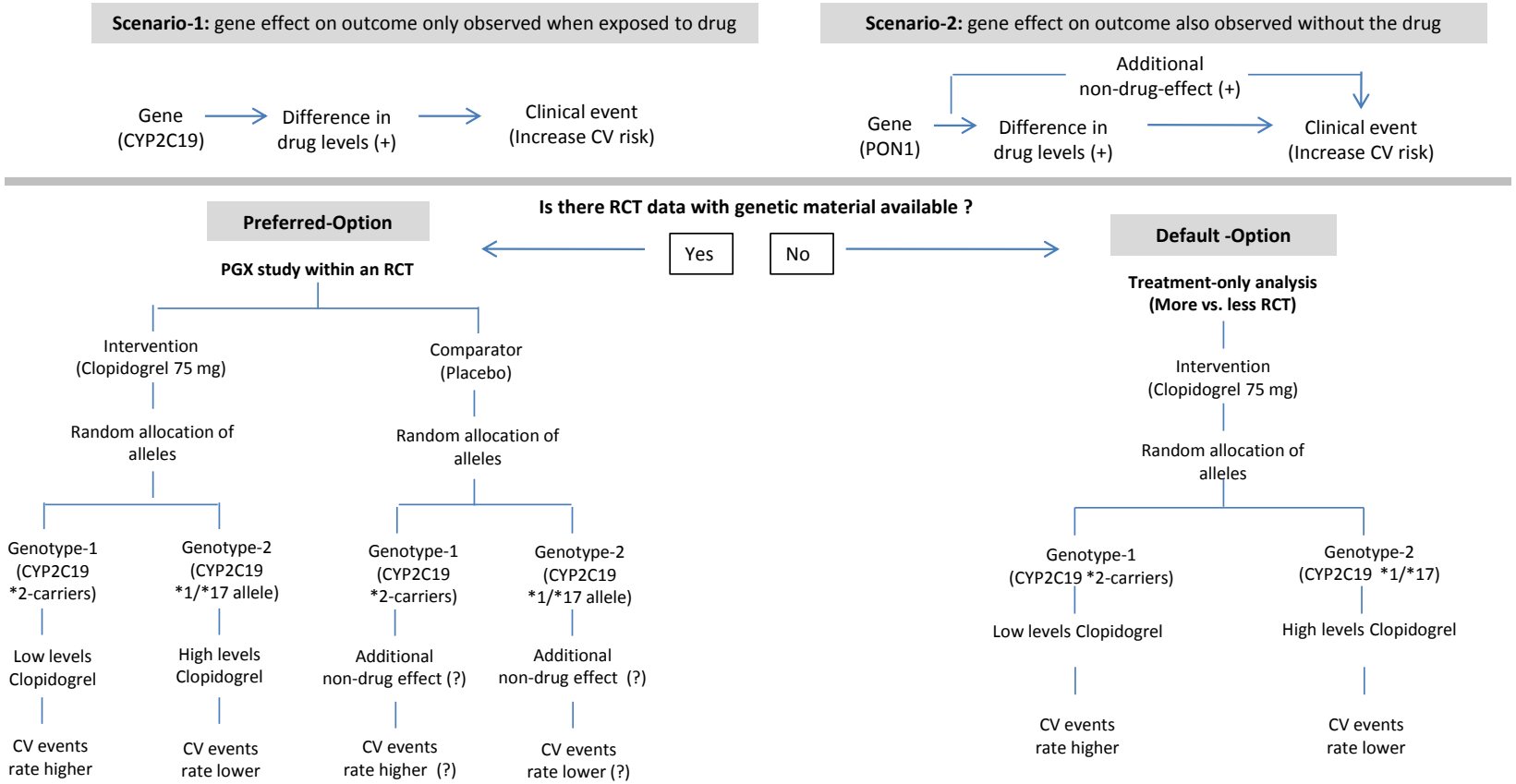
In principle, the investigation of drug effect modification by a genetic variant should not differ from the established approach used for non-genetic variables (such as age, smoking or gender) and should follow methods established for sub-group analysis in a randomized trial (the "Preferred-Option" in Figure 2.12).[184] However, due to a combination of logistic and financial reasons, DNA cannot always be obtained within the setting of large, multi-center trials. Therefore, research teams aiming to investigate potential effect modification conferred by genetic variants on drug response typically use a sub-optimal design ("Default-Option" in Figure 2.12), i.e. observational (prospective cohort or case-control) studies restricted to subjects receiving the medication of interest - a treatment-only option. This type of design would be considered *invalid* for investigating

the potential for treatment effect modification by a non-genetic variable, but there are two reasons that support the popularity of this design for pharmacogenetics, a field where only 5% of studies are set within randomized trials.[110]

The first reason is based on the argument originally described by Motulsky in 1957,[108] that genetic variation in a drug metabolizing enzyme should have no phenotypic consequence (such as an association with cardiovascular events) in the absence of exposure to a medication (in this case, treatment with clopidogrel). The second reason is based on Mendel′s second law - the random allocation of genetic variants from parent to offspring, which should mean that groups categorized by genotype for a drug metabolizing enzyme should not differ systematically except in their response to drug treatment (Figure A.1).[67] These two implicit assumptions make the "treatment-only" design an appealing one. I now critically discuss the validity and implications of these assumptions for the design and interpretation of pharmacogenetic studies that used the treatment-only design.

2.5.2 Assumptions of the "treatment-only" study design in PGx

Studies using the treatment-only design make the assumption that the signal derived from the comparison of 2 genotype groups (e.g. in the case of *CYP2C19*, any ⋆2 to ⋆8 vs. ⋆1 or ⋆17) indirectly evaluates the effect-modification of *CYP2C19* loci on the clopidogrel-cardiovascular events association. However, this approach in statistical terms evaluates the *CYP2C19* variant as a predictor of a clinical event (like a genetic association study) with the feature that all subjects are homogenously exposed to clopidogrel. Several years of genetic research using the hypothesis-free statistical approach of GWAs have indicated that the Motulsky assumption[108] may not always be valid or applicable to all genetic variants within the CYP enzymes. The hepatic CYP450 enzymes form a family of mixed function oxidases that serve multiple functions including, but extending beyond, the metabolism of drugs and xenobiotics.[185] Several CYP enzymes contribute to endogenous steroid hormone and lipid biosynthesis and genetic loci encompassing drug metabolizing CYP genes have recently been identified by genome wide association studies as harboring variants conferring susceptibility to CHD (*CYP17A1*),[186] elevated blood pressure (*CYP1A1*,[187] *CYP1A2*[187] and *CYP17A1*[187, 188]), affecting smoking behavior (*CYP2A6, CYP2B6*)[189] and altering vitamin D level (*CYP2R1*)[190] (Table A.4), all of which may be risk factors for chronic diseases. A candidate gene analysis has also identified associations of *CYP2C19* genotype with inflammation markers[191] which have been linked to increased cardiovascular risk, but to date there have been no large-scale genetic studies implicating *CYP2C19* as a gene with independent associations with cardiovascular events. Thus, associations of genetic variants that encode CYP



Analytical method: sub-group analysis of the effect of the intervention on clinical pre-specified outcomes according to genetic categories.
Assumptions: no confounding across genetic categories. plausible assumption due to Mendel's second law.
Limitation: Larger sample-sizes than those needed to detect the main drug-effect are needed. Limited DNA within trials.

Analytical method: genetic association study limited to drug-users. Ideally using only the outcomes for which the intervention is proven effective.
Assumptions: No presence of an "additional to the drug" effect. No confounding across genetic categories. plausible assumption due to Mendel's second law.
Limitation: Larger sample-sizes needed to detect (often) smaller effects than those observed in placebo-active drug trials

Figure 2.12: Potential scenarios and options for analysis when conducting pharmacogenetic studies in drug metabolizing enzymes.

enzyme with disease risk among individuals exposed to a drug may not necessarily be due to differential drug metabolism.

2.5.3 Scenarios of "treatment-only" studies in pharmacogenetics

To exemplify the assumptions of the treatment-only analysis in more detail, consider three different scenarios (Figure 2.13):

1. in the first, the gene (e.g. *CYP2C19*) alters drug pharmacokinetics which leads to different categories of active drug exposure, but has no effect on disease risk in the absence of drug treatment,
2. in the second example, the gene (e.g. *PON1*, which was recently reported to influence CVD risk in clopidogrel treated patients)[153] affects drug pharmacokinetics but has a potential additional effect on disease risk independent of drug use because the encoded protein may alter HDL-C function;[192] and
3. in the third, the gene (e.g. *APOE*)[193] does not alter drug metabolism or action but is associated with the disease outcome of interest.

In all three scenarios, the genetic variant will be associated with the outcome in a treatment-only design study, but only in the first case would this be the exclusive result of modification of a treatment effect.

In the first scenario, a treatment-only analysis may be a useful alternative if access to genetic information in a randomized trial proves difficult. However, the expected effect of the genotype on treatment outcome should be proportional to the effect of the genetic variant on the effective dose of the medication.

In the second and third examples, the assumption of no association of the genetic variant with a clinical event in non-drug exposed subjects is violated and therefore the treatment-only analysis in this setting is not a true pharmacogenetic analysis, but rather a gene-disease association study in a drug exposed group. Therefore, in the latter two situations, if the investigator is still interested in the potential for these genes to alter drug response, the effect-modification approach is the optimal study design. In the case where randomized trial data are not initially available, an alternative strategy could be to use independent sources of evidence to confirm the lack of association between the genetic locus of interest and the disease outcome before proceeding to an observational study in which all individuals are exposed to the medication of interest. Any subsequent association observed in the treatment-only study should ideally then be verified using prospectively collected data from a randomized trial.

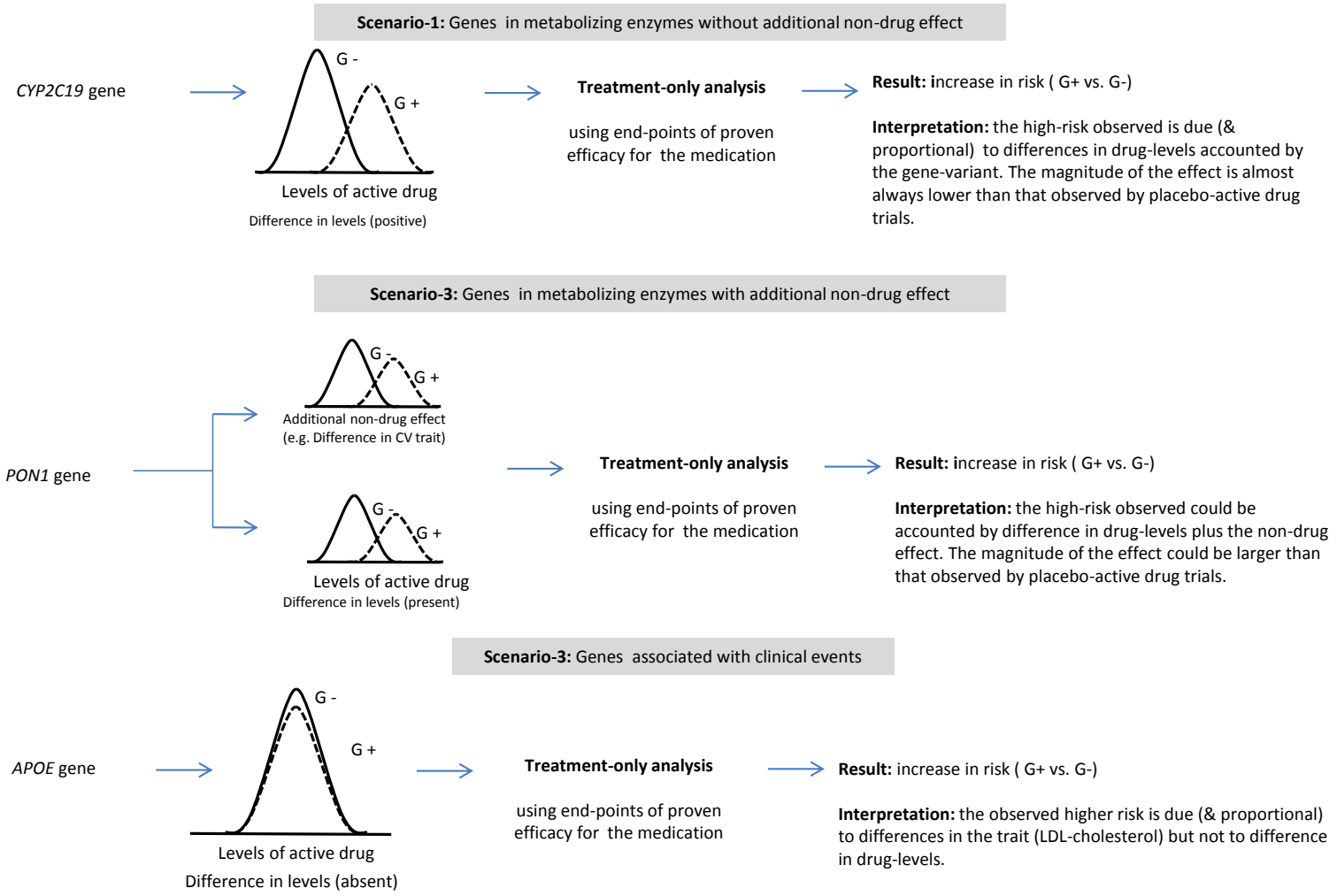


Figure 2.13: Potential scenarios and implications for interpretation when conducting pharmacogenetic studies using the "treatment-only" design.

2.5.4 Implausible effect sizes

A less familiar limitation of the treatment-only design relates to the often implausibly large effects reported by this type of study, in particular when the genetic-variant affects the metabolism of a medication (which makes a treatment-only study similar to a randomized trial of more-versus-less intense dose of medication). As with randomized trials of more-versus-less intensive drug therapy, the expected effect in pharmacogenetic studies evaluating genetic variation on metabolizing enzymes will depend on the difference in concentration of the active medication in the different genotype categories for a given administered dose, and on the position on the dose-response curve (Figure A.5). Mechanistic studies show that the difference in the active form of clopidogrel conferred by the *CYP2C19* genotype, at both 75mg and 600mg was small-to-moderate, with a large overlap of the distributions of AUC of the active metabolite (Figure 2.4). The influence of *CYP2C19* loci on the active form of clopidogrel can yield differing effects on clinical outcomes depending on the location of the administered dose on the dose-response curve (Figure A.5, scenario 1), with a larger effect if the dose under evaluation (e.g. 75 mg of clopidogrel) is located on the linear part of the curve and substantially smaller if it is on the plateau part of the curve (Figure A.5, scenario 2).

Although it is difficult to know with certainty on which part of the dose-response function clopidogrel is located, and indeed the gradient of the linear component of the dose-response curve, it is perhaps safe to assume, based on the modest effect of the *CYP2C19* genotype on enzyme activity, that the relative difference in risk of CVD events between genotype categories in a treatment-only design study should be smaller than the overall relative difference in CVD risk in placebo-controlled trials of 75mg clopidogrel, which is 12% (95%CI: 7%, 17%).[125]

If it is assumed that the association of the concentration of the active metabolite of clopidogrel with CVD events follows a log-linear relationship, the expected effect for fast metabolizers when compared with poor metabolizers (analogous to a RCT comparison of more versus less clopidogrel) will produce a relative risk of 0.95 (obtained by raising the overall estimate of clopidogrel vs. placebo (OR 0.88) to the power of the fractional difference in *CYP2C19* (0.14/0.35) i.e. $0.88^{(0.14/0.35)}$=0.95 (Figure A.5, scenario 1), or even lower if the dose (75mg/day) lies on the dose-response plateau (Figure A.5, scenario 2). This expected effect estimate is closer to null than the summary effect estimate from treatment-only studies reported at the time of the FDA approval (RR 0.82; 95%CI 0.72, 0.93; Figure 2.7), and even the most up-to-date summary effect estimate (RR: 0.87, 95%CI 0.80, 0.95). In summary, the reported effect of *CYP2C19* genotype on CVD risk among clopidogrel treated patients is implausibly large.

Two further pieces of evidence support the conclusion that the effect estimate for treatment-only studies should be smaller than that observed in placebo trials of clopidogrel. Firstly, genetic studies have demonstrated that variants at the *CYP2C19* locus only explain 12% of the variance in clopidogrel response.[140] Secondly, my meta-analysis of randomized trials investigating the effect of more versus less intensive clopidogrel therapy, with a much greater difference in active form of clopidogrel than those differences conferred by *CYP2C19* loci, [194–200] has shown, at most, a small reduction in the risk of major CVD events (RR of 0.90; 95%CI 0.80, 1.00; Figure 2.14). However this value is likely to be an overestimate, since I identified evidence of small-study bias (P-value Harbord test= 0.045).

The most likely explanation for the discrepancy between earlier estimates from pharmacogenetic studies using the treatment-only design and that expected based on the effect of the *CYP2C19* genotype on the level of the active clopidogrel metabolite is small-study bias; by the time of FDA approval of the boxed warning, only one study had more than 200 CVD events (Figure 2.7). The estimate of treatment-only studies after considering the potential impact of small-study bias provided a closer estimate (RR of 0.92 using trim and fill) to the one predicted (RR of 0.95). However, this should be considered an estimate, since methods for small-study bias correction can only approximate the true unbiased effect, and therefore the most credible conclusion is of no effect modification of the *CYP2C19* on clopidogrel response (as observed on restriction to large treatment-only studies $\geq$200 CVD events and on subgroup analysis of effect modification studies).

2.5.5 Separating mechanism-based intended effects from adverse effects

Irrespective of the precise magnitude of effect modification, where the major harms of a drug are mechanism based (as is the case for clopidogrel), any attempt to individualize dose according to genotype to reduce the risk of poor treatment response (i.e. an increased CVD risk) is likely to be offset by an opposing effect on the rate of harm (i.e. bleeding) (Figure A.1). The net benefit of genotyping to adjust dose may therefore not be as great as initially anticipated. In the case where genetic variants under investigation lie in drug metabolizing enzymes, the interaction will likely be quantitative (i.e. differences in degree) rather than qualitative (differences in kind),[184] thus even if a real difference between subgroups is detected, cost-effectiveness analyses would be the decisive test of clinical utility.

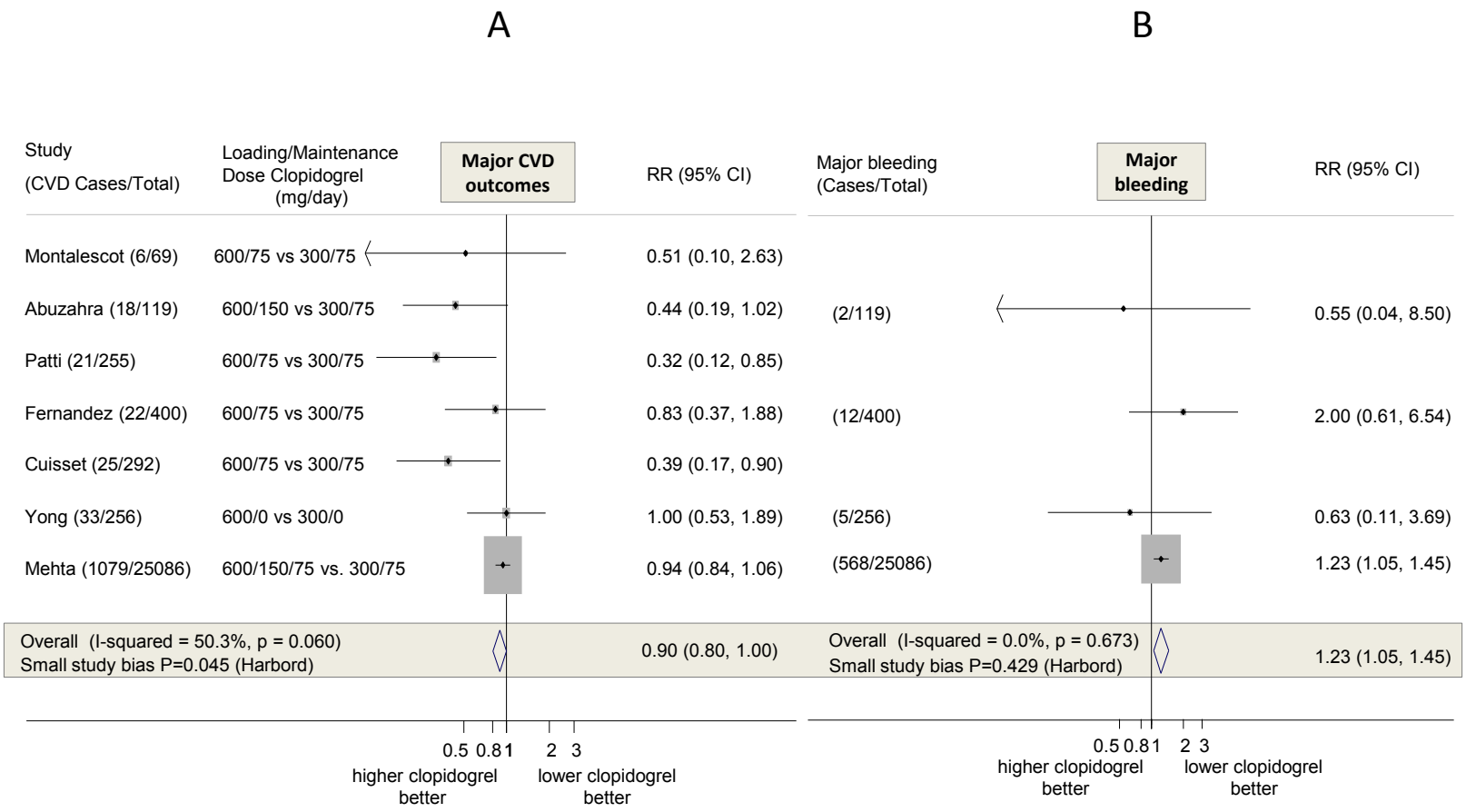


Figure 2.14: Meta-analysis of randomized trials of more-vs-less clopidogrel. Trials compared 600mg vs 300mg loading dose of clopidogrel.

2.5.6 Limitations of the star allele nomenclature

Of the catalogued variant alleles in *CYP2C19*,[201] one or more of nine reduced activity alleles (⋆2, ⋆3, ⋆4, ⋆5, ⋆6, ⋆7, ⋆8, ⋆9, ⋆10), and one increased activity allele (⋆17) was genotyped in the 32 studies of clopidogrel response (Table 2.1). However, the amount of evidence available for each allele differed substantially (Table 2.1). Most studies grouped all patients with presumed low enzyme activity alleles (⋆2 to ⋆8) into a single category, which assumes the degree of functional impairment is similar, or into predicted metabolizer phenotypes (e.g. poor, intermediate, extensive). If a ⋆ allele was not typed at all, the participant was presumed to lack the variant allele in that position. Thus many patients in the ⋆1 category (used to denote an individual lacking all alternative alleles) may have been misclassified. Collectively, these limitations could lead to gene misclassification (a form of measurement error in the exposure), which may result in bias.[202] These limitations could easily be rectified through use of the NCBI Reference Sequences system(RefSeq)[203] to catalogue genetic variants, which is used in all other contexts.

2.5.7 Problems recognized in other fields

Some of the problems unveiled in this appraisal, for example those relating to study design, are specific to pharmacogenetic studies. Other problems, for example small study-bias, are well recognized in other areas of epidemiological research,[204, 205] which are particularly concerning because pharmacogenetics is perceived as a field where advances in genomic medicine could have their earliest translational applications.

2.5.8 Placing these findings into context

The findings I report in this chapter are in stark contrast to three previous reviews,[206–208], but concordant with one appraisal that was published in most recent proximity to this work.[209] Although one of the original systematic reviews of *CYP2C19* and clopidogrel *did* find evidence of small study bias,[206] this was not examined in detail, or given due prominence in the article.

Perhaps the most important prior meta-analysis is that of individual participant data,[207] however, the authors limited the analysis to individuals predominantly receiving percutaneous intervention therapy for ACS.

It is interesting to note that the association of *CYP2C19* loci with clopidogrel response identified from GWAs did not replicate in this analysis for CVD events. Rs1277823 (in LD with the SNP that characterizes *CYP2C19**2, rs4244285 at R^2=0.87) was identified as a GWAs locus for clopidogrel response.[140] I replicated this association in meta-analysis of four studies that investigated the association of *CYP2C19* with platelet function (Figure 2.5). However, despite this replication of platelet function, no association between *CYP2C19* LoF was identified on treatment-only (limited to large studies), or effect modification analysis where the outcome was CVD events. This discrepancy could be explained by platelet function (detected using currently-available assays) not impacting upon cardiovascular events, a hypothesis supported by three recent randomized trails. The GRAVITAS[210], TRIGGER-PCI[211] and ARCTIC[212] randomized trials all failed to identify evidence that acting upon platelet function tests (e.g. through randomized allocation to higher dosing or alternative antiplatelet therapy) altered risk of CVD, casting into doubt whether platelet function is causally linked to CHD risk. Taken together, this suggests that although *CYP2C19* LoF alleles may associate with platelet reactivity following clopidogrel loci, this does not translate into a clinically-meaningful difference in risk of CHD.

Given the high-profile nature of this pharmacogenetic variant, when this analysis was published,[213] it drew high attention from the media, including reports on the television news-channel CNN[214] and popular cardiovascular newsletters including theheart.org[215] and a blog on Nature website.[216]

This analysis and interpretation have been criticized by several papers,[217–221] and high-profile cardiologists claimed the article was 'remarkably misleading'[222] and 'set the field potentially back a bit'[216].

Critics made the following claims:[223, 224]

- that the omission of studies that only reported stent thrombosis from the composite outcome meant we omitted relevant data
- that we included outcomes for which clopidogrel has no evidence of being efficacious (e.g. stroke)
- that the apparent presence of small study bias was due to presence of larger studies being set in patients with stable CHD (for which no evidence exists for clopidogrel), rather than true publication bias

In response[225], the summary estimate was not altered by including the three studies that only reported stent thrombosis (yielding a summary effect estimate of RR 1.21, 95% CI: 1.12, 1.30 for CVD events), and the evidence for small study bias persisted (P<0.01 for Harbord test of small-study effects). Second, we argued that the outcomes included in the analysis were appropriate since clopidogrel *has* been shown to be beneficial to patients with a mixture of stable and unstable CHD,[125] and furthermore, evidence from the ACTIVE-A trial[226] validates the use of clopidogrel to prevent CVD events in patients with atrial fibrillation.

Finally, I responded to the criticism that the small study bias was an artefact generated by larger studies being set in patients without ACS by stratifying the analysis of *CYP2C19* on the CVD event composite by the proportion of individuals with PCI and/or stent insertion at baseline (Figure 2.15), and also by CHD status at baseline (Figure A.3). Neither of these analyses showed evidence of heterogeneity between the strata in each of the subgroups, making this an unlikely explanation.

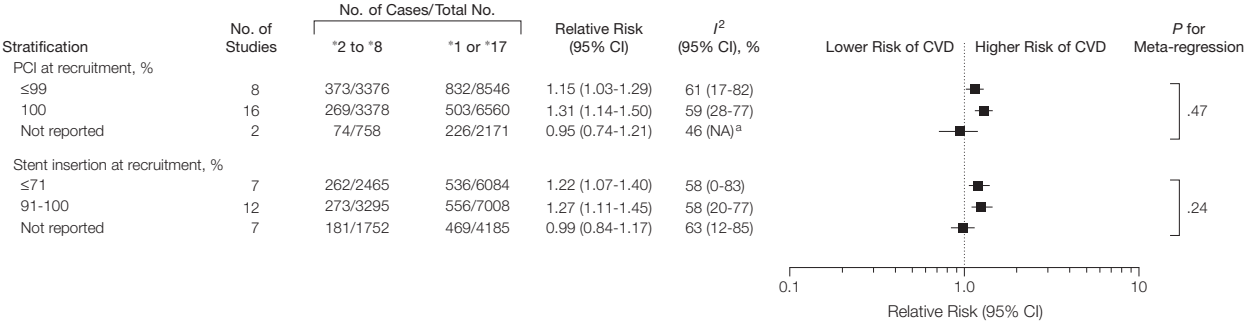


Figure 2.15: Meta-analysis of Studies Evaluating the Effect of *CYP2C19* ⋆2 to ⋆8 vs ⋆1 or ⋆17 on Cardiovascular Disease (CVD) Events.
Data were stratified by the proportion of participants receiving percutaneous coronary intervention (PCI) or stent insertion (fixed effects modeling). The meta-regression was adjusted for study design and sample size. Error bars indicate 95% confidence intervals.
[a]Unable to estimate the 95% confidence interval because only 2 studies contributed to the overall estimate.

2.6 Conclusions

In conclusion, I identified several features that cast into doubt the association between *CYP2C19* genotype and clopidogrel response. Based on these findings, there is no evidence to support the use of *CYP2C19* genotyping in clinical practice to adjust clopidogrel dose or to inform the choice of anti-platelet agent. These problems are unlikely to be exclusive to this particular example of pharmacogenetic research, and as a general rule, pharmacogenetic studies should fully exploit randomized trials to harness robust information on gene-drug interactions. To aid this, pharmaceutical companies ought to

make drug pharmacokinetics and dose-response data available to help researchers prioritise meaningful drug-gene combinations. As funding to support this type of research increases (both UK Medical Research Council and US National Institutes for Health have prioritized this area), so capacity should be built to aid the robust design, conduct, analysis and reporting of pharmacogenetic studies.

Chapter 3

Secretary Phospholipase A_2 and Cardiovascular Disease: Background

3.1 Introduction

3.1.1 Atherosclerosis as the substrate of Coronary Heart Disease

Atherosclerosis is an asymmetrical focal thickening of the inner-most layer of the arterial wall (the tunica intima). The disease process is progressive and when the thickening becomes sufficient that blood flow is occluded through the coronary artery (usually precipitated by plaque rupture and subsequent coronary thrombus formation), the myocardium becomes deprived of oxygen, resulting in myocardial infarction.[227]

The atherosclerotic plaque itself is composed of a necrotic core, consisting of lipids, cellular debris and foam cells (macrophages that have engulfed lipid particles). The necrotic core is surrounded by smooth muscle cells and collagen that creates a cap. Inflammatory cells (consisting of T cells, macrophages and mast cells) infiltrate the area, especially in the region where the atheromatous plaque expands, the so-called 'shoulder' (Figure 3.1).

3.1.1.1 Lipid theory of atherosclerosis

Elevated levels of circulating LDL-C results in activation of arterial endothelial cells and LDL-C infiltration and retention in the tunica intima. The modification of LDL-C in

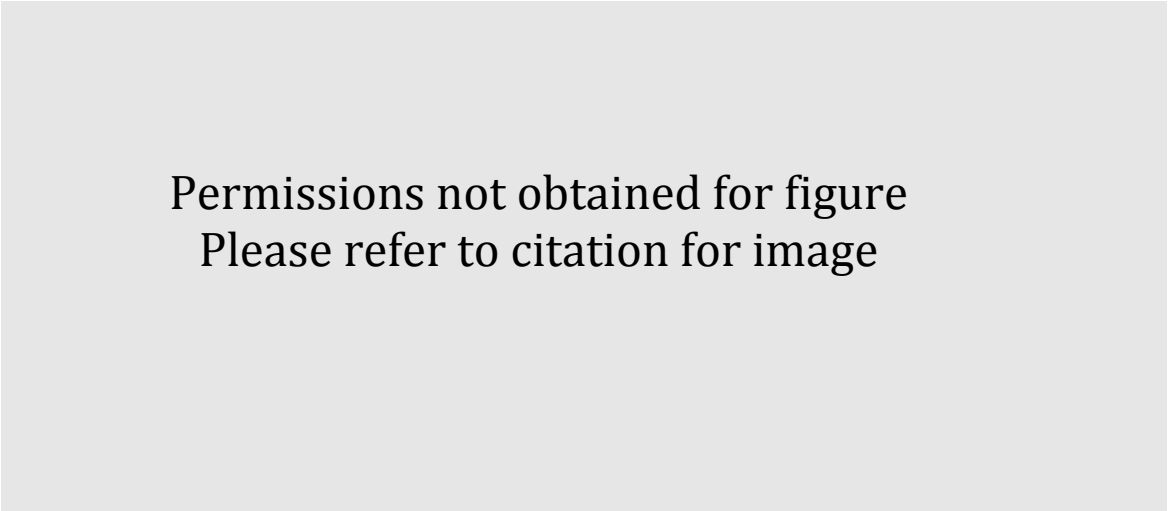


Figure 3.1: Anatomy of an atherosclerotic plaque.
The grey shaded area represents the necrotic core (containing lipids and cellular debris), surrounded by a fibrous cap of smooth muscle cells and collagen. Infiltrating the lesion are inflammatory cells, particularly abundant at the shoulder region. Derived from Hansson and Libby.[228]

the tunica intima encourages endothelial cells to express receptors that further encourages migration and influx of inflammatory cells. This results in migration of circulating monocytes into the tunica intima where they become macrophages and phagocytose the modified LDL-C through the macrophage scavenger receptor resulting in the foam cell, a hallmark feature of the atherosclerotic plaque.[228, 229]

3.1.1.2 Inflammation theory of atherosclerosis

Inflammation has been proposed to play a central role in all stages of the atherosclerotic process.[230, 231] For example, the precursor of the atheromatous plaque is the fatty streak, which is an asymptomatic lesion that can be found in children as young as 3 years of age.[232] Fatty streaks consist predominantly of the inflammatory cells macrophages and T-cells.

Furthermore, inflammatory cells, including macrophage-derived foam cells and T-cells, make a substantial component of cells in the advanced atherosclerotic lesion, (Figure 3.2).

Various sources of evidence provide further support that inflammation is an important determinant of heart disease:

- Chronic systemic inflammation as a stimulus for atherosclerosis: autoimmune diseases such as rheumatoid arthritis, in which there is a chronic inflammatory state,

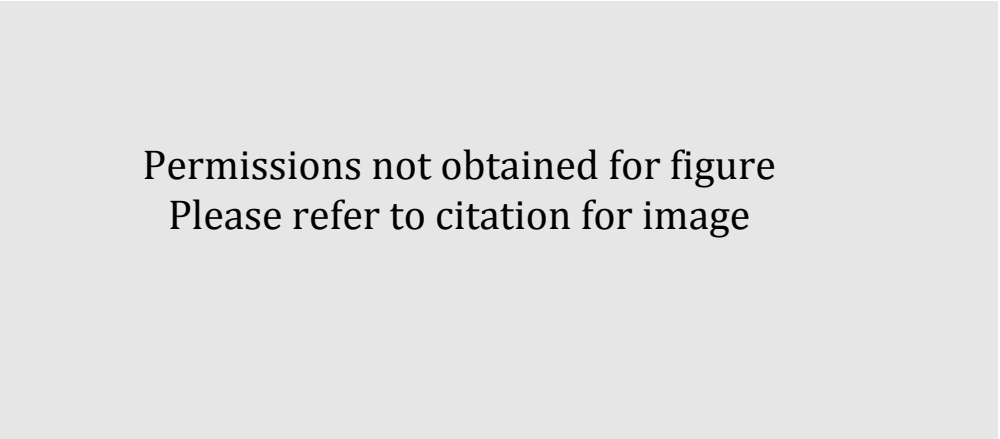


Figure 3.2: Schemata to show the central role of inflammation in the initiation and progression of an atherosclerotic plaque.
Abbreviations for inflammatory cytokines: M-CSF: macrophage colony-stimulating factor; MCP-1:monocyte chemotactic protein 1; MMP: metalloproteinase; PAI-1, plasminogen activator inhibitor 1; PDGF, platelet-derived growth factor; TF tissue factor; UPA urokinase plasminogen activator. Derived from Badimon et al.[233]

are associated with higher risk of coronary heart disease due to accelerated coronary atherosclerosis.[234, 235]

- Acute systematic inflammation as a trigger for acute vascular events: Emerging evidence supports the hypothesis that an acute inflammatory insult arising from e.g. periodontitis[236] or influenza[237], may increase the risk of coronary heart disease.[238] Furthermore, there is weak evidence from randomized trials that influenza vaccination may protect against cardiovascular disease.[239]

3.1.2 Inflammation and Coronary Heart Disease

This model suggests that inflammatory cytokines play a key role in the initiation and progression of atherosclerosis (Figure 3.2).[229] Thus, an appealing means to halt the disease process of atherosclerosis may be to inhibit inflammatory pathways.[231] Much research activity has therefore focussed[240] on delineating the role of candidate inflammatory biomarkers in CHD (see Box 3.1 on page 65 for two contrasting examples of inflammation biomarkers and their potential role in CHD).

Box 3.1: Candidate inflammation biomarkers

C-reactive protein Despite compelling observational evidence on the association between C-reactive protein (CRP) and cardiovascular events,[241] a RCT of a therapy that specifically modulates CRP has not been conducted (due to lack of a specific orally-available drug). Mendelian randomization studies have repeatedly shown that the association between CRP and cardiovascular events, rather than being causal, is likely to arise from reverse causality or confounding.[62] This serves as an important example to the issues of causality discussed in Chapter 1 (association does not equate causation) and to make causal inference, study designs in which randomization is employed to remove confounding is a necessary requirement to be certain of a causal relationship (as no other study design can mitigate this source of error).

Interleukin-6 Interleukin 6 (IL-6) represents another candidate biomarker. IL-6 is secreted by T cells and macrophages[242] and observational investigations provide strong evidence of a monotonic dose-response relationship between circulating IL-6 levels and risk of CHD.[243] No clinical trial has been conducted with a drug that modulates IL-6 levels (or inhibits its receptor) to investigate the role of IL-6 in CVD. A recent large-scale Mendelian randomization study showed that individuals that harbour a genetic variant that alters the ability of IL-6 to bind to its target receptor, the IL-6 receptor (encoded for by the *IL6R* gene), have an altered risk of CVD.[83] Although this does not directly implicate IL-6 *per se* in CVD, by extension a causal role of IL-6 can be extrapolated (assuming specificity of both IL-6 to the IL-6 receptor and *vice-versa*).

3.1.3 The interface between lipids and inflammation

Inflammation is the process by which tissues become swollen and irritated in response to endogenous (e.g. autoimmune disease) or exogenous (e.g. trauma, foreign body, infection) stimuli. Inflammation is characterised by five cardinal signs (*rubor* - redness, *calor* - heat, *tumor* - swelling, *dolor* - pain, and *functio laesa* - loss of function). Whether an inflammatory process persists or not is thought to be determined by the interplay of several cytokines,[244] and the transition from acute to chronic inflammation is regulated by several factors. Two of these regulatory mediators are thought to be prostaglandins and leukotrienes.[245] This is of importance as the inflammation in cardiovascular disease is typified by a chronic disease process (Figure 3.2).[231]

Prostaglandins and leukotrienes have been described as lipid mediators, with potent pro-inflammatory functions.[246, 247]. They have wide-reaching effects on other cells, which ultimately leads to the propagation of the inflammatory cascade.[245]

Prostaglandins and leukotrienes are metabolites derived from arachidonic acid[a] (through the actions of cyclo-oxygenase and 5-lipoxygenase enzymes, respectively). The rate-limiting step for the liberation of arachidonic acid from the bilipid layer is phospholipase enzymes[248] (Figure 3.3).

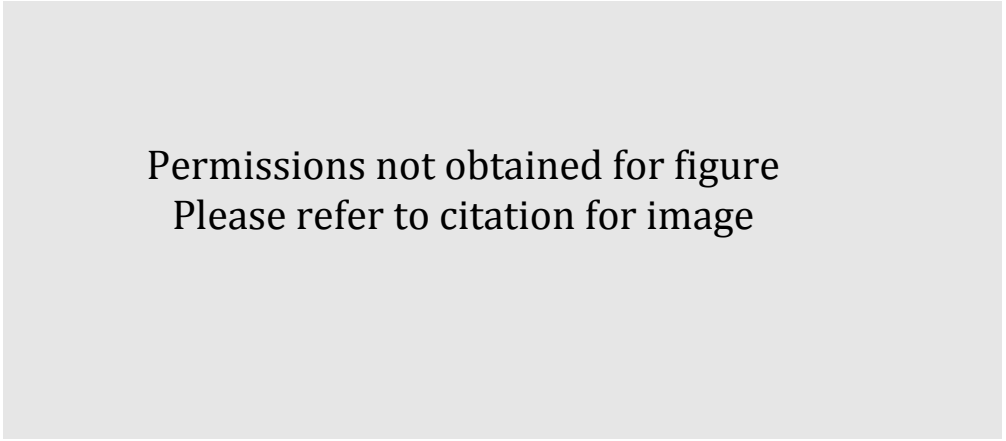


Figure 3.3: Synthesis of inflammatory cytokines from arachidonic acid.
Footnotes: COX: cyclo-oxygenase; 15-HPETE: 15-hydroperoxyeicosatetraenoic acid; 5-HPETE; 5-hydroperoxyeicosatetraenoic acid; LP: lipoxins; LT: leukotrienes; PG; prostaglandins
Derived from Ogawa et al.[249])

3.1.4 Phospholipase enzymes

Phospholipase enzymes are a family of enzymes that share the common function of hydrolyzing phospholipids into fatty acids and other lipophilic substrates.[248] There are four main classes of phospholipase enzymes (A, B, C and D), labelled according to their catalytic function (Figure 3.4).

Figure 3.4: Catalytic activity of phospholipase enzymes on phospholipids.
Phospholipase A1 and A2 cleave the *sn*-1 and *sn*-2 bonds, respectively; B can cleave either one of the *sn*-1 or *sn*-2 bonds; C cleaves the phosphoryl ester at C3 of glycerol; D cleaves phosphodiester bond of the glycerolipid phosphatidylcholine. R1 and R2 represent carbon chains of fatty acids.

[a] arachidonic acid is derived from the cell membrane phospholipids through the action of phospholipase A2 enzymes

- Phospholipase A: (A1 and A2) - cleaves the *sn* bond; A1 and A2 subtypes cleave the *sn*-1 or *sn*-2 bond, respectively. Cleavage of the *sn*-2 bond by PL-A2 releases arachidonic acid
- Phospholipase B: (also known as lysophospholipase) cleaves the acyl chains from both sn-1 and sn-2 positions of a phospholipid
- Phospholipase C: cleaves the phospholipid phosphatidylinositol bisphosphate (PIP2) into diacyl glycerol (DAG) and inositol triphosphate (IP3). IP3 release results in increased intracellular calcium[250], which together with DAG activates protein kinase C, altering cellular activity.
- Phospholipase D: yields phosphatidic acid and free choline from the hydrolysis of the phosphodiester bond of the glycerolipid phosphatidylcholine. Phosphaditic acid is a bioactive lipid and can be converted into diacylgycerol and lysophosphatidic acid. These metabolites are considered important for movement of vesicles, endocytosis and receptor signalling in humans.[251, 252]

3.1.5 Phospholipase A_2 enzymes

Phospholipases A_2 (PLA_2) are a diverse family of enzymes present in most types of cells.[248] The PLA_2 enzymes are involved in a wide range of cellular processes including lipid metabolism, host defence, cell membrane homeostasis and signal transduction.[253, 254] The PLA_2s share a common catalytic function: the hydrolysis of the bond between the *sn*-2 fatty acyl bond of phospholipids present in lipoproteins and cell membranes, releasing arachidonic acid and lysophospholipids (Figure 3.4). As discussed above, arachidonic acid is subsequently metabolised by cyclooxygenase and lipoxygenase to yield the eicosanoids prostaglandins, leukotrienes and thromboxane A_2 (Figure 3.3), important mediators of inflammation.

Phospholipases A_2 can be further classified as secretory, cytosolic and lipoprotein-associated:

secretory phospholipases A_2 ($sPLA_2$) are extracellular, acute phase reactants that are suggested to be pro-atherogenic.[255–257] Eleven $sPLA_2$ isoforms have been identified in mammals (IB, IIA, IIC, IID, IIE, IIF, III, V, X and XIIA and XIIB).[258] $sPLA_2$ is thought to modify LDL-C particles in the circulation and arterial wall, producing pro-inflammatory lysophospholipids and non-esterified free fatty acids. This effect potentially increases aggregation of LDL-C onto vessel wall proteoglycans, promoting foam cell formation and the development of

atherosclerosis. sPLA$_2$-IIA is thought to be the most highly expressed of the sPLA$_2$ enzymes[259] and its mass can be quantified specifically in plasma by ELISA.[260] In contrast, no specific assay exists to quantify the mass of other sPLA$_2$ isoforms. However, an assay exists for 'sPLA$_2$ enzyme activity'[b], which has been reported[259] to represent a composite of sPLA$_2$-IIA, V and X isoforms (see Figure 3.5 for more information).

cytosolic phospholipase A_2 (cPLA$_2$) is intracellular and expressed in macrophages, platelets, neutrophils, and fibroblasts. cPLA$_2$ has a molecular mass of 85 kD,[261] has selective activity for the hydrolysis of arachidonyl phospholipids in the *sn*-2 position, releasing arachidonic acid. cPLA$_2$ is primarily thought to be involved in cell signalling[262]

lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$) is synthesized in macrophages and activated platelets. Lp-PLA$_2$ circulates bound to LDL-C and is expressed in atherosclerotic plaques.[263, 264] Observational analyses reveal strong associations between circulating Lp-PLA$_2$ and CHD, ischaemic stroke, vascular and non-vascular death.[265] Lp-PLA$_2$ therefore represents a potential therapeutic target for CVD prevention, and darapladib [266], an Lp-PLA$_2$ inhibitor is currently in phase III clinical trials (SOLID-TIMI 52[267]: 13,000 patients with ACS with a primary end-point of CV death, nonfatal MI or nonfatal stroke; STABILITY[268]: 16,000 patients with stable CHD followed-up for a primary outcome of major adverse cardiovascular event). Of note, a previous Mendelian randomization study using a SNP in the *PLA2G7* gene identified no association between Lp-PLA$_2$ and CHD[269], and the results of these two clinical trials are eagerly awaited.[270]

3.1.6 Secretory phospholipase A_2-IIA

The sPLA$_2$-IIA isoform is ubiquitous in the human body, including neutrophils and macrophages (where sPLA$_2$ are stored in secretory granules), in tissues including spleen, bone marrow, body fluids and in tears.[274] sPLA$_2$-IIA is a disulfide-rich, stable enzyme with a molecular weight of approximately 16kDa.[254]. The positively charged regions of sPLA$_2$-IIA are thought to enable interaction with glycosaminoglycans on cell membranes.

[b]The sPLA$_2$ enzyme activity assay is based on the decay of a substrate (1,2-dithio analog of diheptanoyl phosphatidylcholine). When the hydrolysis of the substrate at the sn-2 position occurs, free thiols are produced, which is detected using Ellman's reagent(5,5-Dithiobis(2-nitrobenzoic acid)).
Derived from sPLA$_2$ assay kit, Cayman chemical

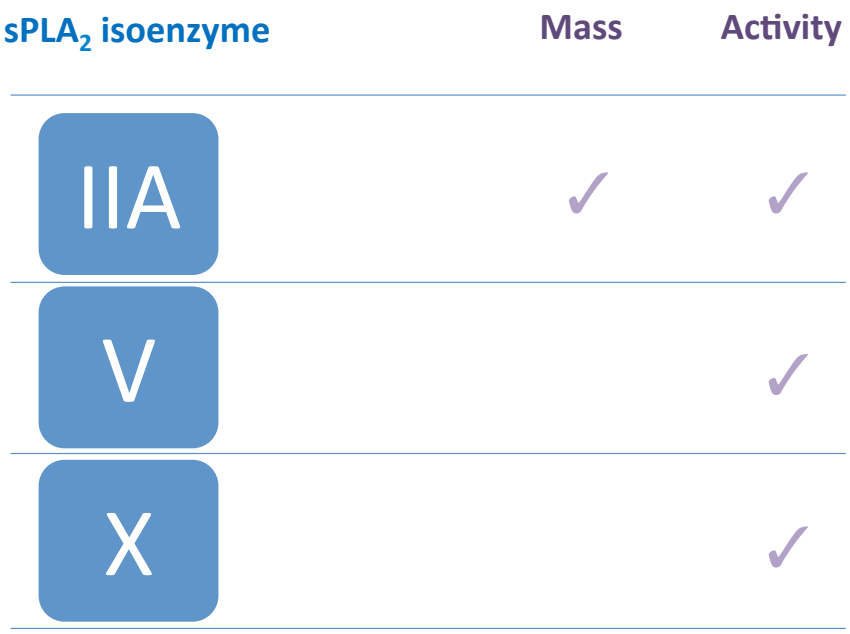


Figure 3.5: Comparison of two measures to quantify circulating $sPLA_2$. $sPLA_2$ enzyme activity (measured by selective fluorometric assay[271, 272]) comprises measures of $sPLA_2$-IIA, $sPLA_2$-V and $sPLA_2$-X, whereas $sPLA_2$-IIA mass can be specifically quantified using an enzyme-linked immunosorbent assay [273].

$sPLA_2$-IIA is an acute phase reactant.[275, 276] In healthy individuals, serum concentrations of $sPLA_2$-IIA are low, and can increase several-fold in response to an inflammatory stimulus, such as myocardial infarction (Figure 3.6).

3.1.7 Divergent roles of $sPLA_2$-IIA

The roles of $sPLA_2$ have been investigated in a wide range of diseases, including cardiovascular,[278] neurodegenerative[279], infectious[280] (including septic shock[281]) respiratory,[282] neoplastic[283, 284], inflammatory and autoimmune diseases (such as pancreatitis, rheumatoid arthritis and inflammatory bowel disease).[285–288] For these diseases, with the exception of infection and neoplasia, higher levels of $sPLA_2$-IIA tend to show association with an increase in disease severity.

In contrast, $sPLA_2$-IIA has been characterised as showing bactericidal effects, with higher levels being protective against infection.[289–292] This potentially brings into question the suitability of inhibiting $sPLA_2$-IIA as a therapeutic goal for cardiovascular disease prevention.[293]

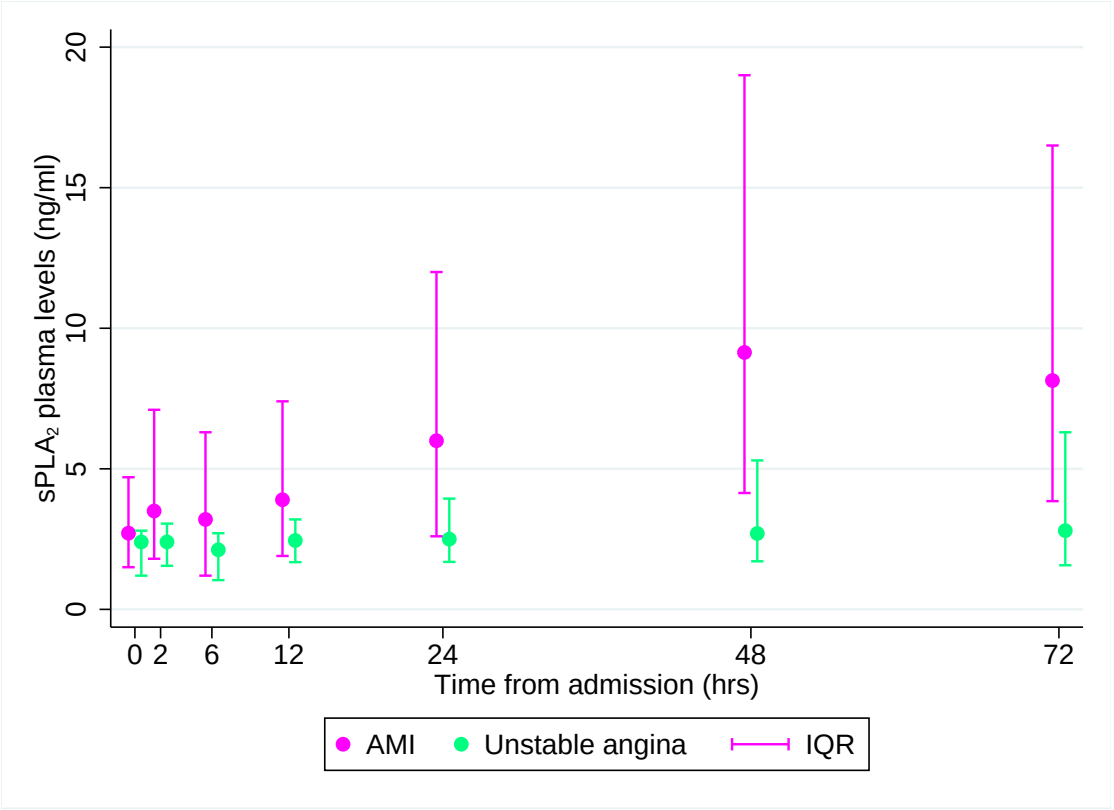


Figure 3.6: Change in sPLA$_2$-IIA mass during acute coronary syndrome. The increase in sPLA$_2$-IIA mass is more pronounced in acute myocardial infarction (AMI) than in unstable angina. Point estimates represent the median and whiskers denote the inter-quartile range (IQR). Figure created from data reported in Nijmeijer *et al*[277]

The role of sPLA$_2$ in tumorigenesis is less clearly defined. sPLA$_2$ levels are elevated in neoplastic tissue (including gastrointestinal, colorectal, prostate and lung tumours)[294–298]. However, small-scale evidence from human cancer tissue does not show evidence that *PLA2G2A* (the gene encoding sPLA$_2$-IIA) is altered in tumour cells.[283]

The disparate evidence on the potentially protective role of sPLA$_2$ in infection and harmful effect of sPLA$_2$ in CVD is worthy of further discussion. Although the focus on my investigation for this thesis is the role of sPLA$_2$ in cardiovascular disease (thus the remainder of this, and the following two chapters that report findings from my investigative work will be focussed on sPLA$_2$ and cardiovascular traits and events), it is important to mention that if a causal association were to be identified between sPLA$_2$ and CVD, meaning that inhibition could be of vascular benefit, any protective effect would need to be considered in light of the potential for harm, if individuals treated with an sPLA$_2$ inhibitor would be at increased risk of infection.[299] The balancing of benefits vs. harms is not unique to this candidate drug for cardiovascular disease prevention, but also exists for some of the most widely drugs available, including aspirin (in which bleeding is a considerable cause of morbidity and mortality)[300] and the lipid-lowering HMG-coA reductase inhibitors (more commonly known as statins, which are

associated with myalgia and an increased risk of type 2 diabetes).[301]

3.1.8 Biological plausibility of sPLA$_2$-IIA in atherosclerosis

In addition to the liberation of arachidonic acid from cell membrane phospholipids (yielding pro-inflammatory metabolites that propagate atherosclerosis, as outlined in Figure 3.2)[302], there are several specific mechanisms by which sPLA$_2$ isoenzymes are considered to be pro-atherogenic (Figure 3.7). These include:

- activation of endothelial cells, which may contribute to 'endothelial dysfunction'[303], increases adhesion molecules such as ICAM (intercellular adhesion molecule 1) and VCAM (vascular cell adhesion molecule),[304] increasing leukocyte migration from the blood lumen into the tunica intima[305], a characteristic of atherosclerotic plaques, as shown in Figure 3.2
- oxidation of LDL-C, which is both directly chemotactic to monocytes and stimulates the release of monocyte chemoattractant protein-1 (MCP-1) from endothelial cells (attracting monocytes into the arterial lesion where they differentiate into macrophages)[306]. Oxidized LDL-C also binds to non-proteoglycan components of the matrix in the atherosclerotic lesion[307]
- modifying circulating LDL to increase the negative charge of LDL particles, making them more pro-atherogenic
- hydrolysis of LDL phospholipid, yielding small, dense LDL-C, a lipid fraction considered to be highly atherogenic[308]
- activating a site on LDL (resulting in a conformational change in apoB100) that makes it more likely to bind to glycosaminoglycans in the proteoglycan matrix located in the tunica intima[309]

3.1.9 Experimental association of sPLA$_2$ with CHD in animal studies

Animal experiments have been used to investigate the relationship between sPLA$_2$-IIA with lipid markers and development of atherosclerosis. Transgenic mice that express human sPLA$_2$-IIA to levels similar to those observed in the acute phase response in humans have lower levels of HDL-C (due to increased catabolism of HDL sub-particles) and increased levels of triglycerides.[310] Thus an increase in sPLA$_2$-IIA associates with a reduction in HDL-C and an increase in triglycerides, a lipid profile consistent with

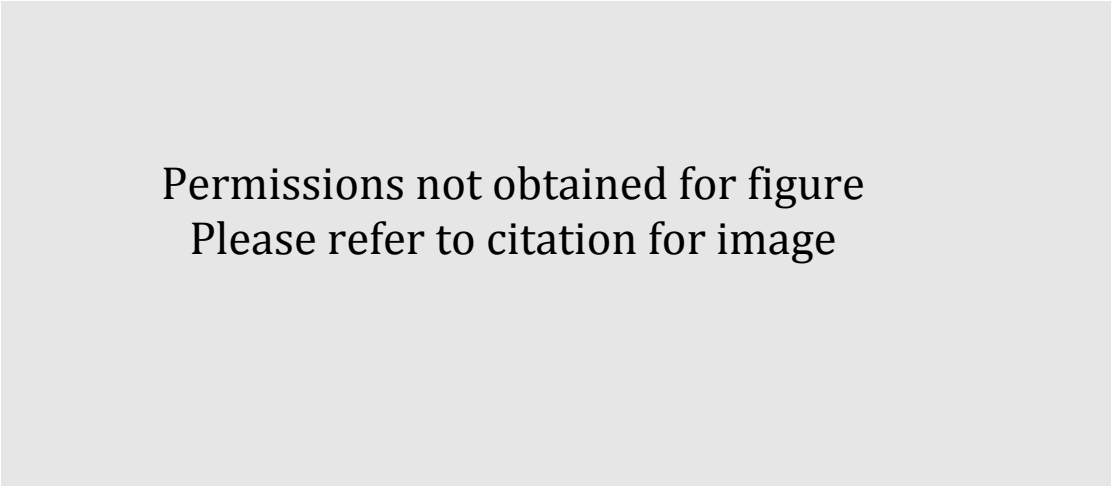


Figure 3.7: Model of propagation of atherosclerosis by $sPLA_2$.
$sPLA_2$ is present circulating in the blood and in tissues. In the tunica intima, $sPLA_2$ is thought to modify LDL-C, which attracts monocytes that migrate into the tunica intima where they become macrophages, and phagocytose the modified LDL-C, yielding foam cells. $sPLA_2$-mediated oxidation of LDL-C is thought to make the LDL-C particles stick to proteoglycans in the atheromatous lesion. Lp-PLA2: lipoprotein-associated phospholipase A2; Lyso-PC: lysophosphatidylcholine; Ox-LDL: oxidized LDL
Reproduced from Mallet et al [259]

higher $sPLA_2$ being detrimental to CVD risk.
Furthermore, when low density lipoprotein receptor deficient ($LDLR^{-/-}$) mice were transplanted with bone marrow from $sPLA_2$-IIA transgenic mice, they showed increased markers of oxidative stress *in vivo*, increased macrophage and foam cell formation in atherosclerotic lesions, and increased atherosclerotic lesion size at the aortic root compared to control mice.[311]
These findings from animal models of atherosclerosis support the hypothesis of $sPLA_2$-IIA being detrimental to health and contributing towards cardiovascular disease.

3.1.10 Observational association of $sPLA_2$ with CVD in humans

Autopsy studies have shown expression of $sPLA_2$ on immunostaining of human atherosclerotic plaques[293, 312, 313], which was particularly highly expressed in foam cells[293], in areas of calcification and in the necrotic core of the plaque. In contrast, cells that did not originate from the diseased carotid intima did not express $sPLA_2$.[312] Furthermore, post-mortem examinations of patients that died following AMI have identified evidence of localisation of $sPLA_2$ in the infarcted myocardium on immunohistochemistry.[277]

However, the presence of $sPLA_2$ in atherosclerotic tissue does not prove cause and effect and $sPLA_2$ may be elevated in response to disease (reverse causality), or the association may arise due to an association with another trait that *is* causally-related (i.e. confounding, as discussed in Chapter 1).[293]

Perhaps two of the most important Bradford-Hill criteria[57] that lend weight to a potential causal association between sPLA$_2$-IIA and CHD are (i) temporality and (ii) dose-response relationships. Evidence in favour of a temporal relationship between the levels of circulating sPLA$_2$ and risk of CHD has emerged from several prospective cohorts in which sPLA$_2$ was measured prior to incident[314, 315] and recurrent[260, 316–319] CHD events. Furthermore, a dose-response relationship has also been identified between levels of sPLA$_2$ and risk of CHD.[260, 314, 320]

However, as was the case for C-reactive protein (see Box 3.1 on page 65), which associates with incident CHD events decades prior to the initial event,[321] the long subclinical phase of atherosclerosis (which lasts several decades) means that measurement even years prior to the clinical event does not rule out reverse causality. And of course the absence of randomization in both animal models and traditional observational epidemiology makes confounding an equally plausible, alternative explanation.[322]

3.1.11 GWAs studies of CHD

At the time of starting this work, no GWAs had identified a SNP in the *PLA2G2A* locus as associated with CHD. However, this did not deter my enthusiasm to pursue a Mendelian randomization investigation of sPLA$_2$-IIA. For example, this could be explained by lack of comprehensive coverage of the *PLA2G2A* locus on GWAs chips (a familiar scenario for the *APOE* locus). Furthermore, lack of association could reflect a type II error arising from inadequate power. Indeed, loci in *HMGCR* have not appeared as GWAs SNPs for CHD, even though RCTs of statins (which inhibit HMG-coA reductase, encoded for by *HMGCR*) have provided conclusive evidence of an association with CHD.

3.1.12 Drug development: sPLA$_2$ as a drug target

On the background of the evidence from animal studies and observational studies in humans, the hypothesis was naturally generated that targeting sPLA$_2$ may represent a novel means to prevent development of atherosclerosis, and thus reduce risk of CHD.[323]

sPLA$_2$ inhibitors have been designed on the basis of the catalytic dyad[c], which differentiates them from cytoplasmic PLA$_2$ and LpPLA$_2$.

[c]The sPLA$_2$ enzymes contain a catalytic dyad at the active site, which consists of aspartic acid and histidine. In contrast, cytoplasmic and lipoprotein-associated PLA$_2$ have a catalytic triad (consisting of aspartic acid, histidine and serine).[324] This subtle difference allows the selective inhibition of sPLA$_2$ enzymes.

The first drug that showed selective inhibition of $sPLA_2$ was reported in 1995.[325] Through use of crystal structures of $sPLA_2$, a panel of drugs was developed with compounds structurally similar to indomethacin (an indole[d] non-steroidal anti-inflammatory). Out of 12 compounds developed, LYS3311727 had the lowest X_i50 (the mole fraction for 50% inhibition of $sPLA_2$ using a chromogenic assay) while retaining specificity for $sPLA_2$.[325]

Further chemical modification of LY311727 to improve binding to $sPLA_2$-IIA yielded varespladib (A-001).[326, 327] Methylation of varespladib into the pro-drug varespladib methyl (A-002), provided an orally-active drug that is metabolised by esterases into the active compound, varespladib.

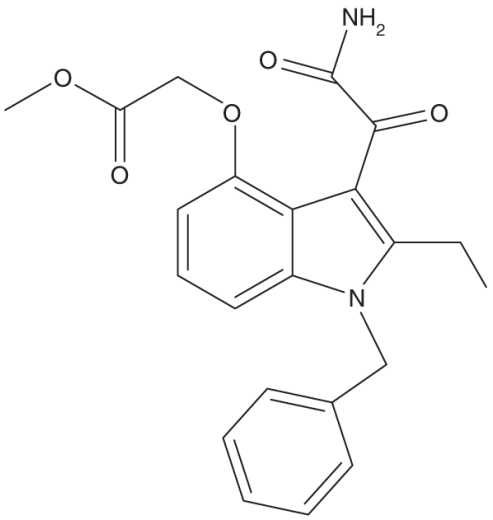
3.1.13 Varespladib

Varespladib (Figure 3.8) was originally designed to be a specific inhibitor of the $sPLA_2$-IIA isoform.[325] However, as was the case for other PLA_2 inhibitors (such as inhibitors of cytosolic PLA_2),[328] as new PLA_2 isoforms have been identified, drugs that were originally considered to be specific to a particular isoform of PLA_2 have shown inhibitory activity for the new isoforms (i.e. exerting non-specific effects).[328]

In the case of varespladib, although it was initially thought to be selective for the IIA isoform of $sPLA_2$, subsequent investigations revealed varespladib also inhibited the -V and -X isoforms[329, 330].

The potency of a compound in inhibiting the activity of a substrate can be quantified as the concentration of drug required to inhibit activity by 50% (denoted as the IC_{50}).[331] Thus to contrast the effects of varespladib on the IIA, III and X isoforms of $sPLA_2$, one method is to compare the IC_{50} estimates for varespladib on these traits. Importantly for varespladib, the IC_{50} estimates for the $sPLA_2$ isoenzymes were obtained from different animal models and using different drug compounds of varespladib, which may muddy the interpretation. Specifically, the mouse model that estimated IC_{50} values for -IIA was a transgenic mouse expressing human $sPLA_2$ and the drug compound used was varespladib (A-001)[329] whereas for IC_{50} values of -V and -X isoforms, a transgenic mouse with human recombinant V and X was studied using the drug compound varespladib methyl (A-002)[330]). Thus the IC_{50} estimates may not be directly comparable with one another. The IC_{50} values from these sources yielded values that were lower for IIA (indicating higher drug efficacy) than for V and X (9, 77 and 15 nM, respectively, Figure 3.10).[329, 330]

[d] Indoles are aromatic heterocyclic organic compounds that contain indole rings, consisting of a benzene ring and nitrogen-containing pyrrole ring



A-002 (varespladib methyl)

Figure 3.8: Structure of the first-in-class orally-available $sPLA_2$ inhibitor for CVD prevention, varespladib methyl

3.1.14 Animal studies of varespladib efficacy

Several animal studies have been conducted to investigate the effects of varespladib on animal models of atherosclerosis.[330, 332] *APOE* $^{-/-}$ mice fed with 16 weeks of a Western diet to induced atherosclerosis, and treated with A-002 had reduced total cholesterol, and considerable reductions in plaque content after 1 month of treatment compared to *APOE* $^{-/-}$ mice fed the same diet but treated with the drug vehicle (lacking varespladib).[330]

Guinea pigs fed a high-fat diet and treated with varespladib had reduced atherosclerotic lesions compared to those treated with placebo.[332]

Thus animal models provide an encouraging indication that varespladib may reduce atherosclerosis. However, the majority of animal models (approximately 90%) that suggest positive findings for predicting the efficacy of treatments fail to translate to treatments that are efficacious at improving outcomes when investigated in man.[51]

3.1.15 Safety of varespladib in humans: data from phase II randomized trials

Four phase II randomized trials of varespladib in patients with heart disease have been conducted.[333–336] Being phase II trials they were not designed or powered to investigate hard (clinical) outcomes, but they did investigate surrogate markers (such as changes in blood lipids and $sPLA_2$ traits), the findings of which are reported in Chapter 5. Of note, side effects occurred at higher rates in varespladib-treated individuals than those receiving placebo. Side effects included headache, [334], nausea,[334] diarrhoea,[334, 335] syncope,[335] arthralgia,[335], and, elevated liver enzymes (alanine transaminase).[333, 334] However, these adverse events were typically mild and no dose-response relationship was identified.[334]

3.1.16 Treatment trials of varespladib for CV efficacy: phase III RCTs

The first phase III randomized trial of $sPLA_2$ inhibition for the prevention of CV events commenced in 2010,[337] which coincided with the start of my investigative work reported in this thesis. The trial, The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16, NCT01130246), was a placebo-controlled clinical trial that recruited patients with ACS and randomized them to receive either 500mg/day varespladib methyl, or placebo for 16 weeks on the background of standard therapy (including atorvastatin). The primary outcome was a composite end-point consisting of cardiovascular death, non-fatal MI, non-fatal stroke or documented unstable angina with objective evidence of ischaemia requiring hospitalisation.[337]. In addition to clinical events, secondary outcomes were investigated, including changes in levels of $sPLA_2$, LDL-C, CRP and IL-6.

The objective was to recruit 6500 individuals in order to accrue 385 outcomes based on a presumed varespladib treatment efficacy of a 25% risk reduction in the event rate. This would achieve 80% power at a 5% type 1 false positive rate.

This trial would therefore provide the first deconfounded evidence for the causal role of $sPLA_2$-IIA in CVD, and more importantly, inform on whether modifying $sPLA_2$-IIA could serve as a new means to address the considerable residual risk of CVD which exists despite current pharmacotherapy.[338]

3.1.17 Genetic variants encoding sPLA$_2$-IIA

sPLA$_2$-IIA mass is encoded for by the *PLA2G2A* gene located on chromosome 1, which contains 6 exons, 4 of which are coding (Figure 3.9). A study that used tagging SNPs in *PLA2G2A* gene identified six SNPs that accounted for the majority of variation within the *PLA2G2A* locus.[339] Two SNPs, rs11573156 and rs3767221, were identified to have strong associations with sPLA$_2$-IIA mass.[339]

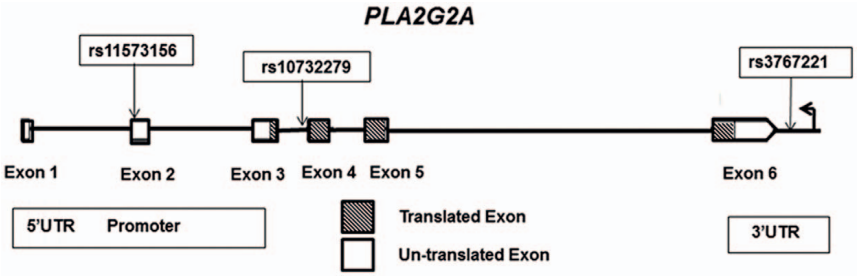


Figure 3.9: Map of the *PLA2G2A* gene
The rs11573156 SNP is thought to be involved in exon skipping of exon 2, and by doing so, influences levels of sPLA$_2$-IIA mass Derived from Exeter et al.[340]

A recent study that characterized these two SNPs identified both to be functional.[340]. Through detailed investigation, the authors suggested that rs11573156 influenced circulating levels of sPLA$_2$-IIA mass through exon skipping (in which exon 2 was differentially expressed according to presence of the minor allele of rs11573156). In contrast, rs3767221 T>G was thought to operate through differential transcription factor binding.

3.1.18 Use of a *PLA2G2A* genetic variant for Mendelian randomization

In Chapter 1, I introduced the concept of Mendelian randomization. Specifically, Figure 1.6 compares the properties of genetic information to that of a randomized clinical trial.

Thus, it is possible to use genetic variation in *PLA2G2A* to conduct experiments in which confounding is minimized to interrogate the causal relationship between sPLA$_2$-IIA mass and CHD.

3.2 Aims

I aimed to undertake a 'natural' Mendelian randomization trial with genetic data, to complement the VISTA-16 trial, and in doing so, to investigate whether sPLA$_2$-IIA is a valid therapeutic target for CHD prevention. Both sources of evidence (RCT design of VISTA-16 and Mendelian randomization from this thesis) would be free from confounding. Just as in the VISTA-16 trial in which individuals were randomly allocated to receive a drug that reduces sPLA$_2$,[337] I used a 'naturally randomly-allocated' genetic variant that also associated with lower levels of sPLA$_2$. The only subtle difference was that the gene variant was specific for the IIA isoform of sPLA$_2$ (the isoenzyme that varespladib was developed with the intention of specifically inhibiting)[323], whereas varespladib has inhibitory activity against the IIA, V and X isoforms. Figure 3.10 contrasts the Mendelian randomization analysis using a genetic variant in *PLA2G2A* with that of the phase III RCT of varespladib.

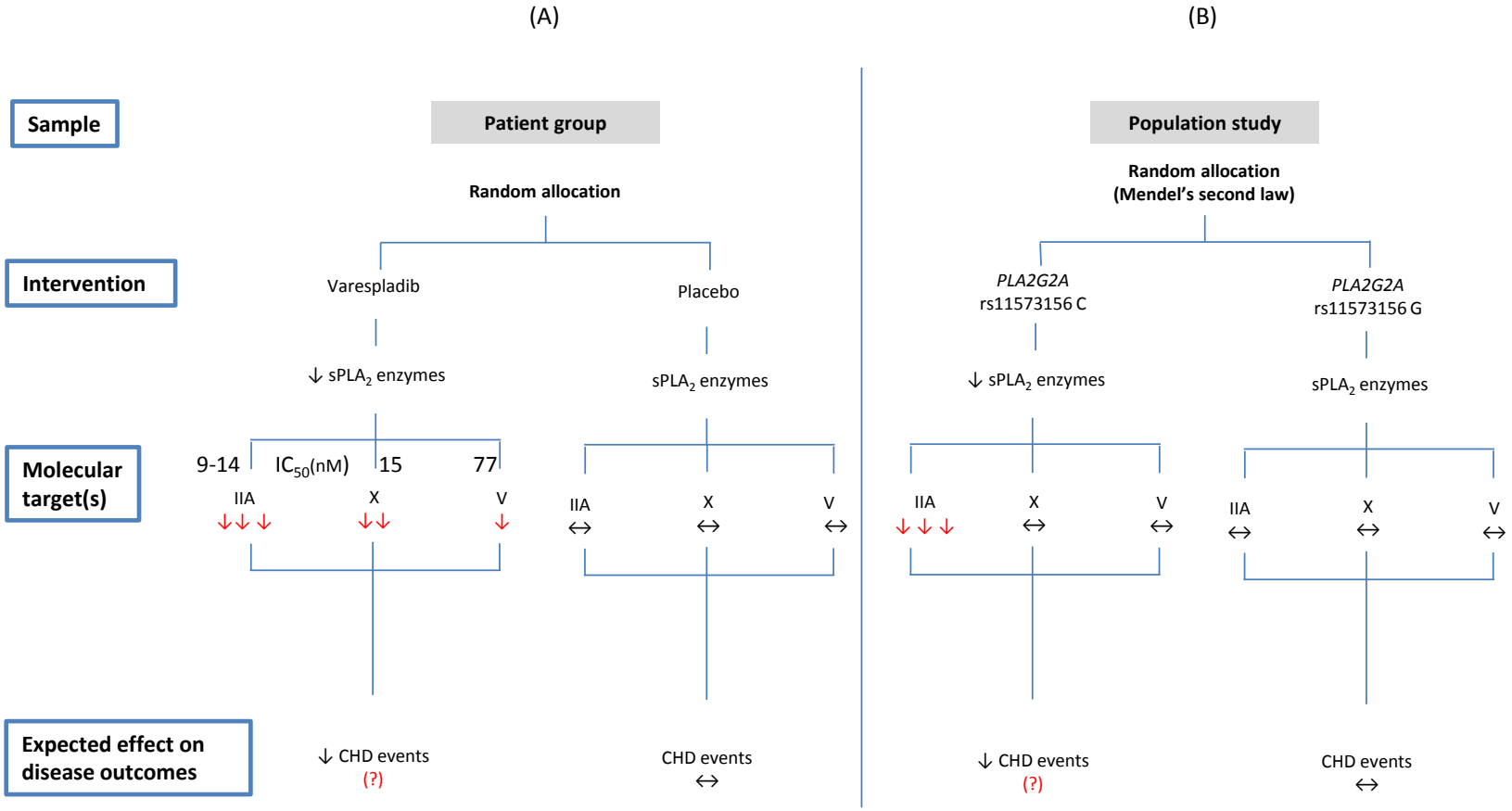


Figure 3.10: Comparison of (A) a randomized trial of varespladib (as in the VISTA-16 trial[337]), with (B) random allocation of *PLA2G2A* rs11573156

Varespladib has greatest inhibitory effect on $sPLA_2$-IIA but additional effects on $sPLA_2$-V and X, whereas *PLA2G2A* rs11573156 is specific for the $sPLA_2$-IIA isoform

The project included the following components:

1. Investigating the observational association between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular risk factors and events in studies set in both the general population and in patients with ACS, reported in Chapter 4
2. Selection of a SNP in *PLA2G2A* as a genetic instrument for Mendelian randomization analysis, reported (together with the remaining items listed below), in Chapter 5
3. Evaluation of the association between the *PLA2G2A* SNP and
   (a) circulating sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity (including an analysis on the association of SNPs in *PLA2G2A* with messenger RNA expression of *PLA2G2A*, *PLA2G5* and *PLA2G10*)
   (b) cardiovascular biomarkers
   (c) cardiovascular events overall and separated by subtype
4. An overview of published randomized clinical trials of varespladib
5. An instrumental variable analysis using the *PLA2G2A* variant to obtain a deconfounded estimate between sPLA$_2$-IIA and CHD through 'Mendelian triangulation'

Chapter 4

$sPLA_2$ and Cardiovascular Traits and Events: Observational Analysis

In this chapter, I will outline the methods and report the findings for the observational association between $sPLA_2$ and cardiovascular biomarkers and events.

4.1 Methods

4.1.1 General overview of studies contributing towards the observational associations between $sPLA_2$ and CHD events

A collaboration of 36 studies was established including 109,179 individuals of European descent (listed in Table B.1). The analyses reported in this chapter were limited to those studies with measures of $sPLA_2$. Approval from relevant ethical committees was obtained for collaborating studies. All analyses, unless otherwise stated, were performed using Stata 12.1 (StataCorp, Texas USA).

4.1.2 Measurement of $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity

$sPLA_2$-IIA mass and $sPLA_2$ enzyme activity were measured in seven and six of the collaborating studies, respectively (Table B.5). I grouped studies according to the population sampled, and the timing of blood sample. Studies were placed into one of two categories: (i) studies set in individuals that were predominantly free from established

CHD (EPIC-Norfolk[341], a nested case-cohort study in the general population and UDACS[342], a cross-sectional study of men with type 2 diabetes), and; (ii) studies set in ACS with samples taken within 48 hours of ACS event (FAST-MI[343], GRACE-France[344], GRACE-Scotland[345] and the MIRACL trial[346]). Owing to the time of blood sampling being greater than one month after the acute coronary event, samples for one study (KAROLA[347]) were not included in the analysis. This was because sPLA$_2$ is an acute phase reactant,[256][276] therefore the association with recurrent events may differ according to the timing of blood sampling.

Details of the assay methods used in each study (including quality control estimates - e.g. information on duplicates) are provided in Table 4.1.

Table 4.1: Characteristics of assays in studies conducted to measure sPLA$_2$ IIA mass and sPLA$_2$ enzyme activity

sPLA$_2$ trait	Parameter	EPIC-Norfolk	FAST-MI	GRACE-France & Scotland	MIRACL	UDACS
sPLA$_2$-IIA mass	**Assay**	ELISA[273] (Cayman Chemical Company, Ann Arbor, Michigan)	Time-resolved fluoroim-munoassay (an ELISA-like assay)[348]	ELISA[273] (Cayman Chemical Company, Ann Arbor, Michigan)	ELISA[273] (Cayman Chemical Company, Ann Arbor, Michigan)	ELISA[273] (Cayman Chemical Company, Ann Arbor, Michigan)
	Duplicates	Intra-assay variation between duplicates was 9.2%	Intra and interassay coefficient of variation was <15%	Intra and interassay coefficient of variation was <10%	Intra-assay variation between duplicates was 9.2%	Intra- and inter- assay coefficients of variation were 6.0 and 10.3%, respectively
	Lower detection limit	0.4 ng/ml	0.5 ng/ml	0.02 ng/ml	0.4 ng/ml	0.02 ng/ml
	Laboratory	CLB/Sanquin Research lab, Amsterdam	Valbonne, France	Paris Cardio-vascular Research Center	Paris Cardio-vascular Research Center	Dr Camejos lab, Gothenburg University, Sweden

Continued on next page

Table 4.1 – *Continued from previous page*

sPLA$_2$ trait	Parameter	EPIC-Norfolk	FAST-MI	GRACE-France & Scotland	MIRACL	UDACS
sPLA$_2$ enzyme activity	**Assay**	Selective fluorometric assay[271, 272]	Selective fluorometric assay[271, 272]	Selective fluorometric assay[271, 272]	Selective fluorometric assay[271, 272]	Not measured
	Duplicates	Intra- and inter-assay coefficient of variation was <10%	Intra- and inter-assay coefficient of variation was <10%	Intra and inter-assay coefficient of variation was <10%	Intra and inter-assay coefficient of variation was <10%	N/A
	Lower detection limit	0.10 nmol/min per ml	0.10 nmol/min per ml	0.10 nmol/min/ml	0.10 nmol/min/ml	N/A
	Laboratory	Paris Cardio-vascular Research Center	Paris Cardio-vascular Research Center	Paris Cardio-vascular Research Center	Paris Cardio-vascular Research Center	N/A

4.1.3 Distribution of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity

I plotted histograms of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity in the study for which I had access to individual participant data (EPIC-Norfolk). Owing to a skewed distribution, I log(e) transformed sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity, which normalized both distributions (Figure 4.1). Thus log(e) transformed sPLA$_2$ was used for all analyses (unless otherwise stated).

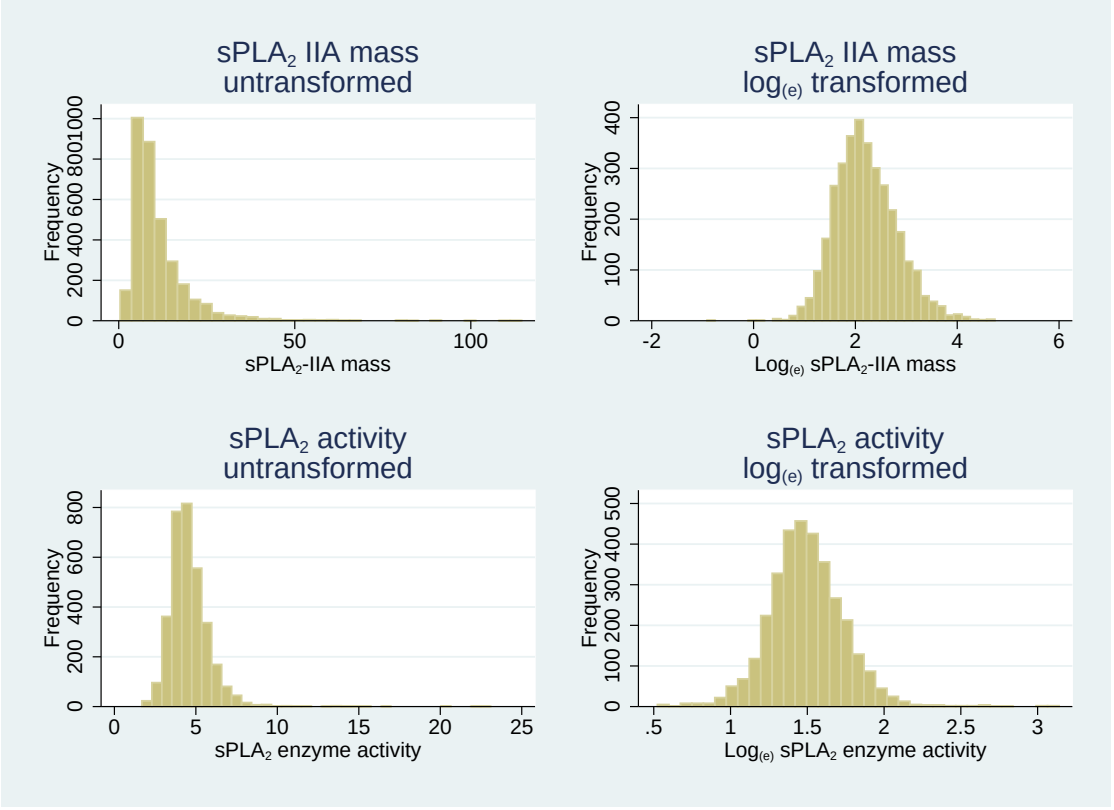


Figure 4.1: Distribution of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity in EPIC-Norfolk
Prior to log(e) transformation (left panes), both sPLA$_2$ traits have a right-skewed distribution. Following log(e) transformation, both sPLA$_2$ traits have a normal (Gaussian) distribution.

4.1.4 Correlation between sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity

I investigated the correlation between log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity in each study that measured both traits using Pearsons correlation coefficient. I plotted the pair-wise values in each study, and super-imposed the regression line of best fit.

4.1.5 Association of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with cardiovascular traits and potential confounders

I evaluated the cross-sectional association between log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity with established and emerging cardiovascular risk factors in the EPIC-Norfolk study. This was conducted for the following traits: age, body mass index (BMI), C-reactive protein (CRP), systolic blood pressure (SBP), diastolic blood pressure (DBP), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), apolipoprotein A1, apolipoprotein B, gender, type 2 diabetes (T2D) and smoking status. First, I created tertiles of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity, and tabulated the mean and standard deviation (SD) for continuous traits and proportion of cases for binary traits for each tertile. Secondly, I performed univariate linear and logistic regression analyses for continuous and binary traits, respectively, using natural logarithm (log[e]) transformed sPLA$_2$ IIA mass and sPLA$_2$ enzyme activity as the explanatory (independent) variables.

4.1.6 Association of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with cardiovascular events

To investigate the association between circulating sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with incident vascular events in general populations, I used the EPIC-Norfolk study and to investigate the association with recurrent events in patients with ACS, I used four ACS cohorts (FAST-MI, GRACE France, GRACE Scotland and MIRACL).

The outcomes for the analysis varied according to availability and study design. In the EPIC-Norfolk nested case-control study, cases were selected for fatal/nonfatal MI and therefore this was the outcome used for the analysis. In contrast, for the ACS studies (GRACE-France, GRACE-Scotland, FAST-MI and MIRACL), the outcome was a composite of all-cause mortality or MI. For further details, please see outcomes defined in Table B.6 and descriptive text on page 215 in Appendix B.

4.1.7 Shape of the association between sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with cardiovascular traits and clinical events

To evaluate the shape of the association between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular events, I used minimally-adjusted logistic regression models with log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity as independent continuous variables (assuming a linear effect). I then added a quadratic term (fitting both [sPLA$_2$ $\times$ sPLA$_2$]

and sPLA$_2$ into the model) and repeated the regression analysis. I tested the null hypothesis, that the linear model explained the data better, by using the likelihood ratio test. This was conducted in all studies individually.

4.1.8 Multivariate analysis to investigate the association of sPLA$_2$ traits and CHD

In order to investigate whether the association between sPLA$_2$ and fatal/nonfatal MI was influenced by confounding, I conducted a step-wise multivariate analysis. In the general population cohort (EPIC-Norfolk), I first created a minimally adjusted model between log sPLA$_2$-IIA mass or log sPLA$_2$ enzyme activity with MI, adjusted only for age and gender. I then added BMI, blood pressure and T2D to the model (but did not include lipids at this stage as they may mediate a potential association between sPLA$_2$ and CVD[256]). In the next model, I also adjusted for LDL-C, HDL-C and TG. Finally, in order to investigate the independent effect of sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with MI, I adjusted for the corresponding (non-index) trait.

For the ACS cohorts, a similar strategy was employed, however the fully-adjusted model consisted of age, gender, BMI and blood pressure.

4.2 Results

4.2.1 Correlation between sPLA$_2$ mass and sPLA$_2$ enzyme activity

Log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity showed strong pair-wise correlation in all four studies (all P values for correlation <0.0001). The Pearsons correlation coefficients between sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity in the ACS studies were 0.50, 0.51 and 0.58 whereas for the general population study, it was 0.18 (Figure 4.2).

4.2.2 Observational Analysis of sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular risk factors

sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity showed associations with several emerging and established cardiovascular risk factors including age, SBP, LDL-C, BMI, CRP and sex (Tables 4.2 and 4.3).

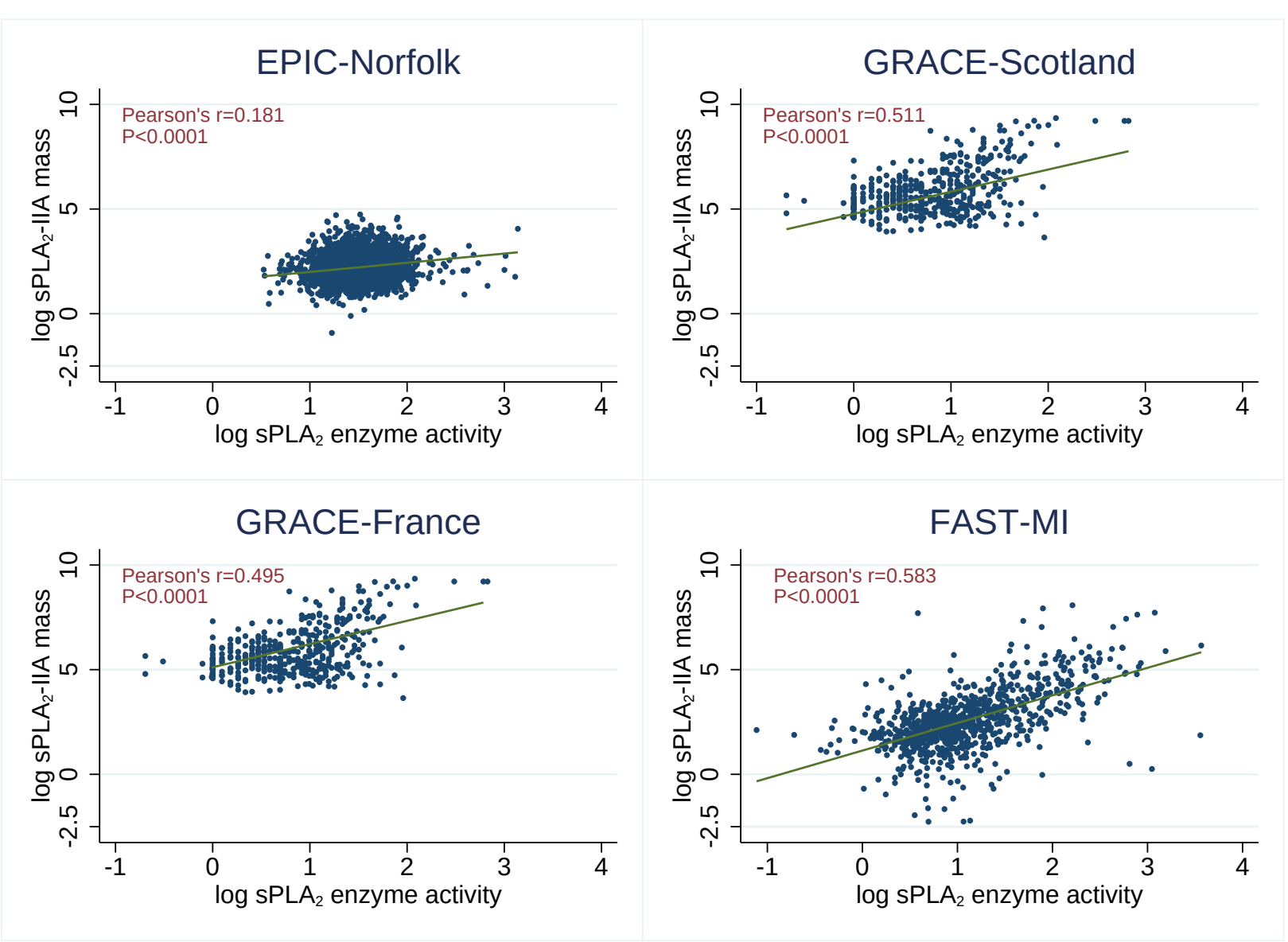


Figure 4.2: Correlation between $sPLA_2$-IIA mass and $sPLA_2$ enzyme activity in studies with both measures

Table 4.2: Association between tertiles of sPLA$_2$-IIA mass and established and emerging cardiovascular risk factors in 3371 individuals in the EPIC-Norfolk nested case control study

	Tertile (min-max, ng/ml) of sPLA$_2$-IIA mass									
		1 (0.4-6.8)			2 (6.8-11.3)			3 (11.3-114.5)		
Continuous traits	N	Mean	SD	N	Mean	SD	N	Mean	SD	P value**
Age (yrs)	1125	63.51	8.24	1124	64.93	7.59	1122	66.07	7.17	1.10×10^{-17}
BMI (kg/m^2)	1125	26.2	3.28	1120	26.69	3.66	1122	27.03	3.99	5.46×10^{-9}
CRP* (mg/dl)	1100	0.22	1.05	1108	0.53	1.15	1104	1.01	1.21	1.67×10^{-74}
Systolic BP (mmHg)	1122	139.54	17.85	1122	140.63	17.64	1120	141.83	19.21	4.36×10^{-3}
Diastolic BP (mmHg)	1122	84.13	11.08	1122	84.49	11.2	1120	84.51	11.99	0.69
HDL-C (mmol/l)	1073	1.31	0.38	1051	1.35	0.4	1059	1.36	0.4	0.03
LDL-C (mmol/l)	1073	4.05	0.98	1052	4.17	1.03	1059	4.2	1.05	0.01
Triglyceride* (mmol/l)	1114	0.57	0.5	1106	0.56	0.51	1105	0.55	0.51	0.06
Apolipoprotein A1 (mg/dl)	977	156.23	28.26	972	160.14	28.58	972	163.27	31.02	9.03×10^{-6}
Apolipoprotein B (mg/dl)	1029	129.79	32.28	1024	133.38	31.28	1038	134.31	33.42	0.07
Binary traits	N	Proportion	SD	N	Proportion	SD	N	Proportion	SD	P value***
Female sex (%)	1125	21.33	40.98	1124	35.68	47.93	1122	53.21	49.92	8.94×10^{-59}
T2D (%)	1124	3.2	17.62	1123	3.03	17.14	1120	4.11	19.85	0.06
Ever smoker (%)	1116	62.01	48.56	1106	62.3	48.49	1113	62.8	48.35	0.78

$*$ log transformed (as skewed distribution) thus units do not apply; $**$ derived from univariate linear regression using log sPLA$_2$-IIA mass as the independent variable; $***$ derived from univariate logistic regression using log sPLA$_2$-IIA mass as the independent variable.

Table 4.3: Association between tertiles of sPLA$_2$ enzyme activity and traditional cardiovascular risk factors in 3371 individuals in EPIC-Norfolk

	Tertile (ηmol/min/ml) of sPLA$_2$ enzyme activity									
	1 (1.7-4.0)			2 (4.0-4.9)			3 (4.9-23.1)			
Continuous traits	N	Mean	SD	N	Mean	SD	N	Mean	SD	P value**
Age (yrs)	1107	64.19	7.84	1107	64.54	7.89	1107	65.8	7.48	$7.17x10^{-7}$
BMI (kg/m^2)	1107	26.02	3.44	1106	26.61	3.75	1104	27.32	3.71	$1.00x10^{-15}$
CRP* (mg/dl)	1086	0.41	1.2	1087	0.53	1.16	1090	0.83	1.14	$1.87x10^{-16}$
Systolic BP (mmHg)	1103	138.62	18.33	1105	139.76	18.13	1106	143.71	18.01	$7.46x10^{-10}$
Diastolic BP (mmHg)	1103	83.22	11.34	1105	83.88	11.02	1106	86.03	11.69	$2.57x10^{-7}$
HDL-C (mmol/l)	1088	1.38	0.4	1070	1.35	0.4	980	1.28	0.38	$2.99x10^{-8}$
LDL-C (mmol/l)	1088	3.93	0.94	1070	4.14	0.99	981	4.37	1.1	$4.26x10^{-21}$
Triglyceride* (mmol/l)	1091	0.29	0.39	1093	0.52	0.44	1092	0.87	0.5	$5.40x10^{-168}$
Apolipoprotein A1 (mg/dl)	976	159.74	28.98	956	160.62	29.45	955	159.18	29.93	0.94
Apolipoprotein B (mg/dl)	1038	120.49	26.91	1024	130.77	29.12	993	146.7	35.2	$5.61x10^{-72}$
Binary traits	N	Proportion	SD	N	Proportion	SD	N	Proportion	SD	P value* **
Female sex (%)	1107	33.06	47.07	1107	35.95	48.01	1107	41.73	49.33	$1.27x10^{-5}$
T2D (%)	1107	2.62	15.98	1105	4.43	20.6	1106	3.35	17.99	0.1
Ever smoker (%)	1095	60	49.01	1098	59.56	49.1	1092	67.58	46.83	$2.42x10^{-4}$

∗ log transformed (as skewed distribution) thus units do not apply; ∗∗ derived from univariate linear regression using log sPLA$_2$ enzyme activity as the independent variable; ∗ ∗ ∗ derived from univariate logistic regression using log sPLA$_2$ enzyme activity as the independent variable.

4.2.3 Shape of the association between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular events

The relationship between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and risk of MI demonstrated a log-linear association (likelihood ratio test for quadratic vs. linear P>0.05 for sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity in all studies; Table 4.4). This indicated a constant proportional decrease in the relative odds per 1 log unit lower sPLA$_2$-IIA mass or sPLA$_2$ enzyme activity.

Table 4.4: Assessment of departure from linearity in the relationship between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular events, adjusted for age and gender.

Study/ Setting	Outcome	One log unit reduction in sPLA$_2$-IIA mass and events			One log unit reduction in sPLA$_2$ enzyme activity and events		
		Total, (n)	Linear OR (95%CI)	LRT P-value	Total, (n)	Linear OR (95%CI)	LRT P-value
General population							
EPIC-Norfolk	Fatal/ nonfatal MI	3371	0.67 (0.59, 0.75)	0.747	3321	0.30 (0.22, 0.41)	0.91
Acute coronary syndrome							
GRACE-Scotland	Death/MI	158	0.77 (0.49, 1.19)	0.737	164	0.46 (0.14, 1.48)	0.1
GRACE-France	Death/MI	277	0.95 (0.64, 1.42)	0.195	278	0.40 (0.16, 1.00)	0.165
FAST-MI	Death/MI	855	1.14 (0.87,1.49)	0.642	1011	0.68 (0.40, 1.14)	0.165

Footnotes: LRT: Likelihood ratio test (test for departure from linearity)

4.2.4 Observational Analysis of sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular events

Lower levels of sPLA$_2$-IIA mass and lower levels of sPLA$_2$ enzyme activity each were associated with a reduced risk of cardiovascular events in the general population with an OR for fatal/nonfatal MI of 0.67 (95%CI: 0.59, 0.75) and 0.30 (95%CI: 0.22, 0.41) per one log unit lower sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity, respectively, after adjustment for age and gender (Figure 4.3 and Table 4.4).

Adjustment for cardiovascular risk factors diminished the association between sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with incident MI in the general population, though

the association persisted following multivariate adjustment (Figure 4.3). Interestingly, both associations (sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with MI) remained after adjustment for one another.

For ACS cohorts, sPLA$_2$-IIA mass did not show association with all-cause mortality/MI on adjustment for age and gender (OR per one log unit reduction in sPLA$_2$-IIA mass: 0.93; 95%CI: 0.84, 1.04).

In contrast, sPLA$_2$ enzyme activity was associated with recurrent events, however the association was weak with an OR of 0.82 (95%CI: 0.69, 0.98) on adjustment for age and gender and OR 0.77 (95%CI 0.64, 0.93) following additional adjustment for BMI and BP (Figure 4.3).

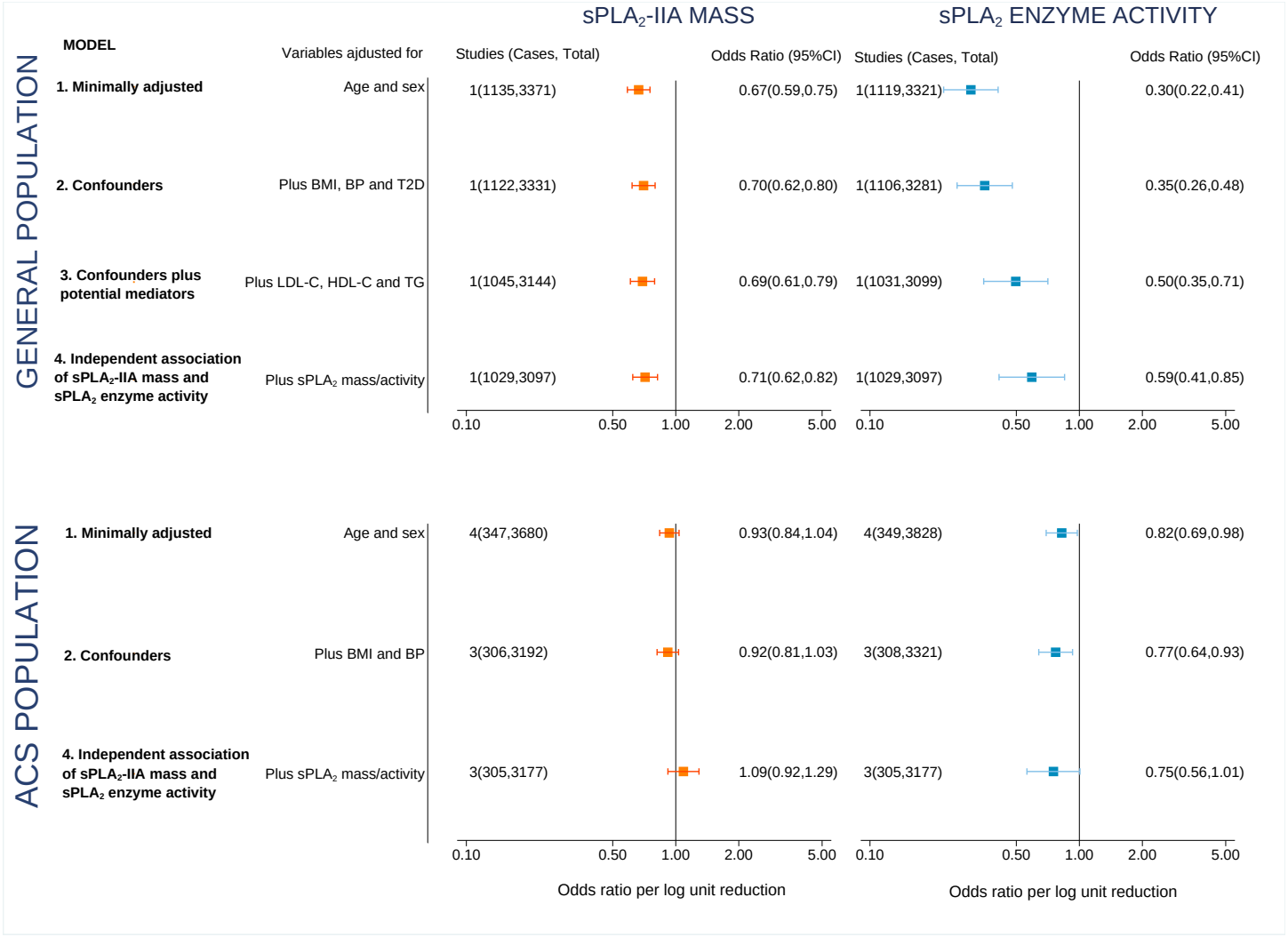


Figure 4.3: Association of a 1 log unit lower $sPLA_2$-IIA mass (orange) or $sPLA_2$ enzyme activity (blue) with fatal/nonfatal MI in general population studies (EPIC-Norfolk) and all-cause mortality/MI in ACS studies (FAST-MI, GRACE Scotland, GRACE France and MIRACL).

Footnotes: In Model 1, only age and gender were introduced as covariates. I then additionally adjusted for covariates (blood pressure, BMI, T2D) that could confound the association between $sPLA_2$ and CHD (Model 2). Because lipids may mediate the association between $sPLA_2$-IIA and CHD, I did not include lipids in model 2, but included them in model 3 (only available in the general population cohort). Finally, to investigate whether there was an independent association between $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and CHD, I additionally included $sPLA_2$ enzyme activity where $sPLA_2$-IIA mass was the explanatory variable (and vice-versa; Model 4).

4.3 Discussion

In chapter 3, I introduced the basis of the inflammation hypothesis for cardiovascular disease. I then described the phospholipase A_2 enzymes, which represent an important component of the inflammatory pathway, liberating arachidonic acid from phospholipid constituents of bilipid membranes.[324] Specifically, secretory phospholipase A_2-IIA, a ubiquitous iso-enzyme, represents a potential new therapeutic target[323] for treating the inflammatory component of cardiovascular disease by hypothetically being involved in the initiation and progression of the atherosclerotic plaque (Figure 3.7).[259]

4.3.1 Correlation between sPLA$_2$ traits

Analysing data from one large general population cohort and four ACS cohorts, I examined the relationship between sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity and found that the two traits were strongly associated with one another ($P<0.0001$ for all associations, Figure 4.2), but that the correlation was low in the general population cohort (Pearson's $r=0.18$)and moderate (Pearson's $r\simeq0.5$) in studies set in ACS.

It is interesting that the correlation between the two sPLA$_2$ traits differed according to clinical setting. Given the assays for both sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity were conducted using similar techniques, and with similar markers of test validity (Table 4.1), this discrepancy is unlikely to arise from differences in experimental technique. One potential explanation is that since sPLA$_2$-IIA is an acute phase reactant,[349] it may account for the majority of sPLA$_2$ enzyme activity during the acute phase of a coronary syndrome. This would therefore increase the apparent correlation between the two sPLA$_2$ measures during ACS (as an acute coronary event involves an increased inflammatory milieu)[350] compared to a general population cohort, in which most individuals would be expected to have only a low level of systemic inflammation.[351]

4.3.2 Association between sPLA$_2$ traits and cardiovascular traits

I identified associations between both sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with established and emerging cardiovascular risk factors, including both non-modifiable risk factors such as age and gender but also established modifiable risk factors for CHD including BMI, SBP and LDL-C (Tables 4.2 and 4.3). This association is important to note for several reasons. First, it introduces the possibility that the association between

sPLA$_2$ with cardiovascular events could be affected by confounding. As discussed in Chapter 1 (Figure 1.5), one of the requisites for a confounder is that an association should exist between the exposure of interest and the potential confounder.

Alternatively, the association between sPLA$_2$ IIA-mass or sPLA$_2$ enzyme activity and risk factors could indicate that they lie on the same causal pathway between sPLA$_2$ and vascular events. For example, data from animal models[352] and randomized trials have suggested that blood lipids could be on the causal pathway between sPLA$_2$ and CHD.[333–335] Indeed, an association between both sPLA$_2$ traits was detected with HDL-C and LDL-C on cross-sectional analysis in the general population cohort (Tables 4.2 and 4.3). This is important because adjustment for blood lipids in the association between sPLA$_2$ and CHD might result in a diminution of effect, but rather than reflecting confounding, this attenuation in the effect estimate could arise from adjustment for a mediator.[353] For this reason, I added blood lipids into the multivariate model (when I tested the association between sPLA$_2$ and CHD) separately to other risk factors (such as age, gender, smoking, which in contrast, are *unlikely* to be on the causal pathway). This allowed a more thorough investigation into the nature of the association between sPLA$_2$ traits and MVE.

4.3.3 Association between sPLA$_2$-IIA mass and vascular events

sPLA$_2$-IIA mass showed association with fatal/nonfatal myocardial infarction in one large general population nested case control study (EPIC-Norfolk), and this association remained robust even after adjusting for the potential confounding effect of other covariates (such as age, gender and cardiovascular traits including blood pressure; Figure 4.3). This 'independent association' (between sPLA$_2$-IIA mass and CHD) is of importance as it supports the possibility that sPLA$_2$-IIA mass may be a causal risk factor for incident CHD.

The association between sPLA$_2$-IIA mass with all-cause mortality/MI in the four ACS cohorts was less clear. No association was identified using the minimally-adjusted model, and this null association persisted when potential confounders were introduced into the model. This is surprising as it is in contrast to published reports of the association of sPLA$_2$ with recurrent events in ACS cohorts.[260, 316, 318, 319, 337, 354] However, it is worthy to note that the dataset used for the analyses in patients with ACS that I report in this chapter is the largest dataset established to date to investigate the nature of the association between sPLA$_2$-IIA and recurrent CHD events. Despite this, with

confidence intervals that span odds ratios from 0.81 to 1.03, I cannot rule out a false negative due to a limited number of events. On the other hand, the lack of association may reflect the pace at which the evidence has emerged. To expand, initial studies often overestimate the effect of a biomarker on a health outcome, and as more evidence accrues the effect may diminish and in some cases, such as this, the effect estimate may disappear altogether; this is known as the winners curse phenomenon.[61, 355]

The discrepancy in the association between sPLA$_2$-IIA mass with CHD events in the general population and ACS cohorts is worthy of discussion. First, there were three-fold more events for the general population cohort compared to the ACS studies, which means the analysis in the ACS cohorts may have been insufficiently powered to detect a true association (creating a false negative or type II error). Alternatively, this discrepancy could reflect the underlying biology: i.e. that sPLA$_2$ may be more important for incident CHD than for recurrent events. A similar suggestion has been made for C-reactive protein (except the opposite way round- i.e. that C-reactive protein is important for recurrent events rather than incident disease)[356], however whether specific risk factors could be important only for incident and not recurrent CHD remains under question.[357]

4.3.4 Association between sPLA$_2$ enzyme activity and vascular events

The association between sPLA$_2$ enzyme activity and fatal/nonfatal MI in general population cohorts was very strong on minimal adjustment (OR per log unit reduction 0.30; 95%CI, 0.22 to 0.41) however this diminished to OR 0.50 in the fully adjusted model, which suggested that the adjusted co-variates may be confounders. However, even in the fully-adjusted model, strong evidence of association persisted.

Again, and as for sPLA$_2$-IIA mass, the association between sPLA$_2$ enzyme activity and cardiovascular events in cohorts of patients with ACS was less clear (Figure 4.3). Both minimally adjusted and fully-adjusted models showed only weak associations with recurrent CHD events. And as for sPLA$_2$-IIA in ACS, this may reflect reduced power to detect an association.

Nonetheless, these findings of (albeit a weak) association between sPLA$_2$ enzyme activity and recurrent CHD do provide some reassurance that sPLA$_2$ may be an important enzyme for CHD, although the question of which of the three sPLA$_2$ isoforms (IIA, V or X) is contributing towards this association is another question that requires further investigation.

4.3.5 Independent association between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and vascular events

Interestingly, there was little alteration in the effect estimate for the association between sPLA$_2$-IIA and CHD when sPLA$_2$ enzyme activity was introduced into the model for the analysis based in the general population. This may indicate that not all the sPLA$_2$-IIA mass variance was detected by sPLA$_2$ enzyme activity.

The same was true when sPLA$_2$-IIA mass was introduced into the model in which sPLA$_2$ enzyme activity was the independent variable (again in the general population setting). This is to be expected since sPLA$_2$ enzyme activity is considered to represent a composite of -IIA, V and X. Adjusting for sPLA$_2$-IIA should therefore not completely attenuate the association as V and X (separate isoforms that are considered to have their own, independent role in atherogenesis[358]) are not measured in the specific sPLA$_2$-IIA assay.

4.4 Conclusions

The findings from this chapter yield important insights into the nature of the association between the sPLA$_2$ traits, cardiovascular traits and events. In summary:

- sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity are strongly associated with each other, but the correlation is higher in studies in which the sample is obtained within 48 hours of an ACS event than in the healthy population
- both sPLA$_2$ measures (IIA-mass and enzyme activity) showed very strong association with several established cardiovascular risk factors, opening up the possibility of confounding and/or a potential mediation effect in the association of sPLA$_2$ with CHD
- sPLA$_2$-IIA mass shows strong association with incident CHD, which remains robust to incorporation of potential confounders
- in contrast, sPLA$_2$-IIA mass did not show association with recurrent CHD events in patients with ACS, both in minimally-adjusted or fully-adjusted analysis

- sPLA$_2$ enzyme activity also showed robust association with incident CHD events, and again the effect persisted with incorporation of potential confounders. However, the point estimate of the effect estimate did diminish on adjustment, suggesting that the covariates may either partially confound the association, or may lie on a causal pathway between sPLA$_2$ enzyme activity and CHD
- sPLA$_2$ enzyme activity showed weak evidence of association with recurrent CHD events in patients with ACS both on minimally and fully adjusted analyses
- in the general population study, there was evidence that both sPLA$_2$ measures had association with CHD that was independent of the other

In the next chapter, I will use a genetic variant for instrumental variable analysis[359] to test whether the observational association of sPLA$_2$ measures with CHD events arises due to a causal relationship, or whether the estimates I report here for incident CHD events have arisen due to residual confounding (from unmeasured or imprecisely measured confounders) and/or reverse causality.

Chapter 5

Randomized Evidence on $sPLA_2$ and Cardiovascular Disease based on Genetic Studies and Trials

In Chapter 4, I investigated the observational association between $sPLA_2$-IIA mass, $sPLA_2$ enzyme activity and cardiovascular traits and disease events and identified an association between $sPLA_2$-IIA mass with incident CHD events, which remained robust to statistical adjustment for potential confounders. This provided evidence of an independent association between $sPLA_2$-IIA mass and CHD that may be suggestive of a causal relationship. However, as outlined in Chapter 1, observational associations in which potential confounding has been minimized through statistical adjustment may still not reflect the 'true' underlying causal relationship due to residual confounding. Such observational associations do, however, generate hypotheses that require formal testing for causality, through use of a randomized design.

In this Chapter, I will describe the Methods and Results for the genetic analysis, including a "Mendelian triangulation" (instrumental variable) analysis. I also report the conduct and results of a systematic review and meta-analysis of randomized trials of the $sPLA_2$-lowering drug, varespladib methyl.

5.1 Methods

5.1.1 General overview of studies participating in the sPLA$_2$ collaboration

An international collaboration was established of 36 studies including 109,179 individuals of European descent (listed in Table B.1). Of the 36 studies, 19 were set in general populations (consisting of 14 cohorts, three nested case-control studies and two case-control studies) and ten studies were of patients with ACS (comprising nine cohorts and one nested case-control study).

In addition, I included four case control studies of coronary artery disease, one cohort of patients with established arterial vascular disease or risk factors for CVD (SMART[360]), and one nested case-control study of coronary artery restenosis in patients with ACS undergoing percutaneous coronary intervention (GENDER[361]). These additional six studies were analysed and reported separately.

Finally, tissue samples from one cohort of patients undergoing aortic valve surgery (ASAP[362]) were used to investigate the association of SNPs in the *PLA2G2A* region with mRNA expression in several tissues (including the aortic adventitia, heart and liver), which was replicated in an external data source comprising 206 transplant donor liver samples.[362]

Approval from relevant ethical committees was obtained for collaborating studies. I used Stata 12.1 (StataCorp, College Station, Texas, USA) to conduct analyses.

5.1.2 Genetic analysis

Access was available to individual participant data in all contributing studies. All studies contributed towards the genetic analysis apart from the MIRACL trial[363], a trial of 3086 participants with Acute Coronary Syndrome that were randomized to atorvastatin or placebo, which was excluded because of lack of availability of genetic data.

A pre-specified Stata script was developed to standardize the genetic analysis in the collaborating studies. I arranged the genotype coding to be directionally concordant with the effects of pharmacological treatment by varespladib - i.e. both the SNP and the drug

lowered sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity. Traits that were not normally distributed (sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity (see Figure 4.1 for histograms pre and post transformation), C-IMT, CRP, triglyceride and IL6 concentrations) were $\log_e$ transformed, and differences between genotype groups were reported as a percentage difference.[364] I obtained the percentage difference estimate by exponentiating the association between *PLA2G2A* with the log transformed trait to obtain the relative difference. I then converted the relative difference to a percentage by subtracting one from the relative difference and multiplying the fraction by 100.

To avoid the potential for established CVD to alter the magnitude of the genotype-trait association, the analysis of associations between genotype and continuous traits was restricted to controls in retrospective case-control studies. I included all individuals from cohort studies and nested case-control studies as measurement of variables at recruitment was made prior to occurrence of the clinical event.

5.1.3 Selection of the genetic instrument and evaluation of its specificity and effect size

5.1.3.1 SNP selection

Six tagging single nucleotide polymorphisms (SNPs) that captured >90% of the genetic variation in *PLA2G2A* (located on chromosome 1) in Europeans[339] were evaluated in three studies (EPIC-Norfolk, GRACE-France and UDACS). The per-allele association of each SNP with sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity was investigated.

Rs11573156 showed strongest association with sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity and was prioritized for analysis in the remaining studies (Figure 5.1). The genotype frequency was consistent across the studies (Figure B.2), and no study deviated from Hardy Weinberg Equilibrium (Table B.4).

5.1.3.2 Specificity of genetic instrument for *PLA2G2A* expression

The Advanced Study of Aortic Pathology (ASAP)[362] was used to investigate the specificity of the SNP for sPLA$_2$-IIA. In ASAP, patients undergoing aortic valve surgery at the Karolinska University Hospital, Stockholm Sweden were recruited.[362] Tissue biopsies were taken from liver, heart, mammary arteries and dilated and non-dilated ascending

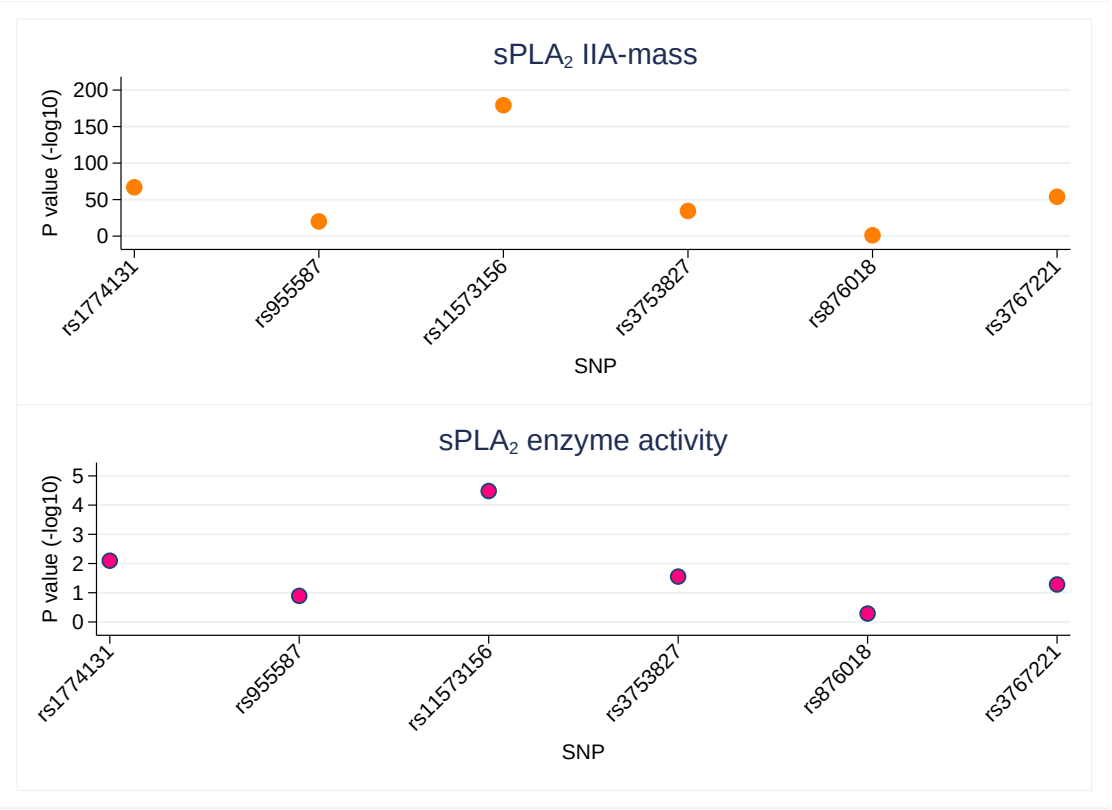


Figure 5.1: P-values for the associations between the 5 tagging SNPs in *PLA2G2A* and sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity.
The analysis was conducted in EPIC-Norfolk, GRACE-France and UDACS and the estimates were pooled across studies. The individual estimates per study are reported in Tables B.2 and B.3

aorta during surgery. A total of 700 tissue samples from 272 different patients were investigated. The medial and adventitial layers of the vascular specimen were separated. All tissue samples were incubated with RNAlater (Ambion, Austin, Texas, USA) and homogenised for mRNA extraction.[362]

Affymetrix GeneChip Human Exon 1.0 ST expression arrays were used to quantify mRNA expression, and participants were also genotyped using Illumina Human 610W-Quad Beadarray (including 101 SNPs in the region 200kb up and downstream from the *PLA2G2A* locus).

Raw data were pre-processed using the RMA algorithm[365] and the core group of metaprobesets, resulting in normalized and log(2) transformed expression data. Gene expression and eQTL effects were evaluated as follows: genotypes were re-coded as 0, 1 and 2 and included as predictors in a linear additive model with gene expression as the response variable. The association analyses of *PLA2G2A* SNPs with mRNA expression

was conducted using R 2.13.0 and Bioconductor.[366] Alignments and plots of exon-level microarray data were performed using the GeneRegionScan package.[367]. For replication of the SNP showing strongest association with *PLA2G2A* mRNA expression, an external data source comprising 206 transplant donor liver samples[368] was used.

These mRNA analyses were conducted by Dr. Lasse Folkerson in collaboration with me.

5.1.3.3 Strength of genetic instrument (rs11573156C>G) on sPLA$_2$

I estimated the association between *PLA2G2A* rs11573156 and sPLA$_2$ measures, as well as the proportion of variance (R^2) of these measures explained by the *PLA2G2A* variant. For each study with traits and genotype available (Table B.5), per C-allele associations of the *PLA2G2A* rs11573156 variant with log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity were estimated.

5.1.3.4 Association between genetic instrument and putative and established cardiovascular risk factors

Twenty studies of individuals in which blood sampling occurred prior to the cardiovascular event were used to test the association of the *PLA2G2A* rs11573156 variant (per C-allele) with cardiovascular risk factors within each study, using linear regression for continuous traits and logistic regression for binary traits. I pooled results using fixed and random effect meta-analysis.

5.1.4 Cardiovascular outcomes

For the general population studies, the composite MVE outcome was separated into prevalent and incident events, whereas for studies of ACS patients, all events after recruitment were included and labelled as recurrent. Prevalent MVE was a composite of nonfatal MI and nonfatal stroke, and incident MVE was a composite of fatal/nonfatal MI and fatal/nonfatal stroke. For ACS cohorts the recurrent MVE were a composite of nonfatal MI, nonfatal stroke and all-cause mortality. Individual components of the MVE outcome were also available for general population studies and ACS cohorts and were reported separately. See Appendix B.1.1 on page 215 for outcomes definitions per study and Table B.6 for study contribution to the composite outcomes.

5.1.5 Association between genetic instrument and major vascular events

I conducted two genetic approaches to evaluate the role of sPLA$_2$ on MVE. First, I performed a genetic association analysis of the *PLA2G2A* rs11573156 variant with MVE, and second, an instrumental variable analysis that quantified a causal effect of a one log unit lower sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity on MVE, under the assumptions of instrumental variable analysis, i.e. of a log-linear association between the biomarker and the outcome.[84]
A total of 26 studies contributed to these two approaches, comprising 17 in general populations and nine[a] studies in patients with ACS.

5.1.5.1 Genetic association analysis

For the genetic association analysis, I quantified the within-study odds ratio (OR) per C allele of *PLA2G2A* rs11573156 with MVE using logistic regression and I pooled the results using fixed and random-effects meta-analysis. I quantified between-study heterogeneity using I^2.[147] Meta-analyses were stratified by clinical setting into general population studies or studies of patients with ACS.

5.1.5.2 Instrumental variable analysis

I used the ratio instrumental variable estimator to estimate the unconfounded effect of log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity on cardiovascular outcomes. For this, I first conducted a fixed-effects meta-analysis of the *PLA2G2A* rs11573156 SNP (per C-allele) on log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity across studies with these phenotypes available separately for general population and ACS studies. Assuming a single (fixed) effect of the *PLA2G2A* SNP on log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity separately for general population and ACS studies, I applied the pooled estimate to studies that did not have information on sPLA$_2$.

Using study-specific associations between the *PLA2G2A* SNP and each outcome, I calculated the instrumental variable estimate for log sPLA$_2$-IIA mass and log sPLA$_2$ enzyme activity by dividing the rs11573156-outcome association by the pooled estimate of the association of rs11573156 and log sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity. This analysis took into account the uncertainty in both the rs11573156-sPLA$_2$ and

[a]The MIRACL trial did not have genetic data available, and thus could not contribute towards the genetic analyses

rs11573156-outcome associations by using the delta method[b] to estimate the standard errors of ratio instrumental variable estimates.[369] I pooled the study specific instrumental variable estimates using fixed effects meta-analysis.

This instrumental variable technique enabled me to include studies that did not have measures of sPLA$_2$-IIA mass or sPLA$_2$ enzyme activity. This should increase the precision of pooled IV estimates. However, this may occur at a loss to the internal consistency of the instrumental variable estimate for studies without measures of sPLA$_2$.[370–372]

Finally, I compared the summary instrumental variable estimates to the expected estimates based on the observational association between sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and cardiovascular events (reported in Chapter 4).

5.1.6 Systematic review of randomized trials of varespladib

In order to be able to quantify the effect of varespladib methyl[c] on sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity and circulating biomarkers, I conducted a meta-analysis of phase II randomized trials. I followed Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines[143] on conducting a systematic review. PubMed was used to search MEDLINE on January 28th 2013. The following search term was used, incorporating both the generic and proprietary drug names and consisting of U.S. National Library of Medicine (NLM) Medical Subject Headings (MeSH)[373] and free text terms `(''varespladib''[Substance Name] OR ''varespladib methyl''[Substance Name] OR varespladib OR A002 OR LY333013)`. This search was supplemented by hand-searching references of recent commentary articles on sPLA$_2$ and varespladib.[256, 293, 323, 337, 374, 374, 375]

The abstracts of all retrieved articles were scrutinized and were preliminarily included if they were randomized trials in which individuals were allocated to varespladib or placebo. The full text of articles satisfying the initial screening were then examined. Trials were included if they reported sPLA$_2$-IIA mass and/or enzyme activity, cardiovascular biomarkers or outcomes.

Articles that were finally included were then examined, and the following values were recorded:

[b] The delta method, also known as the method of propagation of errors, is a way of estimating the variance of a transformation of one or more variables through using a calculus method termed "Taylor series expansion"

[c] Varespladib methyl is the first-in-class sPLA$_2$ inhibitor designed for the prevention of cardiovascular disease

- phase of trial (e.g. phase I, II, III or IV)
- number of individuals randomized
- duration of trial
- dose of varespladib and frequency of dosing
- primary and secondary outcome(s)
- baseline health status (e.g. presence of CVD) of recruited participants
- levels of sPLA$_2$-IIA mass or sPLA$_2$ enzyme activity and cardiovascular biomarkers in the active and control arms at baseline and at follow-up

To facilitate pooling of biomarker data across trials, the most frequently used time point for sampling was identified. In trials that did not measure biomarkers at this preferred time, values reported at a time in closest proximity to the preferred time were used.

I quantified the effect of varespladib on sPLA$_2$-IIA mass or sPLA$_2$ enzyme activity and cardiovascular biomarkers by estimating the difference in each biomarker from baseline to follow-up in the placebo arm and subtracting this estimate from the corresponding value for the active arm. By doing so, I obtained estimates of the effect of the intervention (that were free from confounding).

I estimated the dose-response relationship between varespladib and circulating sPLA$_2$-IIA mass by conducting a meta-regression analysis using the "`metareg`"[376] command in Stata.

5.2 Results

5.2.1 Selection and validation of the genetic instrument for sPLA$_2$-IIA

Of the six identified tagging SNPs in the *PLA2G2A* gene, rs11573156 C>G showed the strongest association with sPLA$_2$-IIA mass (P=5.49x10^{-180}) and sPLA$_2$ enzyme activity (P=3.29x10^{-5}) and was prioritized for analysis in the remaining studies (Figure 5.1).

From the tissues available in ASAP study, *PLA2G2A* was mainly expressed in the liver, aortic adventitia and heart. *PLA2G2A* mRNA levels were considerably higher than those of *PLA2G5* and *PLA2G10* (Figure B.1).

To evaluate the specificity of the genetic instrument (rs11573156), the association of SNPs in *PLA2G2A* with mRNA expression of the different sPLA$_2$ isoforms was analysed, each encoded by separate genes (*PLA2G2A* for sPLA$_2$-IIA, *PLA2G5* for sPLA$_2$-V and *PLA2G10* for sPLA$_2$-X).

The SNP showing strongest association with *PLA2G2A* mRNA expression in liver was rs10732279 (P=8.71x10^{-19}; Figure 5.2 and Figure B.1), in strong LD with rs11573156 (R^2=0.91 in Europeans, HapMap release 21) and explained 31% of the variation in *PLA2G2A* mRNA expression. These findings were replicated in an external data source comprising 206 transplant donor liver samples (P=4.76 x 10^{-8}).[368] In contrast, rs10732279 did not show association with either *PLA2G5* or *PLA2G10* mRNA expression (P=0.04 and P=0.88, respectively) confirming the specificity of the genetic instrument for the sPLA$_2$-IIA sub-type (Figure 5.2).

5.2.2 Association of rs11573156 with sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity

In 3 studies of 1400 individuals with ACS and 2 general population studies of 3533 individuals, an allele dose-dependent association was observed between rs11573156 and sPLA$_2$- IIA mass and enzyme activity (Figure 5.4). For each additional C allele of rs11573156, sPLA$_2$-IIA mass was reduced by 38% (95%CI: 36%, 40%; P=1.2x10^{-216}) in studies of general populations and 44% (95%CI: 37%, 50%; P=1.64x10^{-21}) in studies of ACS patients. The pooled proportion of variance (R^2) of sPLA$_2$-IIA mass explained by rs11573156 was 21% (95%CI: 18%, 23%) in general population and 6% (95%CI: 3%, 9%) in ACS studies, indicating that this variant was a strong genetic instrument for

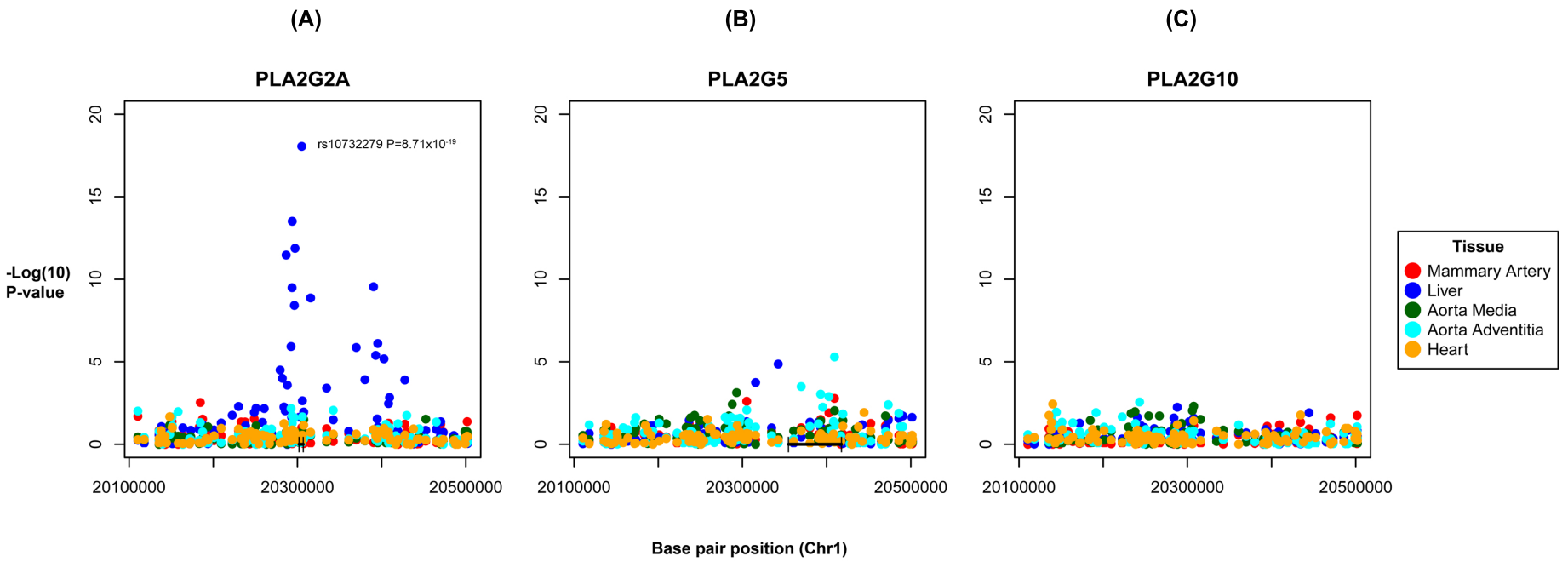


Figure 5.2: Manhattan plots of the association of SNPs in *PLA2G2A* region and expression of: (A) *PLA2G2A* mRNA; (B) *PLA2G5* mRNA, and; (C) *PLA2G10* mRNA, in mammary artery (red dots), liver (blue dots), tunica medica (green dots), tunica adventitia (cyan dots) and heart (brown dots).
Data obtained from the ASAP study[362].

sPLA$_2$-IIA mass.

The effect of the rs11573156 variant on sPLA$_2$ enzyme activity was considerably smaller than that for sPLA$_2$-IIA mass at 3% (95%CI: 1%, 5%; P=1.8x10^{-4}) in studies of general populations and 23% (95%CI: 19%, 27%; P=4.0x10^{-22}) for studies of ACS patients (Figure 5.4).

5.2.3 Systematic review and meta-analysis of the effects of varespladib on circulating sPLA$_2$-IIA, sPLA$_2$ enzyme activity and cardiovascular traits from clinical trials

Four randomized trials were identified (Figure 5.3) [333–336] of 1300 participants with a mean age of 61 yrs. Three trials recruited patients with stable CHD and one recruited patients at the time of ACS (Table 5.1). The primary outcomes were changes in sPLA$_2$-IIA level, LDL-C or cardiac enzymes and follow-up ranged between one and six months.

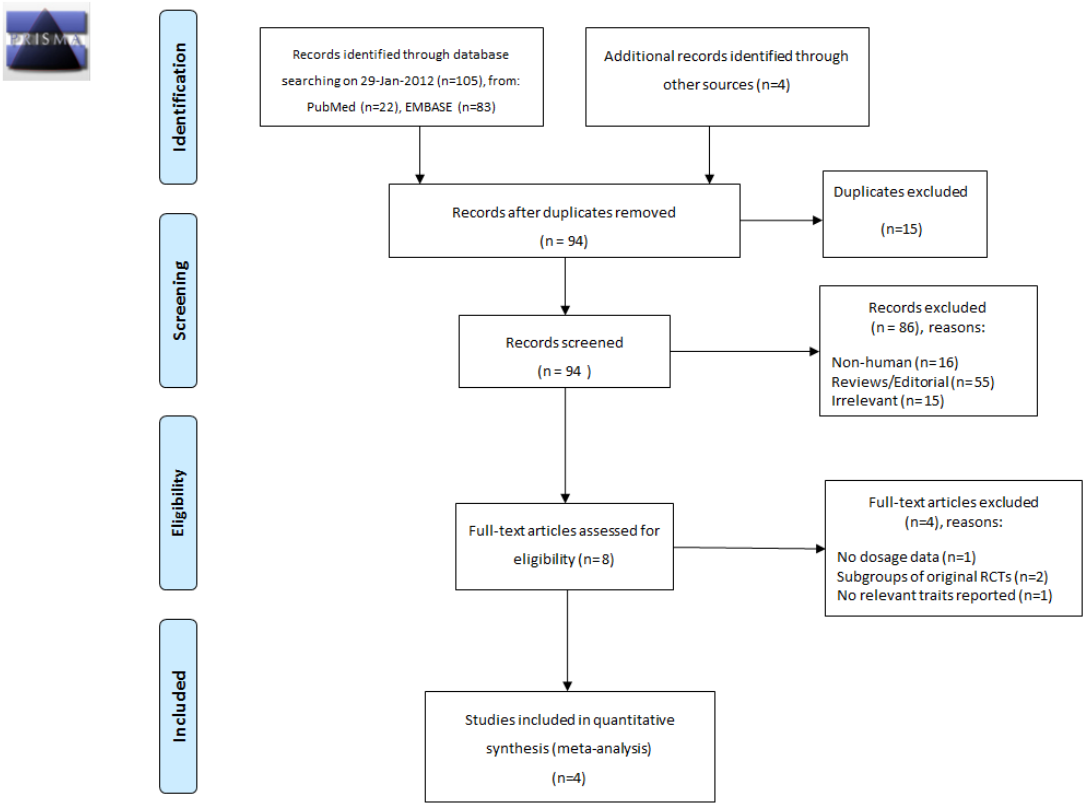


Figure 5.3: PRISMA flow diagram illustrating the search strategy used to identify randomized trials of sPLA$_2$ lowering therapies.

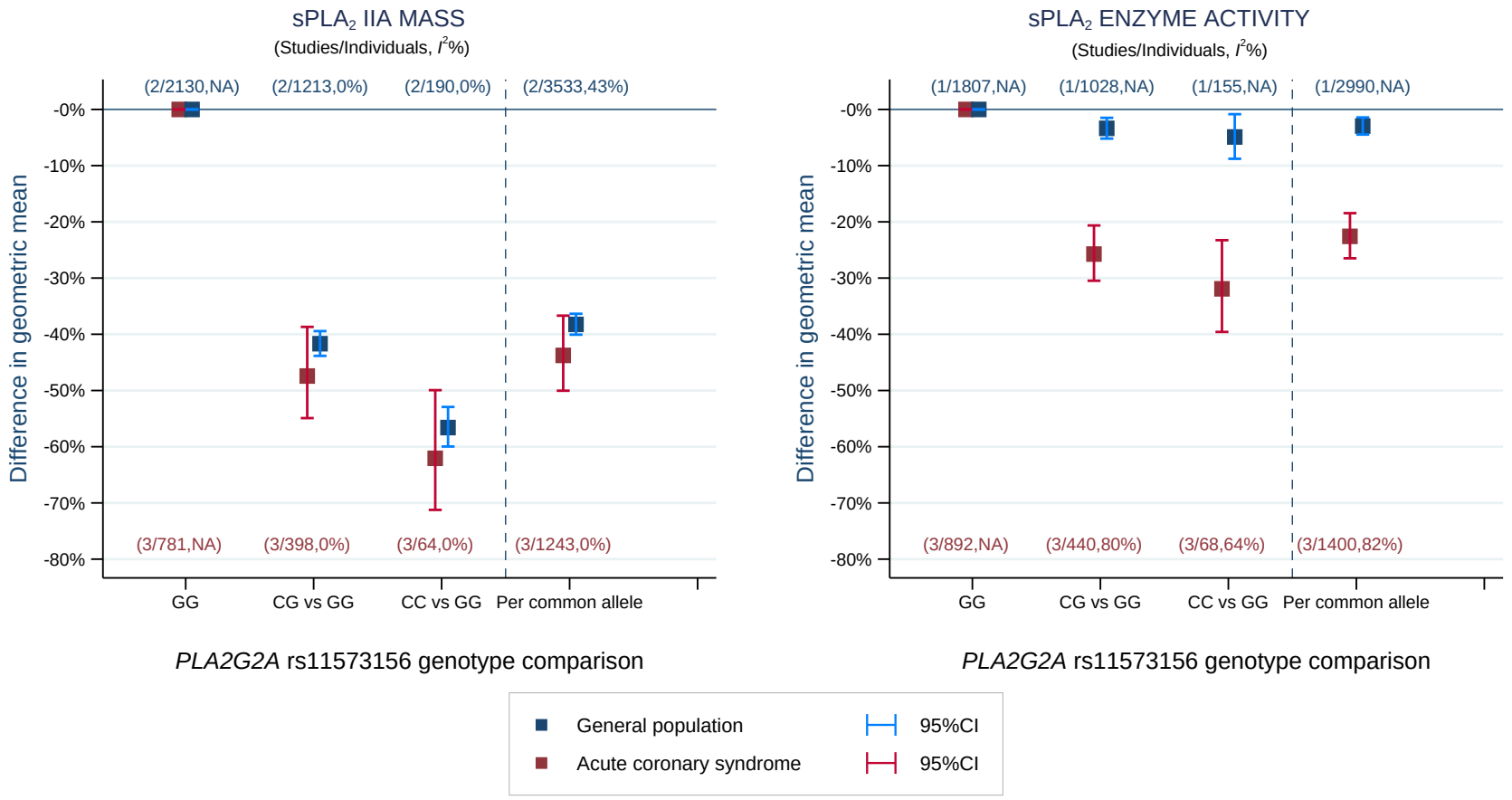


Figure 5.4: Meta-analysis pooled estimates of the association between *PLA2G2A* rs11573156 with (A) sPLA$_2$-IIA mass and (B) sPLA$_2$ enzyme activity, separated by study setting into general populations (EPIC-Norfolk, UDACS; blue) and acute coronary syndrome (GRACE-Scotland, GRACE-France, FAST-MI; red). Numbers in brackets represent the number of studies, participants, and between-study heterogeneity (measured by I^2). NA=not applicable; I^2 values cannot be computed in the presence of <3 studies in Stata

A meta-regression of dose-finding trials indicated a dose-dependent reduction in sPLA$_2$-IIA mass with increasing daily doses of varespladib in the range 100-1000mg/day (P for meta-regression=0.06, Figure 5.5).

I also identified associations between varespladib treatment (500mg/day) and apolipoprotein B (-0.78 SD; 95%CI:-1.05, -0.52) and LDL-C particle size (0.23 nm; 95%CI: 0.19, 0.26; Table 5.4). The effect of varespladib on sPLA$_2$ enzyme activity was not reported in any clinical trial, as levels in the treatment arm were beneath the lower limit of detection of the assay.[333–335]

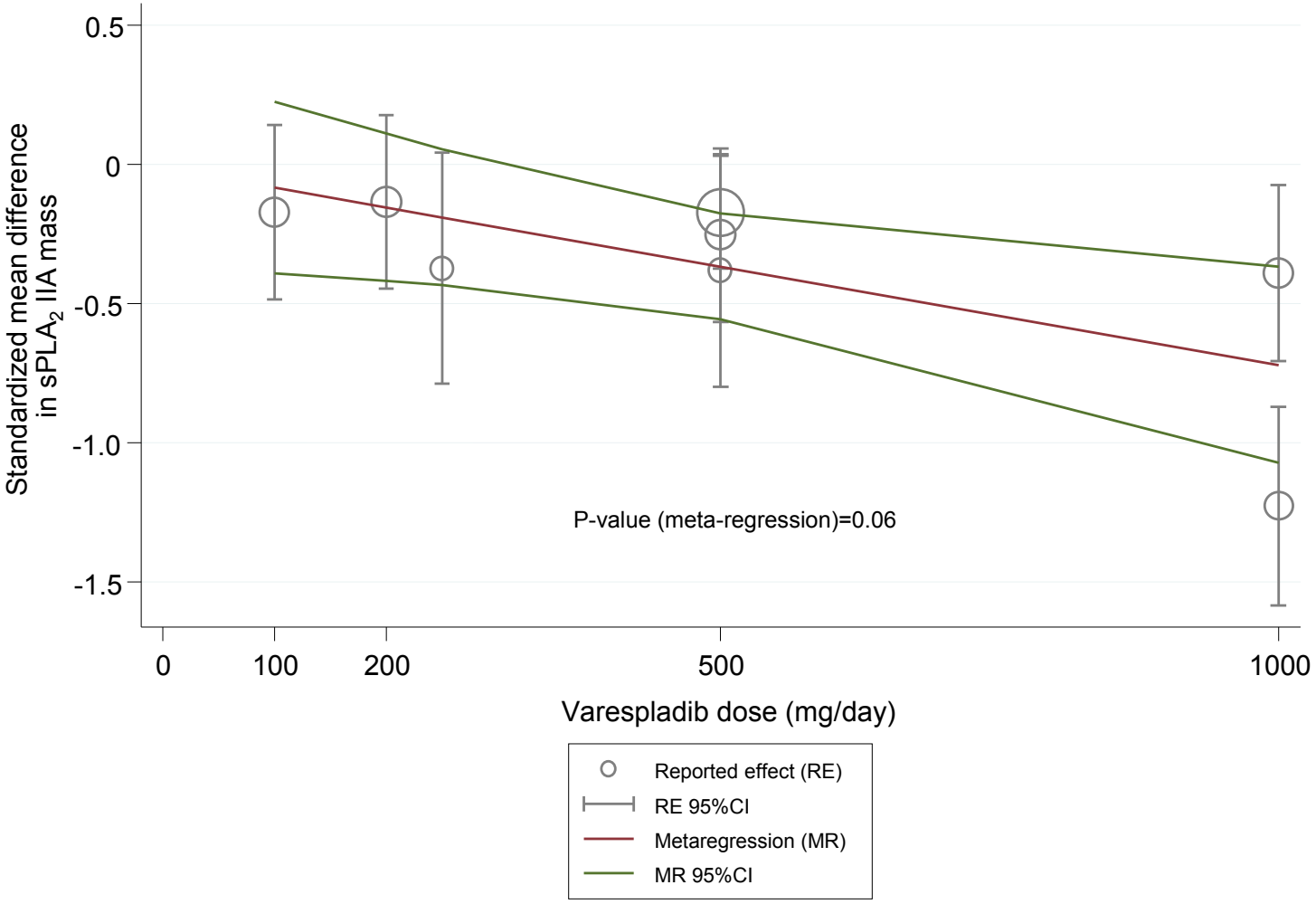


Figure 5.5: Dose-response relationship between varespladib and sPLA$_2$-IIA mass reported in randomized trials.

Footnotes: Grey circles and vertical whiskers represent mean (±95%CI) study-level difference in sPLA$_2$-IIA mass from baseline to 8 weeks comparing varespladib to placebo. Red line represents meta-regression slope (±95%CI, green lines) of dose of varespladib and difference in sPLA$_2$-IIA mass.

Table 5.1: Characteristics of the randomized trials of varespladib identified in the systematic review.

Trial name	RCT phase	Years conducted	Clinical setting	Primary end-point	Age, mean (SD)	Sex, % female	Number randomised	Varespladib dose, mg (dosing frequency/day)	Trial duration, months
FRANCIS-ACS [333]	IIB	2008-2010	ACS	Change in LDL-C from baseline to 8 weeks	59.05 (10.5)	25.3	625	500 (once)	6
PLASMA [334]	II	2007	Stable CHD	Change in sPLA$_2$ concentration or enzyme activity from baseline to 8 weeks	62 (11)	24	393	50/100/250/500 (twice)	2
PLASMA-2 [335]	II	2007	Stable CHD	Change in sPLA$_2$ concentration from baseline to 8 weeks	64 (12)	11	138	250/500 (once)	2
SPIDER-PCI [336]	II	2007-2009	Stable CHD patients undergoing elective PCI	Elevation of cardiac enzymes (CK-MB, Troponin I) up to 24h PCI	63.4 (9.9)	12.5	144	500 (twice)	1

5.2.4 Contextualising the genetic effects on sPLA$_2$-IIA with the sPLA$_2$ inhibitor varespladib

The most common dose of varespladib (500mg/day) reduced sPLA$_2$-IIA by 78% (95%CI: 62%, 94%) (Figure 5.5). This estimate (78%) was about twice that observed for possession of one common (C) allele of rs11573156 compared to none (38% to 44% depending on the population studied), and roughly equivalent to possession of two common (C) alleles of rs11573156 compared to none (what can be considered an "extreme" genotype comparison).

5.2.5 Association between rs11573156 with cardiovascular risk factors and carotid atherosclerosis

In contrast to the observed association between circulating sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity (Tables 4.2 and 4.3), I did not find associations between the C-allele of rs11573156 and cardiovascular risk factors such as blood pressure, LDL-C, glucose and BMI (Tables 5.2, 5.3, and 5.4) or surrogate markers for CHD such as C-IMT. However, I observed associations of a small magnitude between rs11573156 and IL-6 (2.54% per C-allele; 95%CI: 0.68 to 4.44), fibrinogen (0.005% per C-allele; 95%CI: 0.001 to 0.009) and triglycerides (0.78% per C-allele; 95%CI: 0.04 to 1.5) (Table 5.4).

5.2.6 Association between rs11573156 and cardiovascular outcomes in general population studies

In 13 population studies (8021 incident MVE in 56,359 individuals), the summary per C allele OR for the association of rs11573156 with incident MVE (comprising fatal and nonfatal MI or stroke) was 1.02 (95%CI: 0.98, 1.06). The summary OR for the individual outcomes were: incident nonfatal MI 1.04 (95%CI: 0.98, 1.10); incident nonfatal stroke 1.00 (95%CI: 0.93, 1.07), and; incident fatal MI/stroke 1.01 (95%CI: 0.93, 1.10). For details see Figure 5.6 and 5.7.

In 12 studies (7513 prevalent MVE in 55,523 individuals), the summary per C allele OR for the association of rs11573156 with prevalent MVE (MI or stroke) was 0.99 (95%CI: 0.95, 1.03). For the individual outcomes, the summary OR for prevalent MI was 0.98 (95%CI: 0.93, 1.03) and for prevalent stroke 1.03 (95%CI: 0.93, 1.15) (Figure 5.6 and 5.8). Findings from random-effects meta-analysis were similar and are presented in Table B.7.

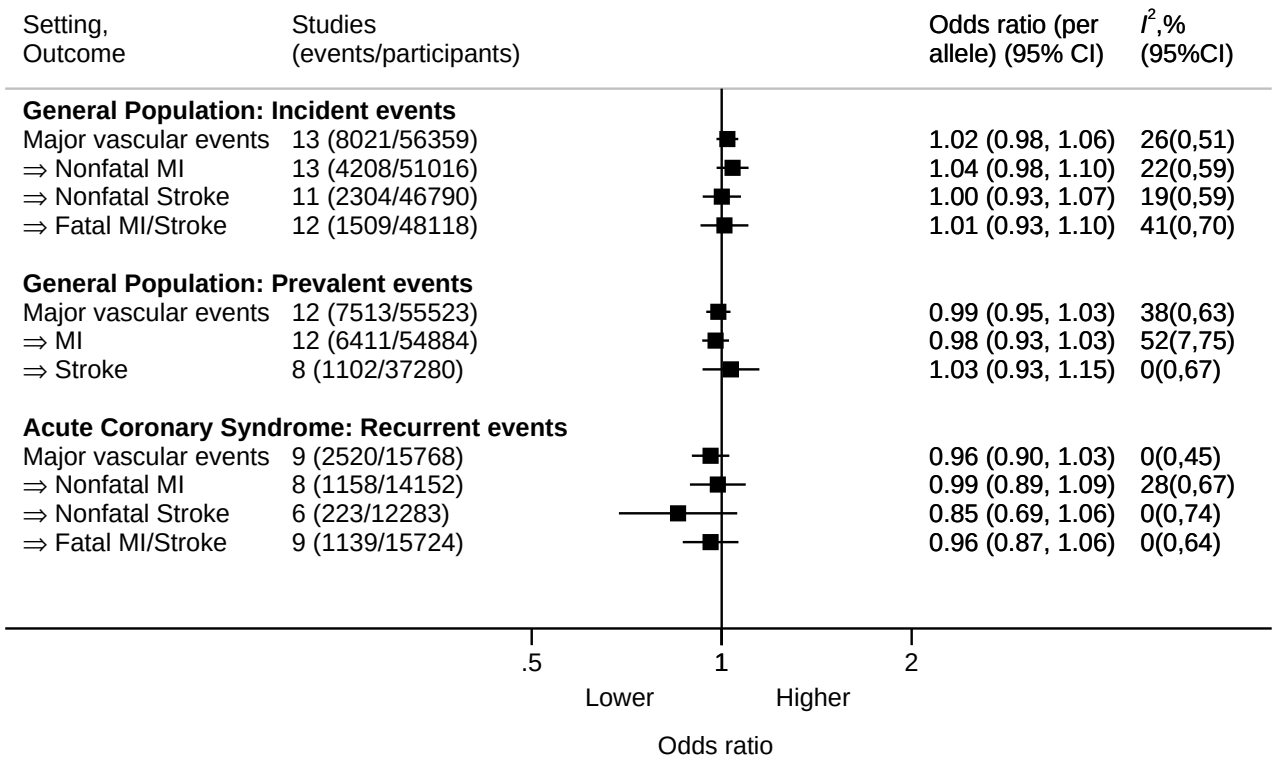


Figure 5.6: Meta-analysis pooled estimates of the association between *PLA2G2A* rs11573156 and major vascular events (including individual components) stratified by clinical setting into general population and ACS patients.

Footnotes: Each plot represents the *PLA2G2A* rs11573156 per C allele OR, with genotype grouping arranged to mimic the effects of pharmacological lowering of sPLA$_2$-IIA. I.e. if lowering of sPLA$_2$-IIA mass were to reduce risk of cardiovascular events, the OR in the plot should be less than one. Major vascular events comprise fatal/nonfatal MI or stroke. Fatal MI/stroke included all-cause mortality for some ACS studies (see Table B.6 for further details).

5.2.7 Association between rs11573156 and recurrent cardiovascular events in patients with acute coronary syndrome

Nine studies (2520 recurrent MVE in 15,768 participants with ACS) contributed towards the analysis, yielding a summary per C allele OR for rs11573156 with recurrent MVE (fatal/nonfatal MI or stroke) of 0.96 (95%CI: 0.90, 1.03). The summary OR for individual components were: nonfatal MI 0.99 (95%CI: 0.89, 1.09); nonfatal stroke 0.85 (95%CI: 0.69, 1.06), and; fatal MI/stroke 0.96 (95%CI: 0.87, 1.06) (Figure 5.6 and 5.9) Findings were consistent in a random-effects meta-analysis (Table B.7).

5.2.8 Extreme genotype comparison

Individuals homozygous for the rs11573156 C allele had a 57%-62% lower sPLA$_2$-IIA mass compared to those homozygous for the G allele (Figure 5.4), which was similar in magnitude to the 78% reduction seen with 500mg/day varespladib dose used in the phase-III VISTA-16 trial. Using this genotype comparison, a null effect was again observed for MVE in all clinical settings: incident (5,175 cases, OR 0.99; 95%CI: 0.89, 1.10); prevalent (3,545 cases, OR: 1.00; 95%CI: 0.88, 1.13), and; recurrent (1,626 cases, OR 0.89; 95%CI: 0.74, 1.06).

5.2.9 Association between rs11573156 and other cardiovascular events

In a meta-analysis including 4 studies (4224 cases in 6619 participants), no association between the C allele of rs11573156 was identified for angiographically-determined coronary artery disease (OR 0.99; 95%CI: 0.91, 1.08; Figure 5.8). In a study set in patients with established vascular disease or high cardiovascular risk (SMART), the association between the C allele of rs11573156 with recurrent MVE (fatal/non-fatal MI or stroke) was OR 0.92 (95%CI: 0.80, 1.07). For the study set in patients with ACS undergoing PCI (GENDER), the association between the C allele of rs11573156 with coronary artery restenosis was OR 1.04 (95%CI: 0.82, 1.31).

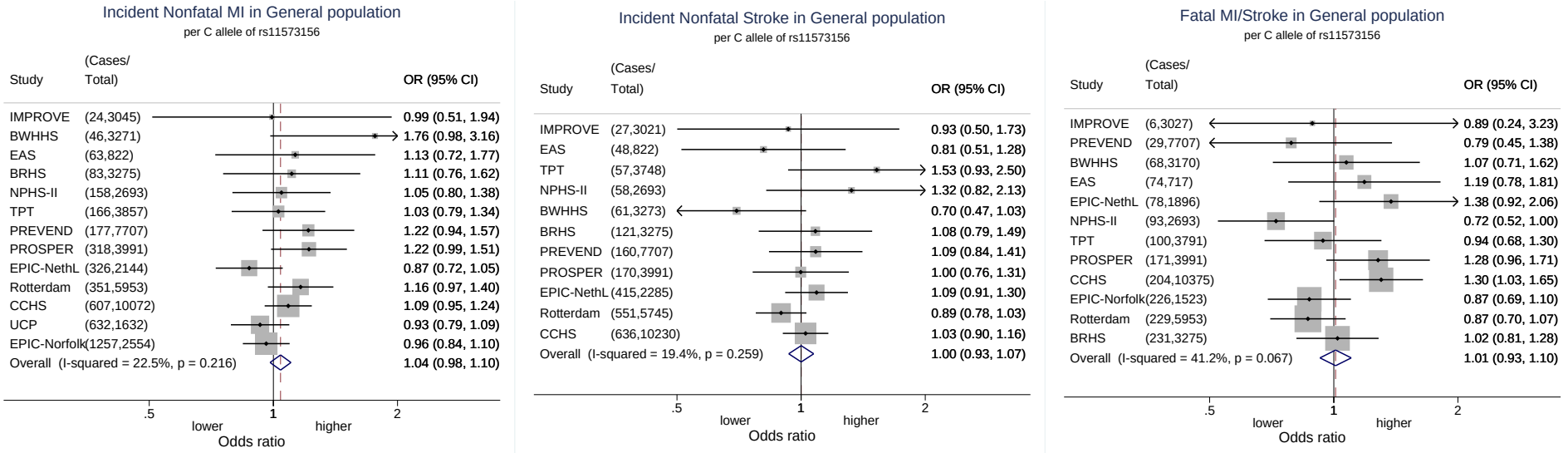
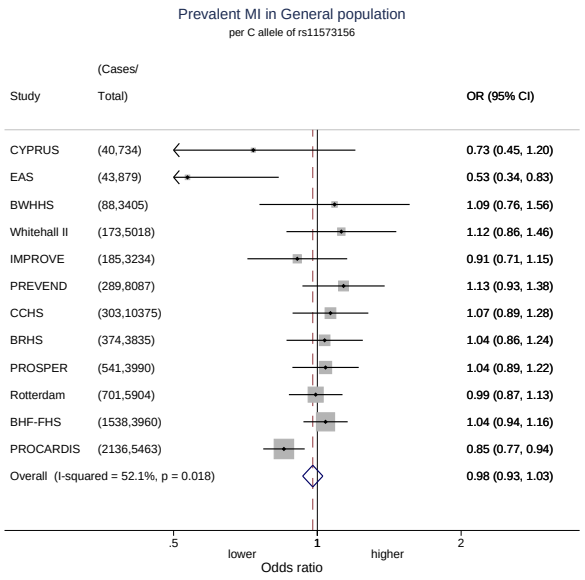
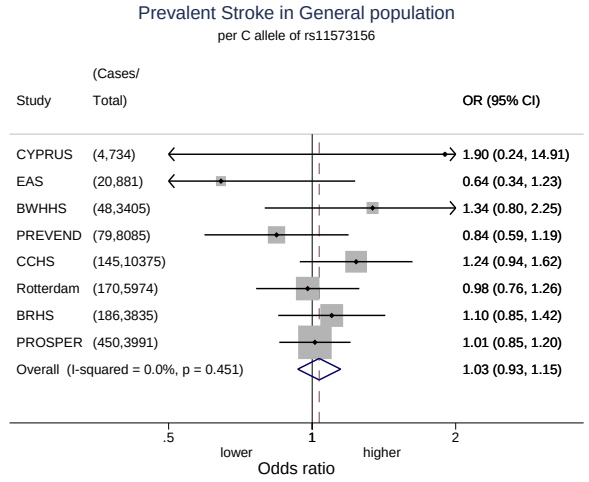


Figure 5.7: Meta-analysis of the effect of *PLA2G2A* rs11573156 in general population studies and risk of: (A) incident nonfatal myocardial infarction (B) incident nonfatal stroke, and; (C) fatal myocardial infarction or stroke.





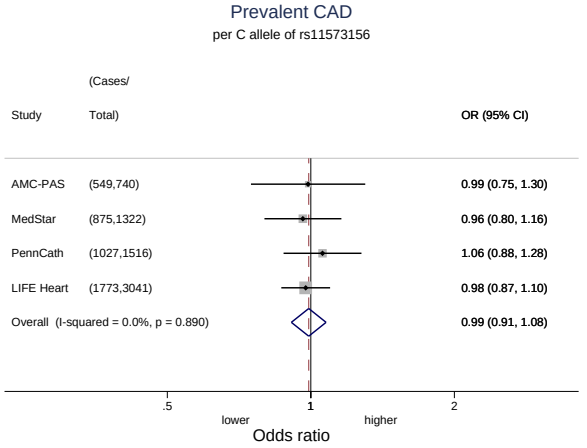


Figure 5.8: Meta-analysis of the effect of *PLA2G2A* rs11573156 in general population studies and risk of: (A) prevalent myocardial infarction; (B) prevalent stroke, and; (C) coronary artery disease

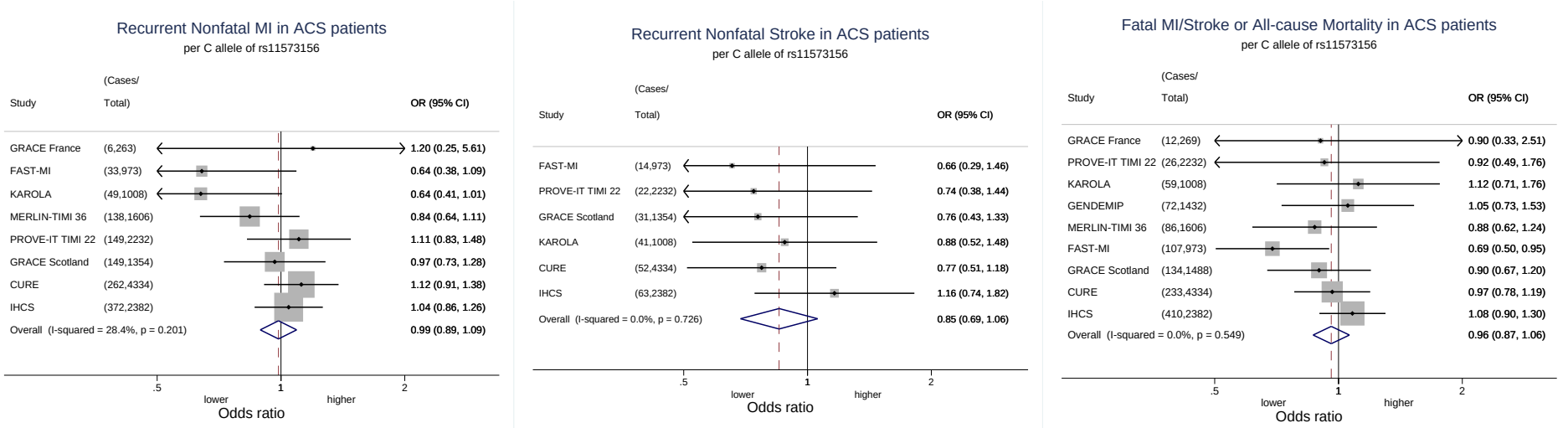


Figure 5.9: Meta-analysis of the effect of *PLA2G2A* rs11573156 in studies of acute coronary syndrome and risk of: (A) incident nonfatal myocardial infarction; (B) incident nonfatal stroke, and; (C) fatal myocardial infarction or stroke.

Table 5.2: Summary associations of *PLA2G2A* rs11573156 per C allele with cardiovascular risk factors and markers of atherosclerosis

	Studies in general populations			**Studies in Acute Coronary Syndrome populations**		
Biomarker (units)	No. of studies (individuals)	Summary effect (95% CI)	P value (I^2, %)	No. of studies (individuals)	Summary effect (95% CI)	P value (I^2, %)
Systolic BP (mmHg)	18 (63,325)	-0.11 (-0.36, 0.14)	0.46 (26)	6 (5530)	-0.66 (-1.63, 0.31)	0.18 (0)
Diastolic BP (mmHg)	18 (63,326)	-0.10 (-0.24, 0.04)	0.17 (30)	6 (5528)	0.05 (-0.51, 0.61)	0.86 (17)
Glucose (mmol/l)	12 (43,186)	0.01 (-0.01, 0.03)	0.32 (12)	5 (4907)	0.09 (-0.04, 0.21)	0.17 (0)
BMI (kg/m^2)	20 (67,764)	0.02 (-0.03, 0.07)	0.39 (18)	5 (5310)	-0.21 (-0.44, 0.02)	0.07 (0)
C-IMT (mm)*	6 (12,237)	-0.34 (-0.91, 0.24)	0.25 (0)	-	-	-

Footnotes Abbreviations: BMI: body mass index; C-IMT: carotid intima medial thickness; DBP: diastolic blood pressure; SBP: systolic blood pressure. * relative units presented as variable log transformed (units do not apply).

Table 5.3: Summary effects of *PLA2G2A* rs11573156 per C allele with potential confounders stratified by study setting

	Studies in general populations			**Studies in Acute Coronary Syndrome populations**		
Trait	No. of studies (cases/non-cases)	Odds Ratio (95% CI)	P value (I^2, %)	No. of studies (cases/non-cases)	Odds Ratio (95% CI)	P value (I^2, %)
Age (yrs)	20 (NA/68,387)	-0.04 (-0.12, 0.05)†	0.36 (15)	6 (6230)	-0.05 (-0.44, 0.35)	0.82 (0)
Gender (female/all)	14 (28,578/57,861)	1.00 (0.98, 1.03)	0.75 (0)	5 (1788/5946)	1.01 (0.92, 1.11)	0.83 (0)
Smoking status (ever smoker/never smoker)	19 (39,790/69,614)	1.00 (0.98, 1.03)	0.78 (6)	4 (2,332/5,113)	0.95 (0.86, 1.06)	0.36 (0)
T2D (prevalent)	18 (5,773/63,097)	1.05 (1.00, 1.10)	0.06 (16)	3 (773/4,045)	0.97 (0.85, 1.11)	0.65 (0)
Statin treatment	13 (8,284/45,509)	1.00 (0.95, 1.05)	0.93 (28)	5 (3,944/5,988)	0.93 (0.84, 1.03)	0.17 (0)
BP-lowering treatment	13 (10,810/48,738)	1.02 (0.98, 1.06)	0.31 (9)	4 (4,269/5,302)	0.92 (0.82, 1.04)	0.18 (0)

Footnotes Abbreviations: T2D: type 2 diabetes; † effect estimate represents beta coefficient for age, with denominator reflecting total number of participants.

Table 5.4: Summary effects of varespladib (500mg/day) and *PLA2G2A* rs11573156 on biomarkers reported in randomized trials

	Randomized trials of varespladib (500mg/day)			**Genetic studies of rs11573156 (per C allele)**			
Biomarker (units)	No. of trials (individuals)	Summary effect (95% CI) *	P value, (I^2, %),	Setting	No. of studies (individuals)	Summary effect (95% CI)	P value, (I^2, %)
			sPLA$_2$ measures				
sPLA$_2$-IIA mass (pmol/L)	3 (624)	-13.13 (-21.99, -4.27)	4 x 10-3 (0)	General population	1 (3035)	-38.62 (-40.49, -36.68)	2.61x10^{-204} (-)
				ACS	3 (1243)	-43.73 (-50.04, -36.68)	1.64x10^{-21} (0)
sPLA$_2$ enzyme activity (nmol/min/L)†	-	-	-	General population	1 (2990)	-2.96 (-4.47, -1.42)	1.8x10^{-4} (-)
				ACS	3 (1400)	-22.59 (-26.51, -18.45)	3.98x10^{-22} (82)
			BIOMARKERS REPORTED IN RCTs WITH ≥500 INDIVIDUALS				
HDL-C (mmol/l)	3 (637)	0.01 (-0.06, 0.07)	0.84 (0)	General population	18 (59,086)	0.000 (-0.005, 0.005)	0.96 (0)
				ACS	4 (3274)	0.01 (-0.01, 0.03)	0.44 (13)
LDL-C (mmol/l)	3 (610)	-0.20 (-0.42, 0.01)	0.063 (0)	General population	17 (53,465)	0.01 (-0.01, 0.02)	0.40 (42)
				ACS	5 (3357)	0.02 (-0.04, 0.07)	0.54 (0)
Triglyceride (mmol/l)†	3 (637)	-0.12 (-0.35, 0.11)	0.32 (0)	General population	15 (53,079)	0.78 (0.04, 1.50)	0.04 (12)
				ACS	4 (3285)	-0.21 (-2.94, 2.58)	0.88 (0)
Interleukin-6 (pmol/L)†	1 (513)	0.00 (-6.34, 6.34)	1 (0)	General population	6 (14,980)	2.54 (0.68, 4.44)	0.01 (0)
				ACS	2 (1895)	-1.82 (-8.08, 4.84)	0.58 (0)

Continued on next page

Table 5.4 – *Continued from previous page*

	Randomized trials of varespladib (500mg/day)			Genetic studies of rs11573156 (per C allele)			
Biomarker (units)	No. of trials (individuals)	Summary effect (95% CI) *	P value, (I^2, %),	Setting	No. of studies (individuals)	Summary effect (95% CI)	P value, (I^2, %)
BIOMARKERS REPORTED IN RCTs WITH <500 INDIVIDUALS							
C-reactive Protein (mg/l)	2 (467)	-0.32 (-3.50, 2.86)	0.84 (0)	General population	15 (52,348)	0.71 (-0.68, 2.12)	0.32 (0)
				ACS	6 (3318)	-0.86 (-6.73, 5.39)	0.78 (0)
Fibrinogen (g/l)†	-	-	-	General population	11 (35,430)	0.005 (0.001, 0.009)	0.02 (0)
				ACS	-	-	-
Apolipoprotein A (g/l)	-	-	-	General population	7 (27,012)	0.001 (-0.005, 0.007)	0.66 (0)
				ACS	1 (931)	-0.22 (-0.98, 0.54)	0.57 (-)
Apolipoprotein B †Ψ	2 (249)	-0.78 (-1.05, -0.52)	8.0x10-9 (94)	General population	7 (27,168)	0.43 (-0.11, 0.98)	0.12 (26)
				ACS	-	-	-
LDL size (nm)	2 (247)	0.23 (0.19, 0.26)	1.4x10-41 (19)	General population	1 (2774)	0.01 (-0.03, 0.05)	0.57 (-)
				ACS	-	-	-
HDL size (μm)	-	-	-	General population	1 (2774)	0.01 (-0.02, 0.04)	0.60 (-)
				ACS	-	-	-
LDL particles (nmol/l)	2 (247)	-19.01 (-516.86, 478.84)	0.94 (0)	General population	1 (2774)	3.70 (-23.19, 30.60)	0.79 (-)
				ACS	-	-	-
HDL particles (umol/l)	1 (157)	0.70 (-8.08, 9.48)	0.88 (-)	General population	1 (2774)	-0.16 (-0.51, 0.19)	0.38 (-)

Continued on next page

Table 5.4 – *Continued from previous page*

	Randomized trials of varespladib (500mg/day)			**Genetic studies of rs11573156 (per C allele)**			
Biomarker (units)	No. of trials (individuals)	Summary effect (95% CI) *	P value, (I^2, %),	Setting	No. of studies (individuals)	Summary effect (95% CI)	P value, (I^2, %)
				ACS	-	-	-

Footnotes Abbreviations: see Glossary. † relative units presented for the genetic analysis as variable log transformed (units do not apply) * represents mean difference in biomarker from baseline to 8 weeks (most parsimonious time-point) comparing varespladib 500mg/day to placebo Ψ RCT summary effect for Apolipoprotein B is the standardized mean difference as different units reported in RCTs.

5.2.10 Instrumental variable analysis of sPLA$_2$-IIA mass, sPLA$_2$ enzyme activity and CVD

5.2.10.1 General population studies

With information on the association of the C-allele of rs11573156 with sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity, and information on the association of the rs11573156 C-allele with MVE, I was able to triangulate the 'causal' effect for a one log unit lower sPLA$_2$-IIA and sPLA$_2$ enzyme activity (Figure 5.10).

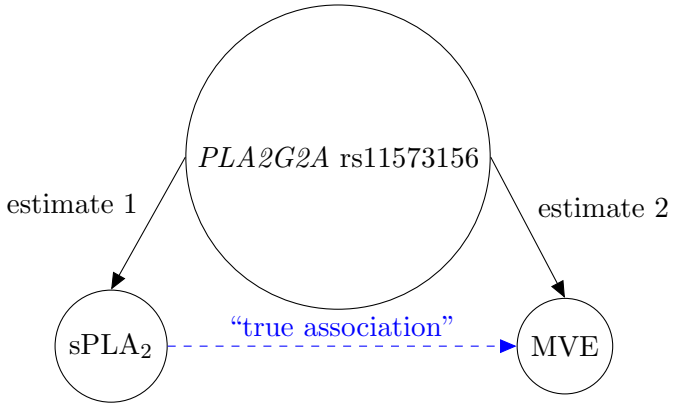


Figure 5.10: Triangulation of the causal association between sPLA$_2$ traits and major vascular events (MVE)
The "true association" is obtained by dividing estimate 2 by estimate 1. The variance of this instrumental variable estimate was obtained using the delta method (as previously described).

For the general population studies, instrumental variable analysis showed a null effect between sPLA$_2$-IIA mass and incident MVE (OR per one log unit lower sPLA$_2$-IIA mass: 1.04; 95%CI: 0.96, 1.13). This was in clear contrast to the expected association based on observational analysis (OR 0.69, 95%CI: 0.61, 0.79) (Figure 5.11).

Similarly, whereas observational studies showed an OR of 0.50 (95%CI: 0.35, 0.71) for incident MVE per one log unit reduction in sPLA$_2$ enzyme activity (Figure 4.3), I obtained only null associations for the instrumental variable estimates for sPLA$_2$ enzyme activity and incident MVE (OR 1.87, 95%CI: 0.47, 7.49). However, in this case the confidence intervals were wide due to the weak effect of the rs11573156 variant on sPLA$_2$ enzyme activity in the general population (as shown in Figure 5.4).

5.2.10.2 Acute Coronary Syndrome studies

For the ACS studies, the instrumental variable estimate for sPLA$_2$-IIA mass and recurrent MVE was null (OR per one log unit lower sPLA$_2$-IIA mass: 0.93; 95%CI: 0.83, 1.05). This was consistent with the null findings from observational studies (OR 0.92; 95%CI: 0.81, 1.03). For sPLA$_2$ enzyme activity, no association was identified for the instrumental variable estimate with MVE (OR 0.86, 95%CI: 0.66, 1.12), but the results were similar to those obtained from observational studies (OR 0.77, 95%CI: 0.64, 0.93) (Figure 5.11).

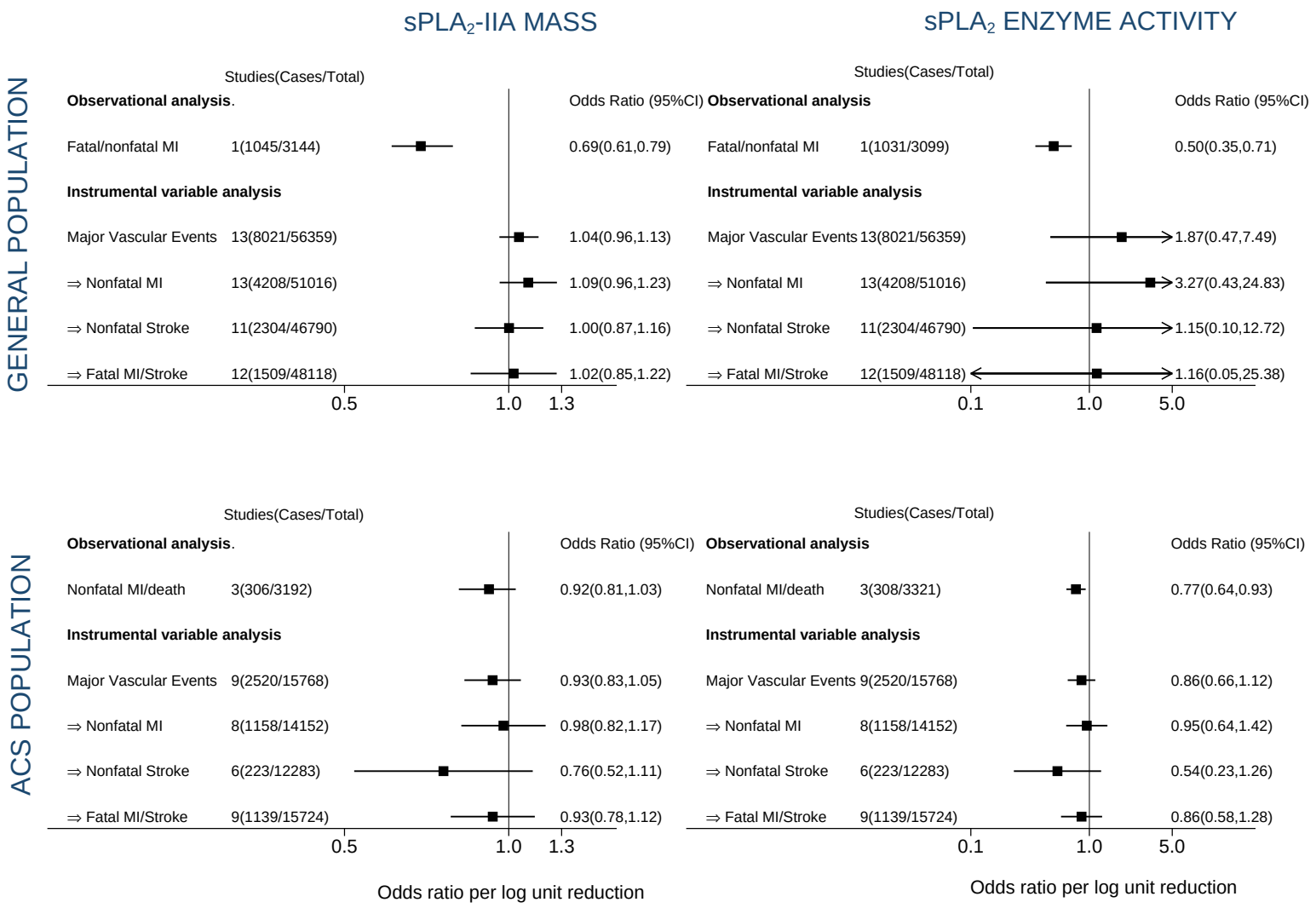


Figure 5.11: Observational and instrumental variable estimates per 1 log unit lower sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with cardiovascular events

The observational analyses were adjusted for age, gender, BMI, blood pressure, T2D, LDL-C, HDL-C, TG in general population cohorts (corresponding to Model 3 in Figure 4.3) and for age, gender, BMI and BP in ACS cohorts (corresponding to Model 2 in Figure 4.3).

5.3 Discussion

In this chapter, I described the methods and presented results from use of a genetic approach to make causal inference on the role of sPLA$_2$-IIA in CVD. This method of investigation provides an un-biased approach to evaluate the causal role of sPLA$_2$-IIA and in doing so, evaluates whether inhibition of sPLA$_2$-IIA might represent a valid therapeutic target for cardiovascular prevention.

5.3.1 SNP selection

For the SNP selection process, out of six SNPs that had been previously identified to detect the majority (≥90%) of variation within the *PLA2GA* gene in individuals of European descent,[339] I selected the SNP that had the largest effect on circulating sPLA$_2$-IIA mass and took it forward to conduct the Mendelian randomization analysis.

5.3.1.1 Specificity of genetic instrument for sPLA$_2$-IIA mass

Using data on the expression of mRNA, I demonstrated the specificity of this rs11573156 SNP (using a proxy SNP with which rs11573156 was in high LD on the Illumina chip, rs10732279) for the sPLA$_2$-IIA isoform, by showing no evidence of association between the proxy SNP with mRNA expression of sPLA$_2$-V or sPLA$_2$-X (Figure 5.2). This was important because if the SNP had shown association with other sPLA$_2$ isoforms, the interpretation of the genetic and instrumental variable analyses might be problematic due to issues of non-specificity that would arise. This could mean that an apparent association may not be due to sPLA$_2$-IIA and/or a negative result could arise by negative confounding from association of the SNP with another trait. Fortunately, the specificity of the SNP for the intermediate phenotype illustrated in the mRNA Manhatten plot meant this was not a likely scenario.

5.3.1.2 Strength of genetic instrument on sPLA$_2$-IIA mass

The effect of the *PLA2G2A* rs11573156 variant on sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity is worthy of comment. The rs11573156 SNP explained between 6% to 21% of the variance of sPLA$_2$-IIA, a value that is several times higher than that observed for all the GWAs hits combined on blood pressure (R^2=1% for 29 SNPs)[377] and similar

to that for 49 SNPs for LDL-C together (R^2=12%).[378] Thus a single SNP explained a huge proportion of variance of the intermediate trait in this case, reducing the risk of weak instrument bias[d].[379]

5.3.2 Comparison of findings from genetic association analysis with findings from observational analysis for continuous traits

In contrast to the observational findings presented in Chapter 4, which revealed strong associations between the two sPLA$_2$ traits (sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity) and several established cardiovascular risk factors in the general population studies, I did not identify corresponding associations between the *PLA2G2A* rs11573156 C-allele variant with these risk factors (Table 5.4). This is important for two reasons. First, it shows the relative specificity of the SNP for sPLA$_2$ phenotypes. Second, it makes it unlikely that these cardiovascular traits could lie on a potential causal pathway between sPLA$_2$ and CVD (this possibility was discussed in the Discussion section of Chapter 4).

I did identify a weak association between the SNP and fibrinogen and interleukin 6 (Table 5.4), however curiously, the direction of effect was opposite to that expected. I.e. the SNP that lowered sPLA$_2$-IIA may be expected to lower inflammatory markers (by having less arachidonic acid yielded stimulating less IL-6[380]), however the opposite was observed and the SNP associated with increases in both IL-6 and fibrinogen. However, these findings require replication.

5.3.3 Comparison of findings from genetic association analysis with findings from observational analysis for major vascular events

Despite the strength of association of the genetic variant on sPLA$_2$-IIA and accruing a collaboration of 32 studies with >8,000 incident, >7,500 prevalent and >2,500 recurrent MVE, I found no association between rs11573156 and prevalent, incident, or recurrent major vascular events on a genetic association (or instrumental variable) analysis. These

[d]The whole purpose of conducting Mendelian randomization analyses is to minimize the bias caused by confounding between the exposure of interest and outcome. However, instrumental variable analyses do suffer from finite sample bias termed weak instrument bias. The bias arising from a weak instrument is in the direction of the observed confounded association. The strength of the genetic instrument can be quantified by the F-statistic (or R^2) obtained from conducting a regression of the intermediate phenotype on the genetic variant. The bias of the instrumental variable estimator is quantified as the relative bias compared to the observational estimate: as approximately 1/F. Thus when the F-statistic is 10, the IV estimate has 10% of the bias of the observational estimate.[379]

findings provide strong evidence that the observational association between sPLA$_2$-IIA mass and cardiovascular events is due to residual confounding or reverse causality.

5.3.4 Placing these findings into context of the VISTA-16 phase III clinical trial

One of the main reasons of conducting a Mendelian randomization experiment is to serve as a proxy for a randomized trial. Fortuitously, at the same time as the Mendelian randomization experiment reported in this Chapter was conducted, a phase III trial was also carried out, which can be considered as a "positive control" (although the drug was not specific for the sPLA$_2$-IIA isoform, whereas the SNP was; Figure 3.10). The VISTA-16[337] trial of varespladib was stopped due to lack of efficacy following a recommendation by the Independent Data Safety Monitoring Board.[381, 382]

The VISTA-16 trial (NCT01130246) had enrolled 5189 individuals out of a planned 8500 (i.e. just over 60% of the intended sample size), and randomized individuals recruited within 96hrs of hospitalization for ACS to 500mg/day varespladib or placebo for 16 weeks duration of therapy. Follow-up was for 6 months with the primary outcome being a composite outcome of cardiovascular death, non-fatal MI, non-fatal stroke or documented unstable angina (i.e. an outcome very similar to the outcome used for MVE in the Mendelian randomization analysis that I present in this Chapter).

The Data Safety Monitoring Board (DSMB) conducted an interim analysis of VISTA-16 when 383 events (of which 212 were adjudicated to be primary composite events) were accrued, relating to approximately 50% of the primary outcome events that were desired according to the power calculation (full details provided on page 234 of Appendix C). Analysis was by intention to treat and found that allocation to varespladib resulted in a hazards ratio of 1.24 (P=0.16) compared to placebo for the primary outcome (fatal CVD, nonfatal MI/stroke, unstable angina). The interim analysis also identified an increased occurrence of nonfatal MI (HR 1.67; P=0.01) in patients randomized to receive varespladib. Given these interim findings, the DSMB recommended the study's premature termination.[381, 382]

It is reasonable to speculate that the null findings from this Mendelian randomization analysis provides an explanation for the lack of efficacy of varespladib in the VISTA-16 trial. I.e. that sPLA$_2$-IIA is not causally involved in CHD aetiopathogenesis.

5.3.5 Lack of association of sPLA$_2$ with MVE on observational analysis in ACS cohorts and implications for drugs targeting sPLA$_2$ for CVD prevention

The lack of observational association between sPLA$_2$ traits with recurrent MVE in the ACS setting may seem to challenge the pursuit of a drug that inhibits sPLA$_2$-IIA mass for the prevention of recurrent events (such as in the VISTA-16 trial). However, two things are worthy of comment. First, when the VISTA-16 trial was initially planned, there was small-scale evidence that sPLA$_2$-IIA mass associated with recurrent CHD events in patients with ACS.[337] Furthermore, the association of sPLA$_2$-IIA mass with recurrent events in patients with *stable* CHD is widely reported.[318, 320, 383] Second, evidence on the observational association between a biomarker and disease is typically initially measured in general population studies[e], while trials of preventative therapies are usually first undertaken in the secondary prevention setting (as these "high-risk" individuals have more clinical events, which increases statistical power and allows for a smaller clinical trial to be conducted).[384]

5.3.6 Limitations

This work has several limitations that are worthy of mention. First, I did not have data from a common set of participants with all three key variables: sPLA$_2$ measures, genetic information and cardiovascular events. However, this is a common scenario with large-scale meta-analyses of Mendelian randomization studies that include novel biomarkers,[371, 385] and the instrumental variable approach I used helps to overcome this problem. Specifically, by assuming a common fixed effect of the SNP on the intermediate phenotypes (sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity), I was able to apply this estimate to studies that did not have sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity measured. This enabled the synthesis of instrumental variable estimates for all contributing studies.

Second, due to the specificity of rs11573156, the genetic analyses do not provide information on the role of sPLA$_2$-V or -X in CVD and cannot exclude a possible causal role for these subtypes. Varespladib was originally designed to inhibit the sPLA$_2$-IIA form.[329], but studies have shown that varespladib has additional effects on subtypes -V and -X,[386] however with 5-10 fold lower inhibitory efficacy.[387] The genetic data *do* however provide strong evidence against a causal role of the sPLA$_2$-IIA isoform in

[e] in this case, there *is* strong evidence of an association between both sPLA$_2$ traits with incident MVE, as shown in Figure 4.3

incident and recurrent MVE. This is further reinforced by the negative findings from the VISTA-16 trial.[381]

Third, given the smaller number of subjects in studies set in ACS, I had reduced power to exclude a small association with recurrent MI or stroke if they were real, as evidenced by the partial overlap of the expected and observed effect estimates for sPLA$_2$-IIA mass and sPLA$_2$ enzyme activity with CVD in this setting. However, the genetic analysis includes 2520 recurrent MVE in patients with ACS, which is more than 6-fold greater than the 385 primary events that VISTA-16 intended to recruit.[337] Furthermore, comparing individuals homozygous for the rs11573156 C allele to those homozygous for the G allele (an "extreme" genotype comparison, excluding individuals heterozygous for rs11573156) resulted in a 62% reduction in sPLA$_2$-IIA mass, similar to the effect of 500mg/day varespladib (78% reduction). Even this extreme genotype comparison failed to show association with incident or recurrent MVE (OR 0.99; 95%CI: 0.89, 1.10 and OR 0.89; 95%CI: 0.74, 1.06, respectively).

5.3.7 Comparison to other Mendelian randomization studies of sPLA$_2$

The findings I report here are in contrast to a previous small Mendelian randomization study, based in the KAROLA study.[383] The KAROLA study contributed towards the genetic analysis of ACS studies presented in this Chapter. Examination of the forrest plot (Figure 5.9) shows KAROLA to have an association consistent with a beneficial effect of sPLA$_2$-IIA reduction in preventing recurrent nonfatal CHD events (OR 0.64, 95%CI, 0.64 to 1.01) however the upper limit of the 95% CI included the null value of 1.0. However, it can be seen from Figure 5.9 that the larger ACS collections (with several-fold more events) had null associations of the rs11573156 SNP with recurrent CHD events, making it likely that the association identified in KAROLA represented a chance finding. Furthermore, the composite of fatal MI/stroke or all-cause mortality in KAROLA yielded an OR of 1.12, which was directionally opposite to that for nonfatal MI, meaning that the estimates were not internally consistent for components of the MVE outcomes in KAROLA (Figure 5.9).

5.3.8 Implications for future Mendelian randomization work in sPLA$_2$ traits

The work I present here has important implications for ongoing development of drugs for other (non-IIA) sPLA$_2$ inhibitors.[388] A Mendelian randomization study using genetic instruments for other sPLA$_2$ isoforms would be an important complement to the current

study and will help to inform the drug-development process of these new compounds, before proceeding into phase III trials. However, identification of a specific instrument for other non-IIA sPLA$_2$ isoforms will require development of assays that quantify their mass to be able to test the strength and specificity of the genetic instrument, an essential step that I conducted in this work. These novel biomarkers would then also need to be validated in large-scale epidemiological studies.

However, conducting a Mendelian randomization analysis for sPLA$_2$-V and sPLA$_2$-X based on sPLA$_2$ enzyme activity (as an intermediate trait) alone would represent an imprecise approach and may lead to the selection of inappropriate genetic instruments that may yield invalid conclusions about the validity of sPLA$_2$-V or sPLA$_2$-X as drug-targets for cardiovascular events.

5.4 Conclusion

In conclusion, this large-scale Mendelian randomization study does not support the hypothesis that sPLA$_2$-IIA mass is causally linked to cardiovascular events and thus sPLA$_2$-IIA does not represent an appropriate drug-target for cardiovascular disease prevention. The concordance of the genetic findings with the lack of efficacy of the varespladib phase III randomized trial[381] supports the use of this genetic approach earlier in drug development to prioritise which drug targets to follow in human randomized trials and reduce the risk of expensive late-stage drug failure.

Chapter 6

Alcohol and cardiovascular disease

6.1 Introduction

6.1.1 Alcohol consumption and the global burden of disease

Alcohol consumption is considered to be a contributory cause to more than 200 ICD-10 conditions (**Box 6.1**) and is most strongly associated with liver cirrhosis and oro-pharangeal cancers.[389, 390]

Box 6.1. Examples of diseases to which alcohol contributes:

- Injuries (accidental and intentional)
- Liver cirrhosis
- Cancer
- Neuropsychiatric disorders
- Low birth-weight

From Rehm *et al*[389]

In the 2010 global burden of disease survey, alcohol consumption was the third leading risk factor for death and disability (after high blood pressure and smoking), accounting for 5.5% of global disability-adjusted life years.[391] Given the considerable worldwide consumption of alcohol (Figure 6.1), even a small increase in disease risk attributable to alcohol could have potentially huge impacts on population health.

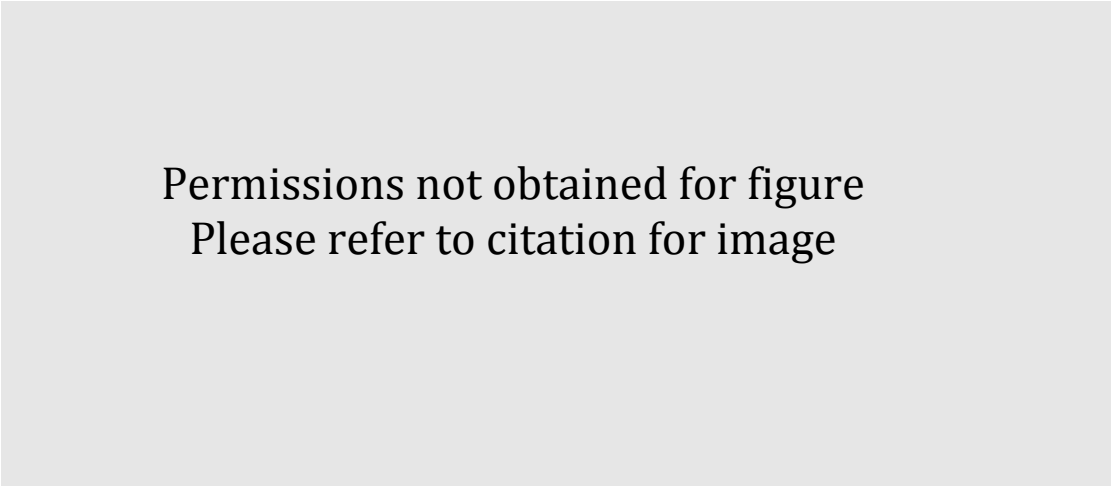


Figure 6.1: Worldwide distribution of alcohol consumption
From Rehm *et al*[389]

6.1.2 Alcohol and coronary heart disease

Despite the un-disputed effects of alcohol on cancer, injuries and mental health, the role of alcohol in cardiovascular health remains the source of ongoing debate.[392–394] Observational studies consistently report that, compared to individuals that abstain, moderate alcohol intake (even up to as much as 90 grams per day) is associated with a reduction in risk of CVD (Figure 6.2).[395, 396]

This potential cardioprotective effect of alcohol is thought to operate through several pathways (**Box 6.2**) including alterations in circulating blood lipid levels, most notably raised HDL-C (which has been proposed to mediate approximately half of the cardioprotective association of alcohol[397, 398]) and reduced platelet aggregation preventing thrombus formation,[398–401] a key step in the manifestation of a myocardial infarction.[402] Observational evidence also suggests that alcohol may reduce the risk of diabetes, through increased insulin sensitivity, mediated through adiponectin.[403] Some studies have suggested that red wine may be particularly cardioprotective due to flavonoid and resveratrol content[404]; however this is controversial, as recent reports suggest that this evidence may have been in part fabricated.[405]

Box 6.2: Associations of alcohol with traits thought to be involved in cardiovascular disorders

- Lipids
  - HDL-C: observational studies show that HDL-C is strongly inversely related to cardiovascular disease,[406] and that alcohol increases HDL-C in a dose-dependent fashion.[407] Therefore, by increasing HDL-C, it is suggested that alcohol may reduce CHD risk. However, the cardioprotective role of HDL-C has been brought into question through a recent Mendelian randomisation study using multiple SNPs, which failed to identify a causal relationship between HDL-C and CHD[408], and a phase III clinical trial of a drug that increased HDL-C (dalcetrapib), which was terminated due to futility.[409]
  - Triglycerides: observational studies indicate that alcohol intake reduces triglycerides, a circulating lipid component that strongly associates with increased cardiovascular risk on observational anlaysis[406]
- Inflammation: alcohol shows a U-shaped association with fibrinogen.[410] This is of potential relevance as atherosclerosis is considered to have an inflammatory component[230] and although fibrinogen is not itself thought to be causally related to CVD[411], it may reflect underlying inflammation. If alcohol acts as a 'general' anti-inflammatory agent, then this could mediate a reduced risk of CVD
- Haemostasis (blood coagulation): alcohol is associated with an improved coagulation diathesis - i.e. observational studies suggest that alcohol makes blood less likely to coagulate as evidenced by increased levels of plasminogen (a circulating blood enzyme that degrades plasma proteins involved in fibrin clots).[401] As described in Chapter 3, a fibrin clot (or thrombus) complicating a ruptured atherosclerotic plaque is often the initiator of an acute myocardial infarction.
- Insulin sensitivity: alcohol shows an inverse dose-response relationship with type 2 diabetes (T2D) risk[412]. It is possible that the mechanism could be mediated via adiponectin.[413–415] Alcohol increases adiponectin, which is inversely associated with risk of T2D.[416] T2D is itself associated with a 3-4 fold increase in CVD risk,[417] thus the prevention of T2D may be another mechanism by which alcohol reduces CVD risk.
- Oestrogens: alcohol intake is associated with higher oestrogen levels.[418] Women are recognized as having a substantially lower risk of CHD than men,[419] and this was thought to be, at least, partially mediated through circulating oestrogen levels. However, the oestrogen-cardioprotection hypothesis was cast into doubt by evidence from RCTs of hormone replacement therapy (drug combinations that include oestrogen) that, contrary to the observational associations,[53] detected that oestrogen-containing hormone replacement therapy increased CVD risk.[54]

Despite the reported beneficial effects of alcohol on cardiovascular health, there is a positive dose-response association between alcohol consumption and blood pressure across

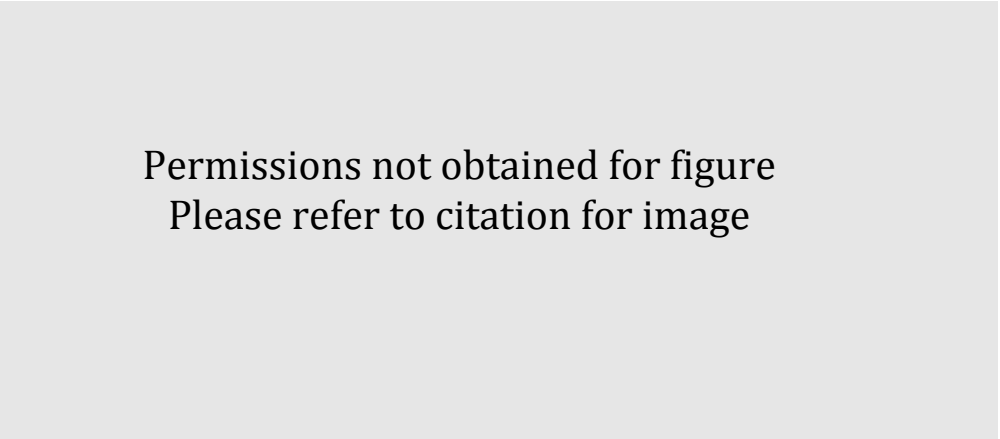


Figure 6.2: Association of alcohol with CHD from 51 observational studies. The nadir is at 25g/day and the point where the relative risk is greater than that of abstainers is 90g/day. The blue dashed horizontal line represent the null effect (relative risk=1.0) where alcohol has no effect on risk of CHD, compared to non-drinkers. Adapted from Corrao *et al*[395]

the whole distribution range of alcohol consumption.[420–422] Therefore if the cardioprotection of alcohol were 'real' (i.e. not due to confounding or bias), alcohol would need to have an effect on other traits that more than offset the blood-pressure raising effect.

6.1.3 Influence of purported effects of alcohol on CHD on UK governmental guidance

This observational association between alcohol and CVD has influenced guidance from the UK government on safe drinking practices.[423] Original guidelines (circa 1987) were based on a Royal College of Physicians report 'The medical consequences of alcohol abuse; a great and growing evil', which stated that there was no safe level of alcohol consumption, and, considering the association of alcohol with various diseases, 'sensible limits' of 21 and 14 British units[a] in men and women per week were advised (including 2 or 3 days without any alcohol).[423]

However, in 1995, a government inter-departmental working group considered the evidence-base that alcohol might be protective to CHD, and revised guidance to suggest that daily or near-daily drinking was permissible, with recommended limits of up to 4 units of alcohol per day.[424] This was highly controversial,[425] with the main criticisms levied that the working group did not contain scientists and that it sanctioned two key behaviours

[a] 1 British unit=10 ml or 7.9g ethanol = 0.57 US units

that were recognized as strongly associated with alcohol-related harm and dependency - namely (i) daily drinking, and (ii) an increase in the recommended safe limit of alcohol volume per week.

More recently, a Science and Technology Committee (appointed by the House of Commons) in 2012 called for an update on the 1995 guidelines. The committee recommended that more emphasis should be placed on the evidence of harm from alcohol, and called for a more measured interpretation of the potential beneficial effects of alcohol on CVD risk.[426] This re-review is currently underway with updated guidance expected in late 2013.[427]

It is interesting that government advice on alcohol consumption should be altered to increase the safe consumption amounts on the basis that it *may* afford cardiovascular protection. Given the vast data accrued that demonstrates the harm from alcohol,[428] it is important to weigh the possible beneficial effects on CVD together with the established harm.[429] For example, it is estimated that alcohol is responsible for 863,300 hospital admissions and 30,000 to 40,000 deaths per annum in the UK with the economic impact of alcohol costing GBP20-55 billion per annum.[430] A recent study that modelled data for benefits (cardiovascular) and harms (cirrhosis and cancer) arising from alcohol using estimates derived solely from observational data showed that, taking the magnitude and directionality of the different associations into account, the optimal population consumption of alcohol should be 5g per day (approximately half a British unit).[431] This is well below the present guidance.

6.1.4 Limitations of observational evidence

To date, most large-scale epidemiological investigations of alcohol intake and its association with human health have been conducted using observational (non-interventional) research, which are limited in the inferences that can be made (see Chapter 1, Introduction).

Some additional limitations are specific for alcohol, and are worthy of mention. These include:

measurement error: most studies rely on self-reported alcohol consumption, which is a subjective trait, thus, accurate measurement may be hindered by recall biases. For example, survey estimates of alcohol consumption substantially underestimate

consumption in relation to data from sales [432] and may be influenced by specific aspects of the question and the reference period.

selection bias: heavy drinking individuals may be under-represented in traditional cohort studies.[433]

reverse causality: one of the potential explanations for the J-shape association between alcohol and CHD (Figure 6.2) is that the relative protection from individuals that consume low amounts of alcohol could arise from individuals classified as abstainers who have in fact stopped drinking due to disease (so-called "sick-quitters"[434, 435]) or they may be ill for other reasons. Contamination of non-drinkers by sick-quitters and use of this group as the reference can spuriously create an apparent protective effect for individuals that drink moderately (a form of reverse causality).

confounding: alcohol is highly correlated with several confounders such as age[436], gender[437], marital status[438], smoking[439], socioeconomic status,[440–442] mental health[443], physical activity[444] and educational attainment[445–447]. If confounding traits are more strongly associated with alcohol at differing levels of alcohol exposure (i.e. non-linear confounding[443, 448]), this could create an apparent J-shape curve.

These features severely limit inference on the causal relevance of alcohol for CVD based on observational evidence alone.

6.1.4.1 The J-shaped curve

The reported J-shape in the association between alcohol and CVD is not unique to this scenario. For example, other J-shaped relationships between exposures and disease outcomes have been reported for:

1. obesity and all cause mortality[449]
2. exercise and CVD[450]
3. sleep and CVD[451, 452]

There has been debate about whether J-shapes can *ever* reflect true causal associations, or whether they fundamentally represent an artifact, and the potential implications that this uncertainty has for approaches to translating such observational data to improvements in population health.[453–457] For example, in all the above cases, a J-shape can

be introduced by reverse causation influencing the baseline group (i.e. analogous to the "sick-quitters" phenomenon) or confounding by social factors in those exposed to low levels of the exposure of interest. For instance, a J-shape was thought to exist between BP and CVD[458, 459], however this was over-turned by subsequent larger studies, that have shown a dose-response relationship and the absence of a plateau (i.e. no "safe" level beneath which a further reduction in BP does not afford further CVD protection).[460]

6.1.5 Experimental and interventional studies

Several interventional studies of alcohol exposure have been conducted to investigate the association of alcohol with cardiometabolic traits. A recent systematic review and meta-analysis summarized data from interventional studies,[461] and identified that alcohol showed strong positive associations with HDL-C, adiponectin and apolipoprotein-A1 and negative associations with LDL-C and fibrinogen. These data provide support for the hypotheses that alcohol increases HDL-C and reduces inflammatory markers, and by doing so, adds credibility that alcohol may be cardioprotective.

However, a few limitations of the data reported in the recent meta-analysis[461] are worthy of comment. Of the 63 interventional studies identified by the systematic review, only 32 (representing 1112 of 1762 participants, 63%) of them were randomized in design. Thus the majority of studies were subject to the usual limitations of non-randomized evidence (i.e. in this case, participant and investigator bias may have played an important source of error). Second, the sample size of the interventional studies was very small, with the median sample size being only 19 (min=5, max=100), as shown by the frequency histogram in Figure 6.3. Finally, all studies were of short duration (range 1 to 8 weeks), making it questionable whether the findings can be extrapolated into long-term differences in exposure to alcohol.

Although the authors reported no evidence of small study bias, in the absence of *any* large studies (i.e. no single study had a sample size greater than 100 individuals), the test of small study bias would only compare the effects between the existing small studies and would not be able to detect presence of bias.[462]

With the preponderance of small studies, potential for bias (both at the study and publishing level) and confounding, and the lack of interventional studies designed to investigate clinical outcomes (such as CHD or stroke), the available experimental evidence of alcohol from interventional studies is inadequate to draw conclusions on the effect of alcohol on risk of cardiovascular disease.[392]

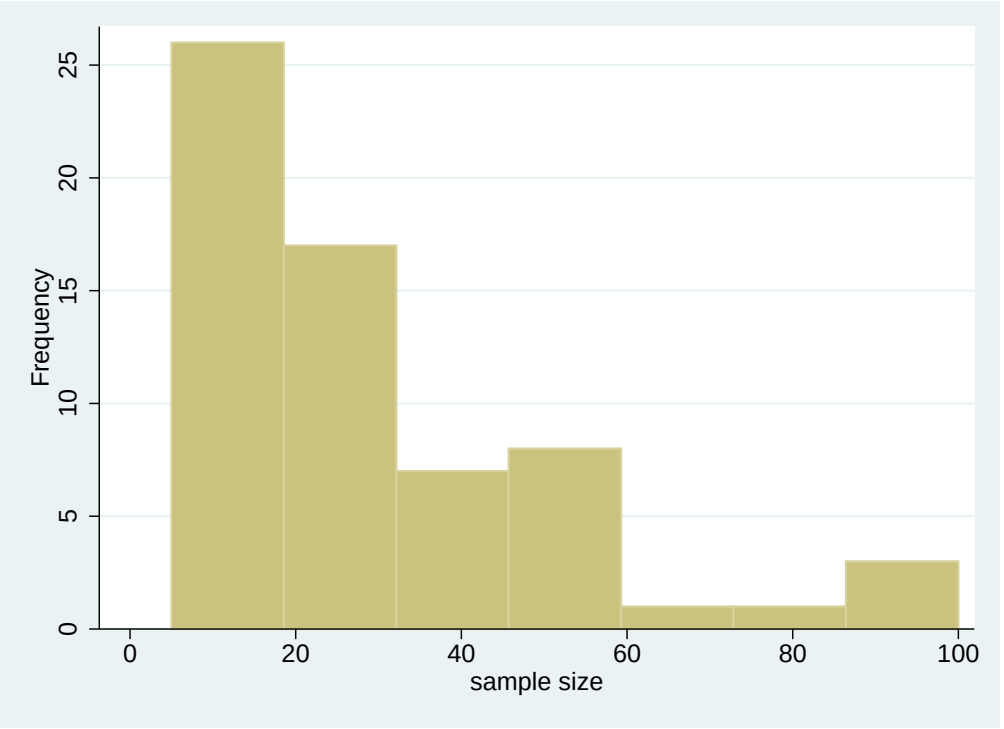


Figure 6.3: Frequency histogram of the sample size of studies included in the meta-analysis of interventional studies of alcohol reported by Brien et al[461]

6.1.6 Ideal study design

The ideal scenario, which would yield the most reliable evidence on the causal relevance of alcohol in CVD, would entail randomizing a large group of individuals to receive differing amounts of alcohol, with participants followed up over several years for incident vascular events. There is sufficient clinical equipoise (i.e. lack of decisive evidence) as to whether alcohol is beneficial at low doses to cardiovascular health to support such a trial. However, the other (non-CVD) harms from alcohol,[389, 390] together with the high-cost of such a trial are likely to make it impractical. Furthermore, maintenance of blinding and challenges in identifying the optimal means to the intervention (e.g. dietary advice vs. a concealed liquid to drink daily) may add further complexities to this trial design.[463]

Fortunately, there is an alternative yet complimentary investigative avenue that provides an opportune means of yielding deconfounded evidence - Mendelian randomisation.

6.1.7 Genetic contribution to alcohol consumption

In order to be able to use a gene as an instrumental variable for alcohol consumption, a proportion of variance of alcohol consumption must be related to the genetic variant.

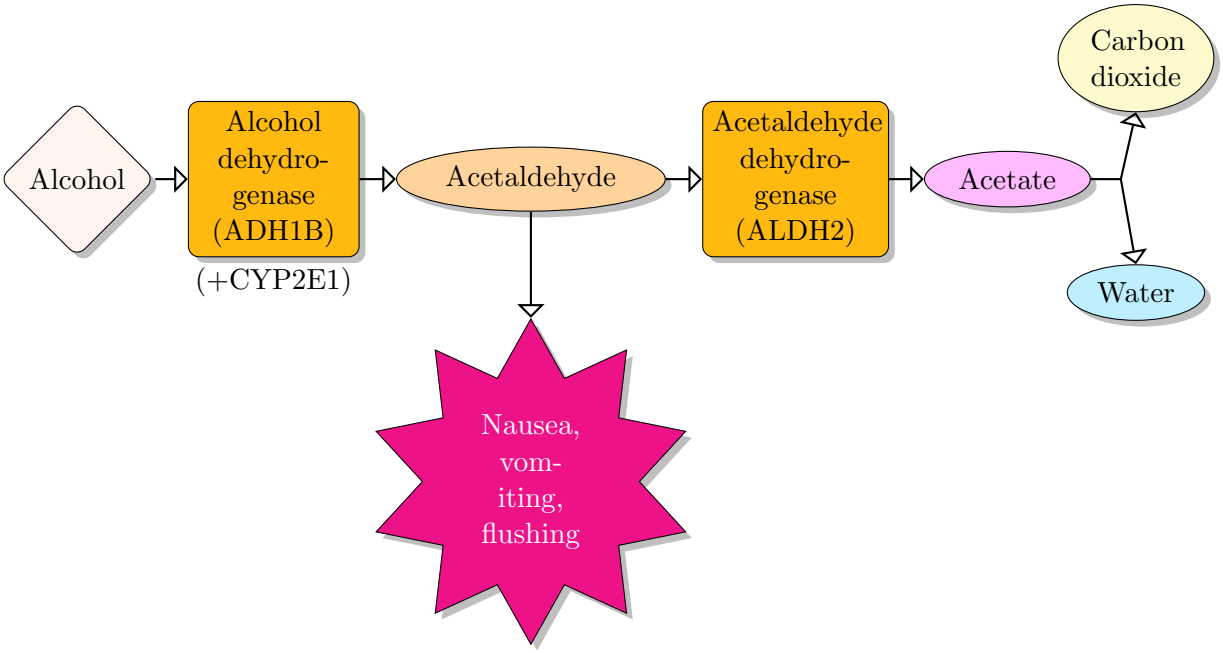


Figure 6.4: Alcohol metabolism. The rectangles represent enzymes involved in metabolism. Acetaldehyde is the first active breakdown product generated during alcohol metabolism. Additional pathways exist through the hepatic enzymes.

A meta-analysis of 72 studies that estimated the heritability of alcohol misuse yielded a weighted mean heritability estimate of 0.12 (95%CI: 0.11, 0.12).[464] This indicates that a reasonable proportion of variability in alcohol consumption arises from genetic predisposition, suggesting that it may be possible to use Mendelian randomization to investigate causal relationships between alcohol and vascular disease.

6.1.8 Use of Mendelian randomization to investigate alcohol consumption

Unlike 'traditional' Mendelian randomization that uses a gene variant that has specificity for an endogenous biomarker such as $sPLA_2$-IIA, as I described in Chapter 5 (Randomized Evidence on $sPLA_2$ and Cardiovascular Disease based on Genetic Studies and Trials), alcohol is an exogenous exposure and its consumption is a behavioural characteristic that is determined by many social and cultural factors including age, education, smoking status, diet, religion and gender.[465, 466] Thus alcohol consumption per se is not *directly* encoded by any single gene. However, the metabolism of alcohol provides an opportunity to use a SNP involved in the metabolic pathway as a proxy for alcohol consumption.

6.1.9 Metabolism of alcohol

The metabolism of alcohol occurs principally in the liver.[467] Alcohol is metabolised by several enzymes (Figure 6.4),[468] the first of which is alcohol dehydrogenase, which oxidizes alcohol and yields acetaldehyde. Acetaldehyde is a metabolite that yields particularly unpleasant symptoms of flushing, tachycardia and nausea, and a correlation has been reported between the level of circulating acetaldehyde and the symptomatic flushing response to alcohol consumption.[469]

Acetaldehyde is then oxidized to acetate, a step that is catalysed by aldehyde dehydrogenase. Acetate is finally metabolized in tissues into carbon dioxide (expired in the breath) and water.

In addition to this primary metabolic pathway, other enzymes such as the hepatic cytochrome P450 CYP2E1 enzyme can play a role in alcohol metabolism.[470]

Variants in genes encoding the enzymes involved in alcohol metabolism may alter the catalytic properties of the encoded enzyme, which can impact upon alcohol metabolism, and in particular influence levels of acetaldehyde.[471] Individuals that possess genetic polymorphisms that associate with higher acetaldehyde exposure in response to alcohol consumption are therefore less inclined to consume alcoholic beverages - a form of negative feedback.[471, 472]

6.1.10 Alcohol dehydrogenase (ADH1B)

Alcohol dehydrogenase catalyses the oxidation of alcohol to acetate and forms the initial step in the metabolic pathway of alcohol (Figure 6.4). The ADH1B enzyme was first characterised in 1977, when it was termed II-ADH.[473] The gene that encodes the ADH1B enzyme (*alcohol dehydrogenase 1B (class I), beta polypeptide*, *ADH1B*), is located on chromosome 4 and contains 9 exons (Figure 6.5).

The *ADH1B* rs1229984 SNP is bi-allelic with the two variants being G and A. The A allele has an allele frequency of approximately 2-5% in Europeans.[474, 475] Possession of the A-allele results in a change in amino acid from Arginine to Histidine. This amino acid alteration results in increased ADH1B enzyme activity - activity is increased by 40 to 100 times (as measured by Vmax and Km, respectively for ethanol oxidation).[476, 477]

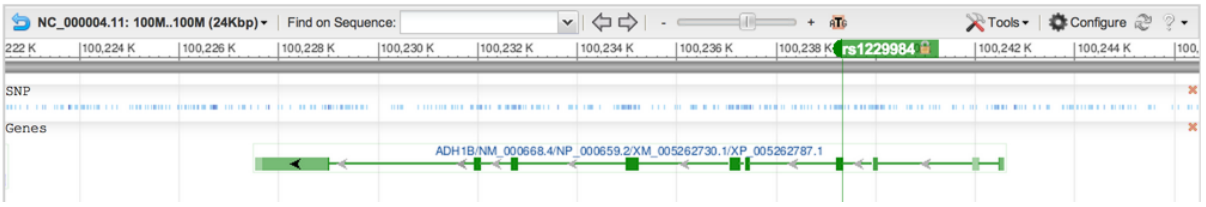


Figure 6.5: *ADH1B* gene with exons illustrated as boxes.
The location of rs1229984 SNP is also shown. Obtained from dbSNP.

6.1.10.1 Association of *ADH1B* rs1229984 with alcohol indices

Several studies have examined the effect of the *ADH1B* rs1229984 allele on measures of alcohol consumption.

For example, compared to individuals homozygous for the G-allele, individuals carrying the rare (A-allele) variant at the rs1229984 locus:

- have a higher maximum rate of alcohol metabolism (Vmax)[478]. This fits with the hypothesis that A-allele carriers, who have higher ADH1B enzyme activity, have an increased conversion of alcohol to acetaldehyde. This increased acetaldehyde concentration for a given exposure to alcohol would be expected to reduce exposure to alcohol consumption on average when compared to individuals who do not carry the A-allele
- report subjective feelings of flushing in response to alcohol[471, 479]
- are less likely to report alcohol dependence.[480–484]
- consume fewer units of alcohol per week[485]
- are more likely to report abstention from alcohol[485]

Collectively, the evidence supports the hypothesis that individuals that carry the A-allele at the rs1229984 locus are exposed to less alcohol consumption.

6.1.10.2 Association of *ADH1B* rs1229984 with recognized associations of alcohol

One way to test that the genetic variant is serving as a proxy for alcohol consumption is to examine the association of the variant with a 'known' outcome for alcohol - for example oesophageal cancer.

Previous investigations of *ADH1B* rs1229984 have shown association of the A-allele with a reduced risk of alcohol-related diseases including upper aerodigestive cancer [486] and esophageal cancer[487] (and this is in the expected direction as rs1229984 A-allele carriers consume less alcohol).

Prior to 2013, the association of *ADH1B* with cardiovascular outcomes and biomarkers was limited to single studies[488, 489] and/or meta-analyses[490] of small numbers of studies lacking adequate statistical power to detect meaningful effects.

Thus a natural extension to previous work is to build up a large-scale collaboration to use the rs1229984 variant to investigate the role of alcohol in CHD.

6.1.11 Use of *ADH1B* rs1229984 to investigate CHD

When using a genetic variant to investigate the association of alcohol with CHD, it is worthy to consider the underlying observational association and the implications this has for the interpretation of an estimate obtained from a genetic analysis. Recall that in Figure 6.2 the observational association between alcohol and CHD was curvilinear, with a nadir at 25g/day.

In Figure 6.6, two potential scenarios are contrasted. First, on the left we have individuals that consume low amounts of alcohol. We can see that if an individual is an A-allele carrier, because they are expected to drink a lower amount of alcohol than an individual that is homozygous for the G-allele (owing to the underlying genetic variant, the implications this has on alcohol metabolism and the feedback this has on alcohol-related behaviours), the A-allele carrier would be expected to have a higher relative risk of CHD (compared to the GG carrier). This is because the left hand side of the figure is to the left of the nadir (the point at which the RR of CHD is lowest compared to non-drinkers), and therefore any reduction in alcohol (e.g. resulting from carriage of the A-allele of rs1229984) is associated with an increased risk of CHD.
In contrast, when we examine the right of Figure 6.6, we are now on the right side of the minimum. Individuals that possess the A-allele (that drink less alcohol than GG carriers) would be expected to have a *lower* risk of CHD compared to individuals that are homozygous for the GG variant. This is because at this part of the curve, a reduction in alcohol is associated with a reduction in CHD risk.

The implication is that, if the curvilinear association is 'true', the association of the *ADH1B* rs1229984 on CHD risk will vary according to the amount of alcohol consumed, and where this lies on the curve.
In contrast, if the 'true' association is linear then the effect of the SNP will be consistent at all levels of alcohol intake.

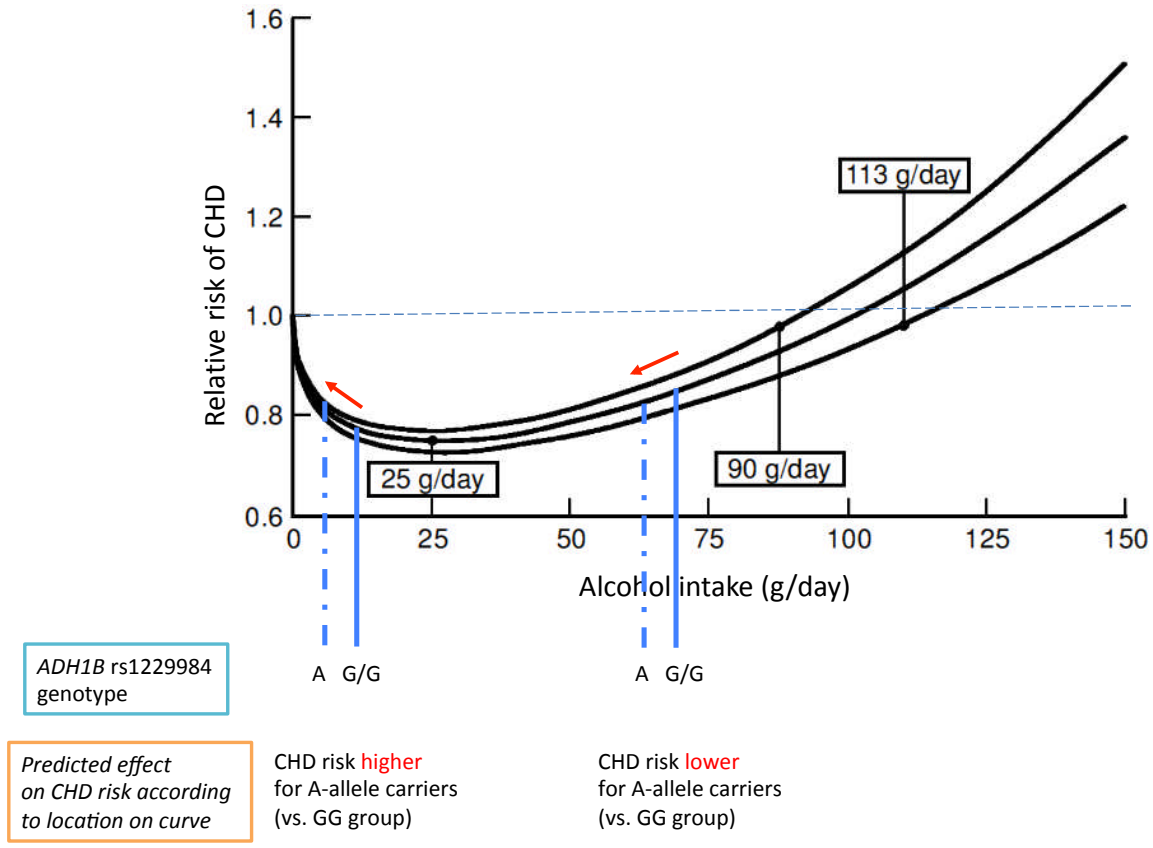


Figure 6.6: Implications of the observational association between alcohol and CHD on the interpretation of genetic associations of *ADH1B* with CHD

6.1.12 Consideration of worldwide *ADH1B* genotype frequency

The *ADH1B* genotype frequency is strongly associated with geographical location (Figure 6.7), which probably represents the pattern of population migration from Africa to Europe and North America (Figure 6.8). However, alternative explanations include the geographical variation in consumption of fermented rice.[491]

This worldwide difference in *ADH1B* genotype frequency introduces the potential for confounding by population stratification, especially as geographical region is associated with both alcohol intake (Figure 6.1)[492] and cardiovascular events.[493]

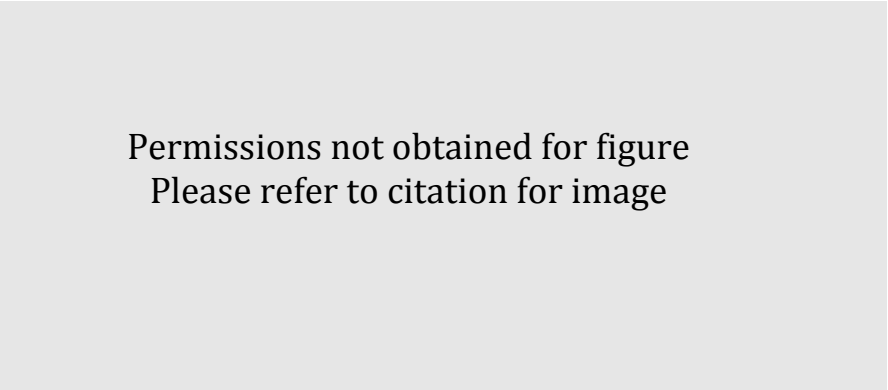


Figure 6.7: Contour plot of the allele frequency of *ADH1B* worldwide. The darker the shading, the higher the proportion of individuals that carry the *ADH1B* rs1229984 A-allele variant. Reproduced from Li et al.[494]

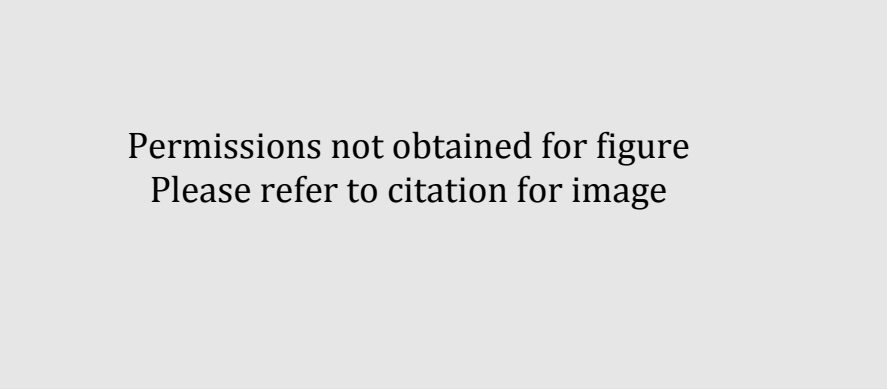


Figure 6.8: Routes of migration of *ADH1B* genotype. The letters (H1-H7) refer to haplotypes. Reproduced from Li et al.[495]

Fortunately, practical solutions to this problem exist (as outlined in Chapter 1), including limiting analysis to European individuals, interrogating the genotype frequency of included studies (to identify evidence of non-European admixture), and adjusting for population stratification where possible using principal components analysis.[94]

6.1.13 Mendelian randomization investigations of alcohol and human health using *ALDH2* genotype

Aldehyde dehydrogenase is an enzyme that is responsible for catalysing the oxidation of acetaldehyde into acetate, the second step in the metabolic pathway of alcohol (Figure 6.4). The *ALDH2* rs671 variant, a non-synonymous SNP, is located on chromosome 12. The A variant (Glu504Lys in exon 12, also termed $*2$) results in a change from a glutamate at amino acid 487 to lysine, which results in decreased enzymatic activity of ALDH2. Individuals homozygous for the A allele have almost no enzyme function and individuals with one copy of the A-allele have 60% lower enzyme activity than those

homozygous for the G allele.[496]

ALDH2 rs671 genotype associates with several alcohol-related phenotypes including the flush response to alcohol intake (a consequence of acetaldehyde),[497] volume of alcohol intake[498], including binge drinking[499] and alcoholic liver disease.[498]

To date, Mendelian randomization studies have predominantly used a SNP in the *aldehyde dehydrogenase 2 family* gene (*ALDH2* rs671, also known as ⋆2) as an instrument for alcohol consumption.[487, 489, 500–524] Robust associations have been reported between *ALDH2* rs6712 and several alcohol-related disorders including hypertension, esophageal cancer and head and neck cancer.[511, 525, 526] These serve as positive controls since the causal role of alcohol in these diseases is not disputed. However, the genetic variant is monomorphic in individuals of European descent, and therefore cannot be used in these populations for instrumental variable analysis.

6.2 *ADH1B*-CVD project

6.2.1 Rationale

The rationale for this project was to interrogate the causal relationship between alcohol consumption and cardiovascular risk factors and disease outcomes using a SNP in *ADH1B* as a proxy for alcohol intake using the principles of Mendelian randomization.

6.2.2 Aims

The project had several objectives:

1. to assemble an international collaboration to yield data with sufficient numbers of individuals in order to be able to make meaningful inferences about the relationship between alcohol consumption and cardiovascular traits and outcomes
2. to investigate the observational association between alcohol consumption and cardiovascular biomarkers and risk factors as well as potential confounders
3. to validate the use of *ADH1B* rs1229984 as an instrument for alcohol exposure in populations of European descent
4. to investigate the association of *ADH1B* rs1229984 with CVD biomarkers, risk factors, potential confounders and CVD events

5. to use instrumental variables analysis to estimate the un-biased (causal) associations between alcohol consumption and cardiovascular traits

Chapter 7

ADH1B-Cardiovascular Disease Collaboration: Overview and Observational Associations between Alcohol and Cardiovascular Traits

7.1 Introduction

In this chapter, I will introduce the observational component of the *ADH1B*-CVD collaboration. The rationale for the observational analyses was to validate the dataset as showing associations consistent with the published literature, but also to add to the scientific literature on the relationship between alcohol consumption and emerging cardiovascular risk factors, such as IL-6 and C-IMT.

7.2 Methods

7.2.1 International collaboration

An international collaboration was established by inviting cohorts and large case-control studies with information on alcohol consumption, *ADH1B* rs1229984 genotype and cardiovascular traits to participate (details of invitation letter provided in Appendix C, on page 305). The collaboration commenced in 2009, and over the period from 2009 to

2013 expanded to include 56 studies with over 260,000 participants.
Studies contributing towards the observational analyses are denoted in Table C.1.

7.2.2 Measures of alcohol consumption

Several measures of alcohol consumption were evaluated:

Units per week the principle alcohol trait of interest was weekly volume of alcohol in British units (1 British unit = 7.9 grams pure ethanol or half a pint of beer, a small glass of wine or a single measure of spirits). For studies in which this variable was not already present, I either calculated weekly volume of alcohol by summing the individual components of beverage-specific drink questions, or by converting alcohol recorded in grams/week into British units.

Binge drinking binge drinking was defined as consumption of five or more drinks in a single occasion in the past month, or the closest possible definition to this. Please refer to Table C.2 for specific information on this measure in participating studies.

Self-reported abstainer definitions of self-reported abstaining varied between studies from lifelong abstention to individuals who had been abstaining for the past six months (see Table C.2 for details of study-specific definitions).

Top-tertile per study Individuals were grouped into tertiles in each study, separately for men and women, based on their alcohol consumption in units/week. Individuals grouped in the top-tertile of alcohol consumption were compared to the lower two tertiles.

GGT plasma level of gamma-glutamyltransferase (GGT), a liver enzyme that is increased in a dose-dependent fashion in relation to alcohol consumption,[527, 528] was used as a marker of heavy alcohol consumption.

7.2.3 Data handling

Non-normally distributed continuous variables, including weekly units of alcohol, were logarithmic transformed on the natural scale. In order to include individuals who declared drinking zero weekly units alcohol, a value of one was added prior to transformation (otherwise this transformation would have rendered them as missing[a]). Non-high density lipoprotein cholesterol (non-HDL-C) cholesterol was derived by subtracting HDL-C from total cholesterol. Hypertension was defined as a systolic BP of $\geq$140mmHg and/or a diastolic BP of $\geq$90mmHg.[529]

[a] as log(0)=undefined

7.2.4 Statistical analysis

In order to be able to compare the effect of alcohol on different traits, all continuous traits were standardized prior to the observational analysis. To assess the shape of the association between log weekly alcohol consumption units and each trait, in a total of 28 studies (131,490 individuals), statistical models were constructed using individual participant data (IPD). In each study, each cardiovascular trait was treated as the dependent variable and linear and quadratic terms were fitted for alcohol consumption, adjusted for age and gender. The study-specific beta coefficients and 95%CI for the linear and quadratic terms for alcohol consumption across the studies were pooled using fixed-effects (inverse variance) meta-analysis. If the 95%CI of the pooled quadratic term did not include the null value (of 0), I used this as evidence to suggest a non-linear relationship was the appropriate model to reflect the association between alcohol and the trait. Plots were generated of the pooled estimates using the summary regression coefficients as a function to illustrate the shape of the observational association between alcohol and each trait across studies.

7.2.5 Proportion of variance explained by alcohol

For each of the continuous traits, I estimated the proportion of variance (R^2) explained by alcohol consumption. The purpose was to try and gauge whether negative results from the genetic analysis could be ascribed to lack of power. In the pooled IPD dataset, I estimated the R^2 and 95%CI using bootstrapping (with n=1000 repetitions) adjusted for age, gender and study. I then repeated the model without the log weekly units/week alcohol variable and subtracted the R^2 estimate from the second model from the first, in order to obtain the R^2 from alcohol alone. The lower boundary of the lower 95%CI estimate of the derived R^2 was limited to zero.

7.3 Results

Twenty-eight studies with 131,490 individuals provided individual participant data that contributed towards the observational analysis (Table C.1). Of the 26 traits, summary estimates for the studies contributing towards the observational analysis for 24 of 26 traits were similar to the studies that did not contribute (Figures C.2 to C.18).

7.3.1 Availability of traits and proportion of variance of traits explained by alcohol on observational analysis

Figure 7.1 shows the availability of different variables across the studies contributing towards the collaboration, ordered by the proportion of all individuals (n=261,991) with data available. The trait with the greatest number of individuals was body mass index, with 232,570 individuals and the trait with the least was cotinine (with 9378 individuals). Alcohol explained the greatest proportion of variance of HDL-C R^2=3.75% (95%CI, 2.94, 4.57).

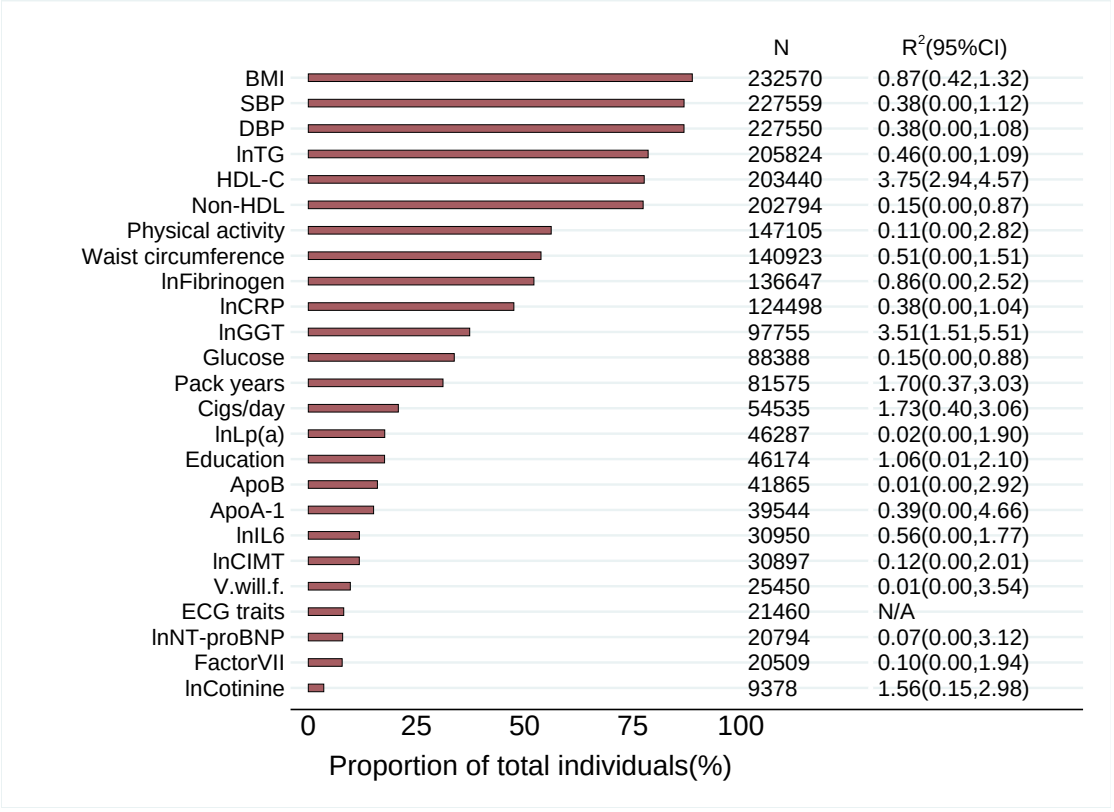


Figure 7.1: Total number of individuals for each trait and the proportion of variance (R^2) of each trait explained by alcohol in the collaborating studies. R^2 values are expressed as a percentage.

7.3.2 Observational analysis between alcohol consumption and cardiovascular biomarkers

Observational analysis revealed that with exception of lipoprotein(a) and BNP, all other cardiovascular biomarkers, life-style and social variables were associated with alcohol consumption (Figures 7.2, 7.3, 7.4 and 7.5), with most relationships being curvilinear in nature (Table C.9).

All coagulation and inflammation biomarkers showed a curvilinear association with alcohol consumption. In general, light-to-moderate drinkers had the lowest values and heavy drinkers had the highest values compared to non-drinkers. Similar curvilinear associations were observed for systolic blood pressure (BP), glucose, and carotid intima medial thickness (C-IMT).

Light-to-moderate drinkers showed the lowest levels of measures of smoking habit, body mass index (BMI) and waist circumference and the highest levels of education and physical activity.

Associations of alcohol consumption with lipids and apolipoproteins varied in shape and strength. Apolipoprotein A1 and HDL-C showed the strongest positive dose-response with alcohol consumption, with no evidence of a plateau, and with heavy drinkers (>50 units/wk) having the highest mean values by 0.8 SD compared to non-drinkers (Figure 7.2). In contrast, the nature of the association between alcohol with non-HDL-C was more shallow.

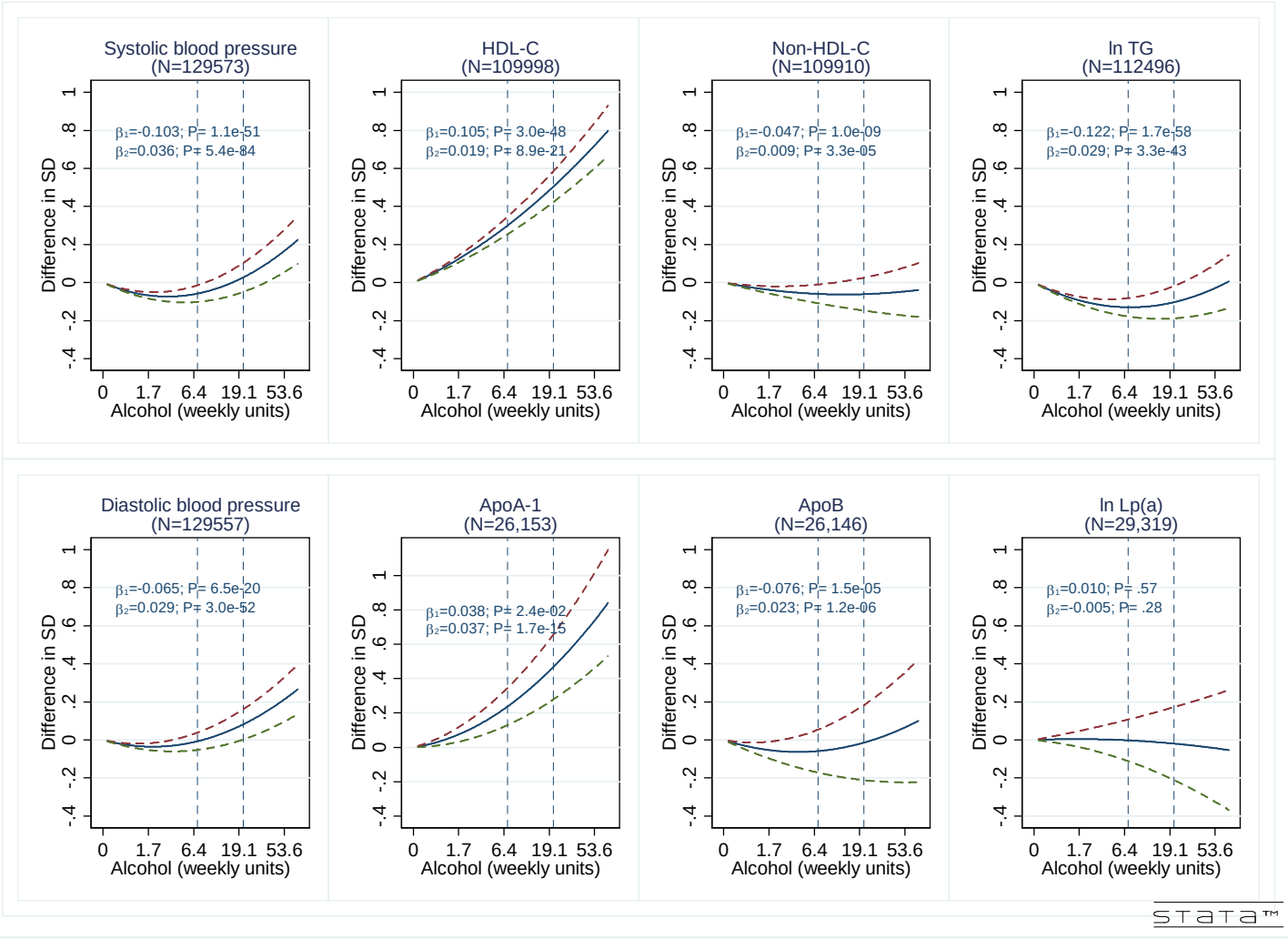


Figure 7.2: Observational association between alcohol consumption and other variables (1)

Footnotes: The dose response relationship is derived from the best fit model obtained from each comparison. The values plotted represent the predicted estimates derived from the regression model that includes a linear and quadratic term for alcohol consumption (log units/week). The vertical dotted lines represent the cut-points for the main alcohol categories used in the analysis: 7 and 21 units/week.

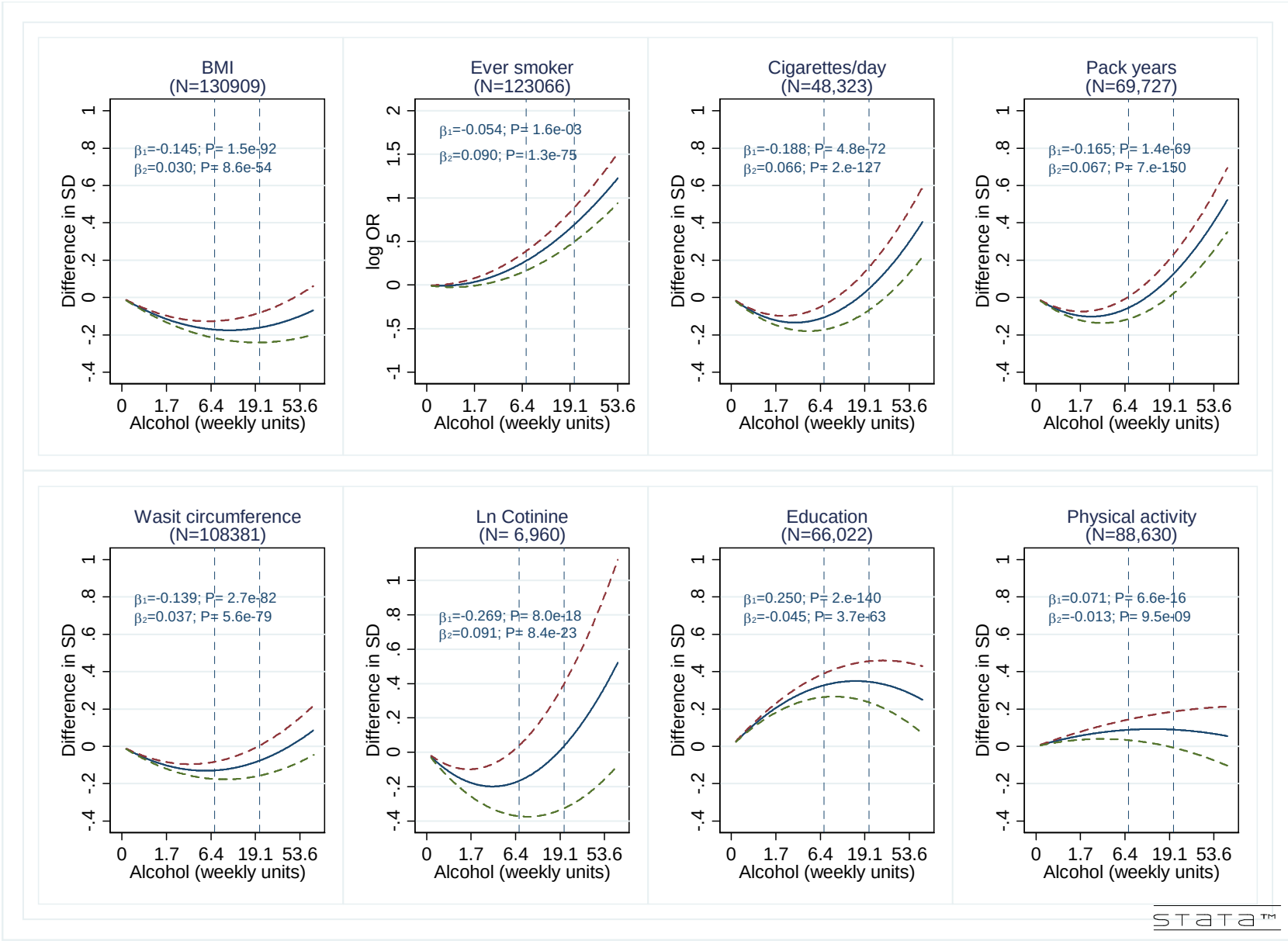


Figure 7.3: Observational association between alcohol consumption and other variables (2)
See footnote to Figure 7.2 for further details.

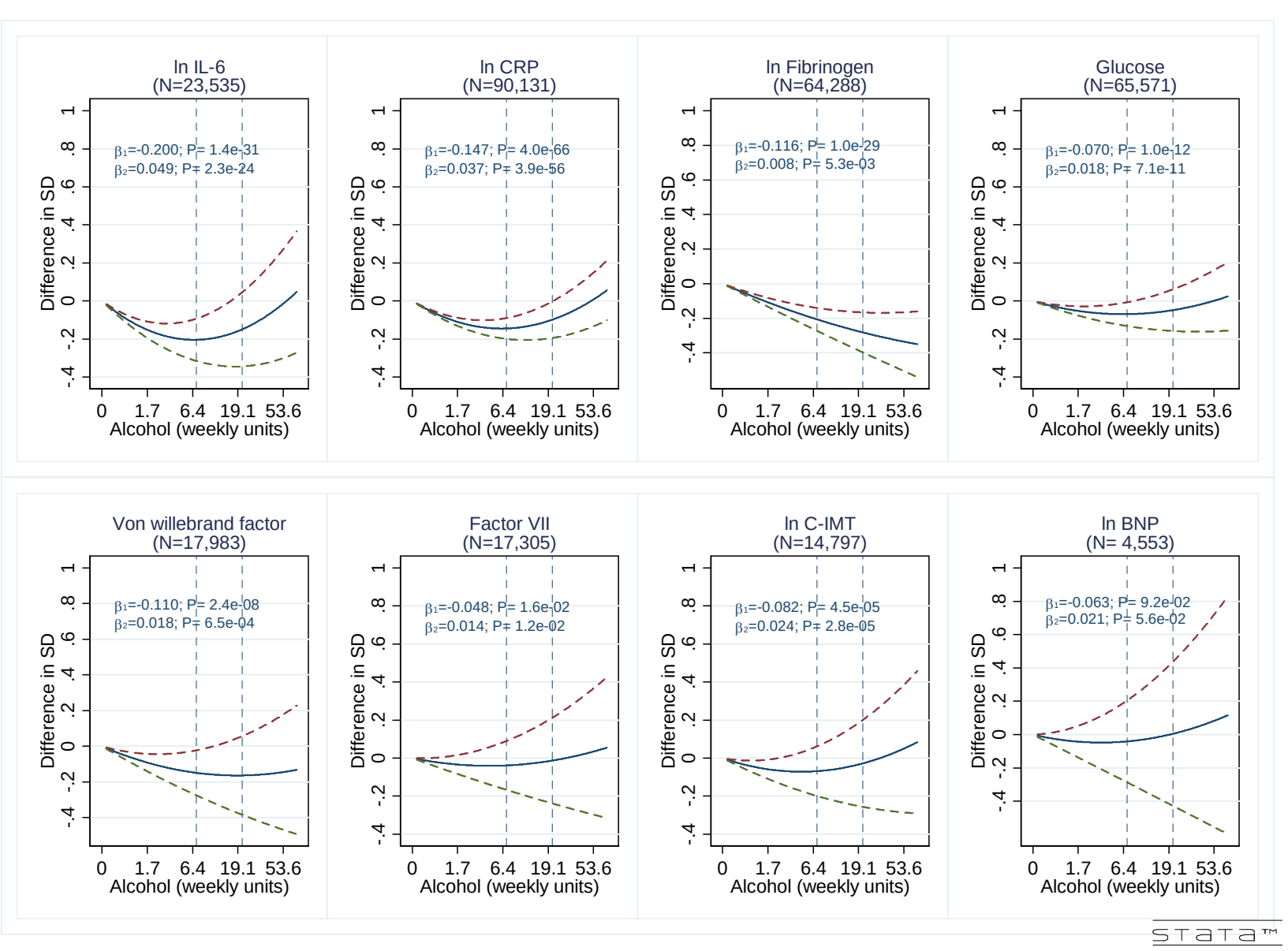


Figure 7.4: Observational association between alcohol consumption and other variables (3)
See footnote to Figure 7.2 for further details.

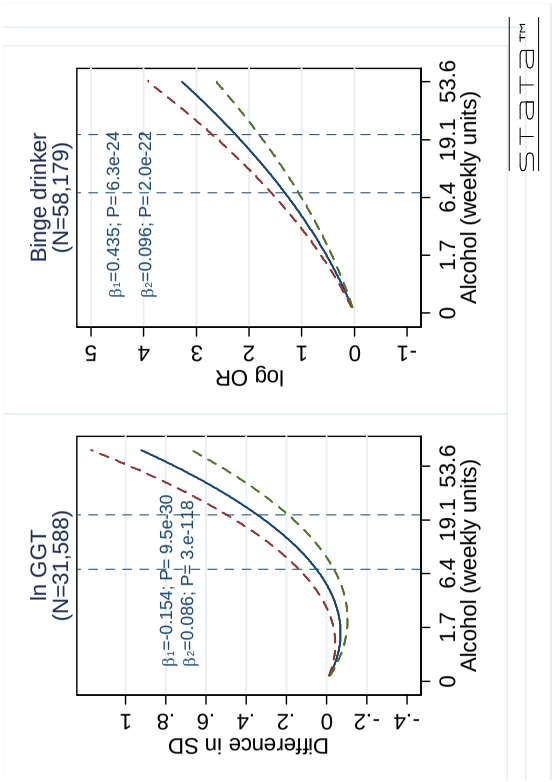


Figure 7.5: Observational association between alcohol consumption and other variables (4)
See footnote to Figure 7.2 for further details.

7.4 Discussion

The data I present in this Chapter serve to illustrate the observational association of alcohol consumption with multiple cardiovascular risk factors, biomarkers and potential confounders. Of the 28 traits I investigated, alcohol showed strong association with all but two of them and the vast majority (24 of 26) of associations were curvilinear in nature.

This is important for several reasons. First, the curvilinear association of alcohol with established and emerging risk factors (e.g. SBP[31], non-HDL-C[530], smoking-related traits, IL-6[83]) introduces the possibility that one or more could act as potential non-linear confounders of the association of alcohol consumption with risk of CVD. Alternatively it is possible that some of these traits might mediate a causal association between alcohol consumption and CHD risk. For example, if we look at the plots for SBP, non-HDL-C and IL-6, they all have evidence of non-linear positive associations with alcohol consumption, similar to the relationship of alcohol with CHD shown in Figure 6.2 in Chapter 6. Thus, if we assume all three traits (SBP, non-HDL-C and/or IL-6) were to mediate the alcohol association and that the J-shape was a true reflection of the relationship between alcohol and these traits (i.e. not influenced by error from bias or confounding) then it is conceivable to think that the true association between alcohol and risk of CVD may also be J-shaped.

These findings also validate the dataset for the Mendelian randomization analysis I present in the next Chapter. For example, the association with SBP is similar to that previously reported.[421] Second, the association with HDL-C appears to be almost linear, and this positive dose-response relationship is in agreement with the estimates reported from prior meta-analyses of interventional studies.[401, 461] Interestingly, I identified strong evidence of association between alcohol and other biomarkers (e.g. TG, CRP, IL-6 and fibrinogen) that did not show association with alcohol in the interventional meta-analyses.[401, 461] However, the sample size reported in this Chapter is many-fold greater (see Figure 6.3) than in those prior studies, meaning the results from interventional studies could well represent Type II (false negative) errors.

These data also increase understanding to the relationships of alcohol with cardiovascular biomarkers. For example, the association of alcohol with C-IMT provides novel insights into the underlying relationship, and importantly, mimics the same association

that has been reported with CHD (Figure 6.2). As C-IMT is recognized as an important surrogate marker for CHD[531, 532], it is perhaps expected that it should share the same *observational* association with alcohol as CHD (i.e. J-shaped in nature). Of course, this does not help delineate the underlying *causal* association as the same confounding factors could influence the relationship between alcohol and C-IMT as for CHD.

Another characteristic of the observational plots presented in this Chapter is the concordance of directionality between biologically related, but independently-measured traits. For example, there was concordance in the nature of the association for the following pairs of traits: SBP and DBP; HDL-C and Apolipoprotein-A1; LDL-C and Apolipoprotein-B; CRP and IL-6. This consistency helps to affirm the nature of the association of alcohol with blood pressure, lipid and lipoproteins, and inflammatory markers.

7.5 Conclusion

In this Chapter, I reported the observational associations between alcohol and cardiovascular biomarkers and lifestyle variables. These analyses illustrate the wide-reaching associations of alcohol with numerous measures that could represent potential confounders and/or mediators in the pathway between alcohol and CHD. This has implications on the credibility of using observational evidence on the association of alcohol with CHD to make health-related policy judgements. Fundamentally, it shows the necessity to use evidence from an unbiased source to make causal inference.

This is the basis for the next Chapter, which will investigate the genetic association between a SNP that encodes an enzyme central to alcohol metabolism (ADH1B), used as a proxy for alcohol intake, and CHD events.

Chapter 8

ADH1B-Cardiovascular Disease Collaboration: Genetic and Instrumental Variable Associations

In Chapter 6, I described the outline of the *ADH1B*-CVD project and in Chapter 7 presented the observational associations between alcohol consumption, cardiovascular biomarkers, and other covariates that could either confound or mediate the association of alcohol with CVD events.

In this chapter, I will present an investigation of the causal role of alcohol in cardiovascular disease using *ADH1B* rs1229984 as an instrument for alcohol intake. This analysis had two components: first I conducted a genetic association analysis (using the *ADH1B* rs1229984 SNP) with alcohol consumption, lifestyle factors and cardiovascular biomarkers and disease events and; second, I conducted a formal instrumental variable analyses to "triangulate" the true (causal) effect of alcohol consumption on cardiovascular biomarkers.

8.1 Methods

8.1.1 Genetic analysis

A standardized script was written and deployed in all participating studies. This homogenized the analysis procedure, preventing subtle differences between analyses affecting

the overall meta-analysis estimates.

For the genetic analysis, I used a dominant model for the analysis: i.e. one and two copy carriers of the A allele were grouped and compared to individuals homozygous for the G-allele (reference group).

The association between *ADH1B* rs1229984 A-allele and each continuous trait was estimated by obtaining the means and standard deviations separately for rs1229984 A-allele carriers and non-carriers. For binary variables, the univariate log odds ratios and standard errors comparing the A-allele carriers to non-carriers were obtained. All effect estimates were pooled using fixed-effects and random-effects modelling. I quantified heterogeneity using the I^2 statistic.[533]

In a subset of studies, information was available on lipid lowering and blood pressure lowering treatment. For these studies, individuals who were documented as receiving anti-hypertensive medications had 15mmHg added to their values of systolic blood pressure (SBP) and 10mmHg to diastolic blood pressure (DBP).[534] For individuals on lipid-lowering drugs, 2.096 mmol/l was added to total cholesterol and 0.461 mmol/l to triglycerides (constants derived from Whitehall II study[535] following methods described by Tobin).[534, 536]. The association of the SNP with medication adjusted traits was used as a sensitivity analysis. As no difference was identified between medication-adjusted and unadjusted values, the analyses presented thereafter were based on unadjusted values.

All participants with non-missing data contributed towards analysis of continuous traits as the majority of study participants were free from coronary heart disease (CHD) at recruitment. The only exception was the SMART study[537] (a cohort of patients with established cardiovascular disease or with multiple risk factors), which only provided data for carotid intima medial thickness (C-IMT) and N-terminal fragment B-type natriuretic peptide (BNP).

8.1.2 Meta-analysis of rs1229984 A-allele and cardiometabolic events

I used counts of cases and non-cases per genotype group to generate the corresponding log odds ratio and standard error. A continuity correction was not used, therefore if a study contained a "0" count for any binary trait, the study was excluded from the meta-analysis. I conducted meta-analyses using inverse variance weighting for the fixed effects models, and DerSimonian and Laird for random effects models.

8.1.3 Subgroup analyses

I investigated differences in the overall association between the gene variant and each variable by conducting subgroup analyses based on individual-level and study-level characteristics.

The principal subgroup analysis of interest was alcohol consumption (an individual-level characteristic), with study participants grouped according to their weekly volume of alcohol consumption into none, >0 to <21 and ≥21 units/week. The rationale for this sub-group analysis was two-fold. First, it allowed evaluation of the effect of the gene variant in individuals not consuming alcohol. Because the rs1229984 SNP is expected to have an effect only when an individual is exposed to alcohol, in individuals consuming no alcohol, I anticipated there should be no association between the SNP and cardiovascular biomarkers and disease outcomes. Second, in individuals that consumed alcohol, stratifying by volume of alcohol permitted the investigation of a dose-response relationship between alcohol and cardiovascular biomarkers and outcomes. The limitation of this approach is that stratifying on an intermediate trait may introduce "collider bias"[538] by introducing confounding via a back-door pathway (described in Figure C.19 on page 304).[64]
To investigate whether collider bias was likely to influence the association between *ADH1B* rs1229984 on analyses stratified by alcohol, I also stratified the association of *ADH1B* rs1229984 with potential measured confounders (with the caveat that this analysis would be limited only to measured confounders). Thus, by stratifying the confounders by alcohol, if an association were to be identified (between alcohol and a confounder that was not present on the unstratified analysis), this would suggest that such stratified analyses should not be conducted.[539]

In addition to alcohol volume, the following characteristics at the individual/study level were chosen *a priori* for secondary subgroup analyses:

- gender (women, men)
- geographical region (North Europe, West Europe/Australia, Central/South Europe, East Europe, USA)
- age (mean age of study participants, dichotomized into <60 years, ≥60 years)
- Hardy Weinberg Equilibrium (dichotomized into studies not showing evidence of Hardy Weinberg Disequilibrium (P>0.001), and those showing evidence of Hardy Weinberg Disequilibrium (P≤0.001))

- genotyping platform (dichotomized into those that genotyped *ADH1B* rs1229984 on a single SNP platform, or as part of a chip or array)
- year of blood sampling for DNA extraction (split into 1980 to <1990, ≥ 1990 to <2000, and ≥2000 to present)
- whether the study contributed towards the observational analysis presented in Chapter 7.

8.1.3.1 Investigation of subgroup analyses using metaregression

For the subgroup analysis based on alcohol consumption, I organised alcohol categories into a logical order (0, >0 to <21, ≥21 units/week). This categorical variable was entered into a metaregression analysis. Each group was assigned the corresponding median alcohol intake in units/week obtained from a pooled individual participant dataset using the following values: group >0 to <21: 6.24 units/wk; ≥21: 32.88 units/wk. The P value for heterogeneity that I report for alcohol subgroups was derived from this meta-regression analysis. For all other (non-alcohol) subgroup analyses, I tested for presence of heterogeneity between strata using unordered categorical metaregression, with the '`i.`' prefix for the variable name in the metaregression model. All meta-regressions were conducted using the metareg command in Stata.

8.1.4 Sensitivity analysis

For volume of alcohol in units/week (the main alcohol phenotype), I performed a measurement error sensitivity analysis according to the level of information obtained from the self-reported alcohol questions in the original study questionnaires. If the original alcohol intake questions recorded information on beverage-specific consumption (i.e. consumption questions were asked separately for beer, wine and spirits rather than all beverages combined), the study was labelled "specific" in relation to the subgroup analysis by alcohol questionnaire, otherwise the study was labelled "non specific". I used this information to perform a sensitivity analysis according to the type of questionnaire used in each study.

8.1.5 Investigating potential for confounding by population stratification

In 11 studies of over 40,000 individuals, principal component analysis was possible based on the IBC CardioChip data.[540] In these studies, to investigate the possibility of

residual confounding by population stratification, I conducted two analyses. First, I conducted univariate linear or logistic regression analyses with each cardiovascular trait and disease outcome as the dependent variable, and rs1229984 A-allele carrier status as the independent variable. In the second analysis, I incorporated the first three principal components traits into each of the models.

I tested for evidence of an association between available PC traits with log weekly units of alcohol in the largest dataset that I had access to individual participant data and included only PC traits that showed association. Since each of the three PC traits demonstrated association with log weekly units of alcohol (at $P<0.05$), I incorporated all three PC traits in analyses to adjust for potential residual population stratification.

I compared the beta-coefficient or log odds estimates derived from the univariate and principal component adjusted models for evidence that adjustment for principal components attenuated any of the estimates. A difference between the two models (unadjusted and adjusted) would suggest that the unadjusted association was influenced by residual population stratification.

8.1.6 Instrumental variable analysis

In order to estimate the de-confounded effects of alcohol on cardiovascular traits, I used two stage least squares instrumental variable (IV) analysis. However, this made the strong assumption of a linear association between alcohol and cardiovascular traits. The instrumental variable analysis was conducted for all traits that had a nominally significant ($P<0.05$) association with *ADH1B* rs1229984 on genetic association analysis.

8.1.6.1 Linear instrumental variable meta-analysis

I used the ratio instrumental variable (IV) estimator to estimate the unconfounded effect of log weekly units of alcohol on biomarkers. For this, I conducted a fixed-effects meta-analysis of *ADH1B* rs1229984 on log weekly units of alcohol across studies that had information on alcohol in units/week. I used this pooled estimate for the IV analysis, thus assuming a fixed effect of *ADH1B* rs1229984 on log units/wk alcohol intake across studies. This also enabled the incorporation of studies that did not have alcohol measured to contribute towards the IV analysis.

Using study-specific associations between *ADH1B* rs1229984 and each continuous trait and outcome, I calculated the instrumental variable effect estimate for alcohol by dividing the *ADH1B*-trait association by the pooled estimate of the association of *ADH1B* and log units/wk of alcohol consumption.[84, 370] This analysis took into account the

uncertainty in both the *ADH1B*-trait and *ADH1B*-alcohol association by using the delta method to estimate the standard errors of ratio instrumental variable estimates.[541] I incorporated studies that did not have information on alcohol into the instrumental variable analysis by applying the pooled effect estimate of the *ADH1B*-log units/wk association to them.[370, 372, 541] I pooled study-specific instrumental variable estimates using fixed- (inverse variance) and random-effects (DerSimonian and Laird) meta-analysis.

8.2 Results

8.2.0.2 Studies included in the collaboration

Of the 56 studies included in the collaboration, there were 38 cohorts, 6 nested case-control studies, 3 case-cohort, 4 randomized trials, 4 case-control and 1 cross-sectional study. The studies originated from Europe (n=44), North America (n=11) and Australasia (n=1) (Appendix C).

There were a total 261,991 participants in the analysis, of which 48% were female, and the mean age was 58 years (range 26, 75) (Table C.1). Characteristics of the alcohol questionnaires in each study are summarized in Table C.2. The median number of alcohol units consumed in each study is shown in Table C.4. There were 20,259 CHD events, 10,164 combined subtypes of stroke cases, 4339 ischemic stroke cases and 14,549 type 2 diabetes cases (Table C.5, defined in Table C.3). Means and distributions for continuous traits in all studies are presented in Tables C.6, C.7 and C.8.

8.2.1 Allele frequency of *ADH1B* rs1229984 SNP

The *ADH1B* rs1229984 SNP was directly genotyped in all collaborating studies (Table C.1). When I grouped studies by geographical location, the allele frequency was consistent in all geographical regions (Figure C.1). The call rate (the proportion of individuals assigned a genotype out of all individuals who were genotyped) was >90% in all contributing studies (Table C.1). For 9 studies, the Hardy Weinberg Equilibrium P-value was ≤ 0.001 (Table C.1). Stratifying the effect estimates in the relationship between *ADH1B* rs1229984 with each of the traits by studies that were and were not in Hardy Weinberg Equilibrium did not show evidence of heterogeneity (Figures C.2 to C.18).

8.2.2 Association of *ADH1B* with alcohol phenotypes

To validate the use of rs1229984 as an instrument for alcohol, I investigated the association between the rs1229984 SNP and the following alcohol consumption measures: self-reported volume, abstention, top third of drinking per study, binge drinking and measures of the liver enzyme GGT.

When I compared carriers of the rs1229984 A-allele to non-carriers, carriers consumed fewer units of alcohol per week (-17.5% units/wk; 95%CI, -19.1 to -15.8) and had lower

odds of being in the top third of drinking volume (OR 0.70; 95%CI, 0.67 to 0.73). Furthermore, carriers of the rs1229984 A-allele had lower odds of binge drinking (OR 0.78; 95%CI, 0.73 to 0.84), increased odds of being self-reported abstainers (OR 1.27; 95%CI, 1.21 to 1.34) and lower levels of GGT (-1.8%; 95%CI, -3.4 to -0.3) (Table 8.1). The association with alcohol volume remained unaltered when stratified by laboratory procedures or study characteristics (Figure C.2).

Table 8.1: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and indices of alcohol consumption.

Trait (units)	Studies, Cases/Individuals	Effect estimate (95% confidence interval)	P value	I^2, % (95%CI)
		% difference		
Volume (ln units/wk)	46, NA/219752	-17.47 (-19.10, -15.80)	1.4×10^{-78}	66 (54, 75)
Ln GGT (U/L)	15, NA/97755	-1.84 (-3.40, -0.26)	0.02	36 (0, 65)
		Odds Ratio		
Top tertile of alcohol intake	45, 69229/222332	0.70 (0.67, 0.73)	5.9×10^{-69}	60 (45,71)
Binge drinker	21, 22198/131290	0.78 (0.73, 0.84)	1.4×10^{-12}	47 (13, 68)
Alcohol abstainer	32, 24482/189854	1.27 (1.21, 1.34)	2.6×10^{-19}	73 (62, 81)

Footnote: For definitions of binge drinker and alcohol abstainer, please refer to Table C.2. GGT: Gamma-glutamyl transferase; NA: not applicable

8.2.3 Association of *ADH1B* rs1229984 with cardiovascular biomarkers

Carriers of the rs1229984 A-allele had lower systolic BP (-0.88 mmHg; 95%CI, -1.19 to -0.56) compared to non-carriers (Table 8.2).

Concordant with this, rs1229984 A-allele carriers had lower odds of hypertension (104,570 cases; OR 0.94; 95%CI, 0.91 to 0.98) compared to non-carriers. Rs1229984 A-allele carriers had lower levels of interleukin-6 (IL-6) (-5.2%; 95%CI, -7.8 to -2.4), C-reactive protein (CRP) (3.4%; 95%CI, -5.7 to -1.1), BMI (-0.17kg/m^2; 95%CI, -0.24 to -0.10) and waist circumference (-0.34cm; 95%CI, -0.58 to -0.10). Rs1229984 A-allele carriers also had lower non-HDL-C (-0.03 mg/dl; 95%CI, -0.05 to -0.01) (Table 8.2).

When the analysis was stratified by alcohol consumption, a more pronounced effect of rs1229984 on these cardiovascular traits was observed in individuals with higher alcohol consumption (P<0.05 for most comparisons) (Figure 8.1). In contrast, the effect of rs1229984 on these traits did not differ systematically according to exploratory subgroup

Table 8.2: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) with cardiovascular biomarkers irrespective of alcohol intake.

Trait (units)	Studies, Individuals	Mean difference (95%CI)	P-value	I^2, % (95%CI)
Blood pressure				
SBP (mmHg)	48, 227559	-0.88 (-1.19, -0.56)	4.1 x 10-8	26(0,48)
Anthropometric				
BMI (kg/m^2)	51, 232570	-0.17 (-0.24, -0.10)	3.4 x 10^{-6}	52(33,65)
Waist circumference (cm)	42, 140923	-0.34 (-0.58, -0.10)	6.2 x 10^{-3}	41(14,59)
Inflammation				
ln IL-6 (pg/ml)	17, 30950	-5.15 (-7.82,-2.40)	2.90 x 10^{-4}	33(0,62)
ln CRP (mg/l)	42, 124498	-3.40 (-5.68,-1.05)	4.60 x 10^{-3}	1(0,36)
Lipids				
Non-HDL-C (mmol/l)	46, 202794	-0.03 (-0.05, -0.01)	5.1 x 10^{-3}	25(0,48)
ln TG (mmol/l)	46, 205824	1.61 (0.66,2.57)	8.90 x 10^{-4}	36(8,55)
HDL-C (mmol/l)	46, 203440	-0.004 (-0.012, 0.003)	0.259	54(37,67)

Footnote: For log(e) transformed traits, the percentage difference in the geometric mean is reported rather than the mean difference

analyses by laboratory procedures or major study characteristics (P>0.05 for 51 of 57 comparisons; Figure C.5, C.6, C.7, C.8 and C.11). Although I observed that rs1229984 A-allele carriers had higher triglyceride levels (1.6%; 95%CI, 0.7 to 2.6), this effect was not modified by alcohol categories (P value for heterogeneity=0.69, Figure 8.1).

There were no overall differences in HDL-C (-0.004 mmol/l; 95%CI, -0.012 to 0.003) between rs1229984 A-allele carriers and non-carriers. However, an association between rs1229984 A-allele carriage with HDL-C was observed in the highest category of alcohol consumption (0.03 mmol/l; 95%CI, 0.01 to 0.05), but in the opposite direction to that expected from observational findings. In subgroup analysis by laboratory procedures and major study characteristics, rs1229984 A-allele carriers from Northern Europe had lower levels of HDL-C (-0.04 mmol/l; 95%CI, -0.05 to -0.02). Since this geographical specificity could reflect residual population stratification in samples outside Northern Europe, we adjusted for principal components in a subset of individuals not from North Europe. The unadjusted model for the association between rs1229984 A-allele and HDL-C (0.02 difference in SD; 95%CI, -0.02 to 0.06) did not differ from the model adjusted for population structure (0.01 difference in SD; 95%CI, -0.03, 0.05) (Figure 8.3). Similar null results were observed for Apo-A1 (Table 8.3).

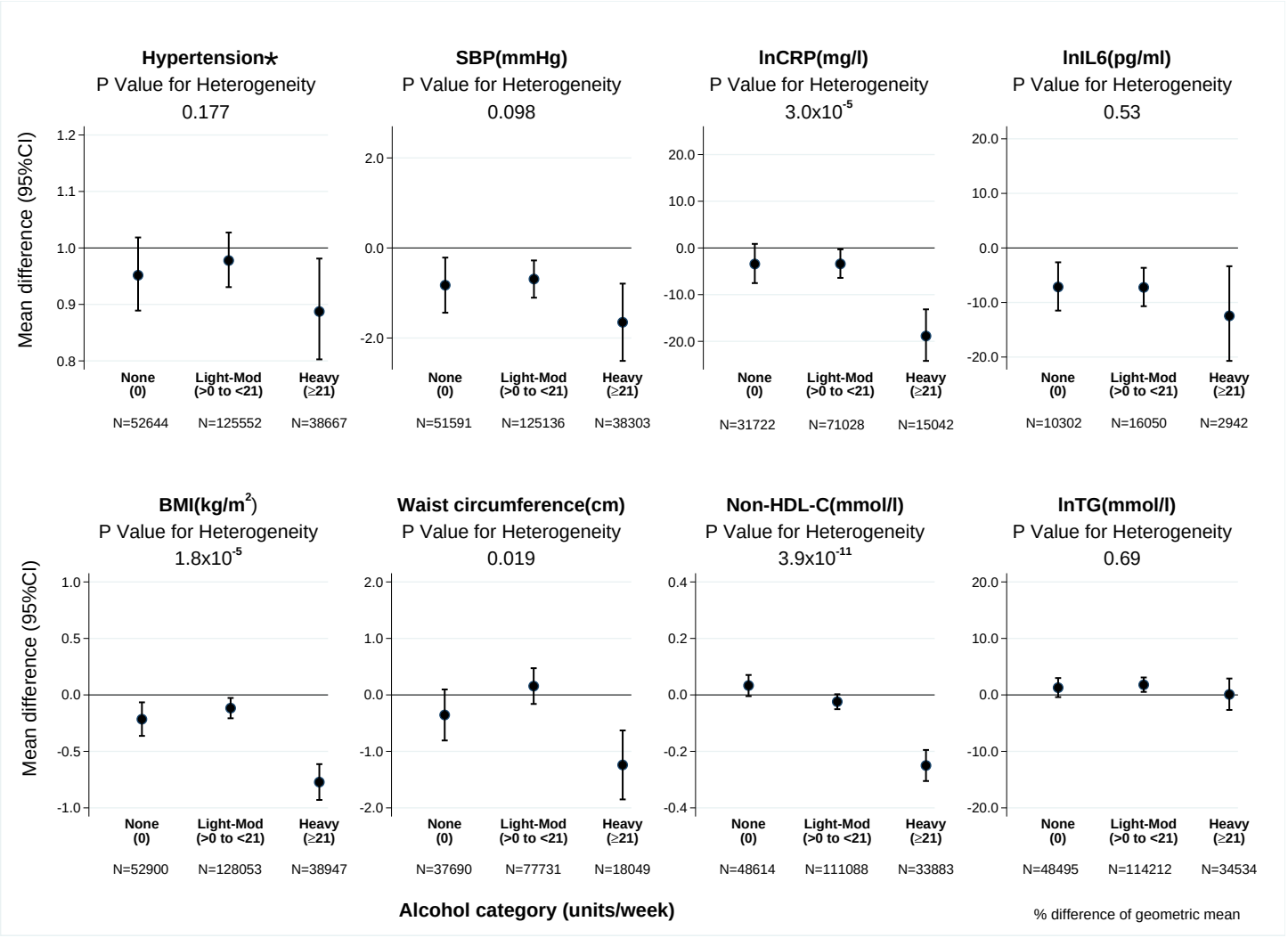


Figure 8.1: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular disease biomarkers showing association on crude analysis, stratified by alcohol intake.

Footnote: for log transformed traits, the percentage difference in the geometric mean is reported rather than the mean difference. To convert from British to US units, divide by 1.75 (i.e. 1 British unit=10 ml or 7.9g ethanol = 0.57 US units). The test for trend was conducted by meta-regression. ∗ For hypertension the plotted values represent the odds ratio (95%CI)

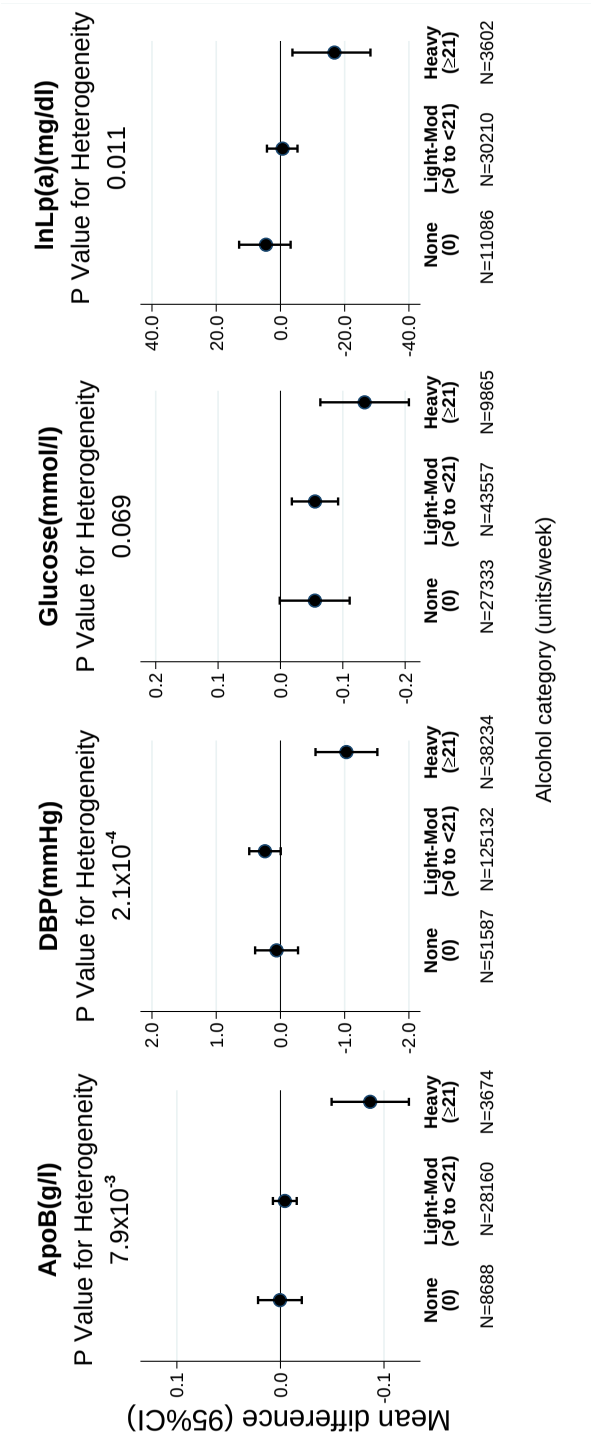


Figure 8.2: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular disease biomarkers not showing association on crude analysis, stratified by alcohol intake.

Footnote: for log transformed traits, the percentage difference in the geometric mean is reported rather than the mean difference. To convert from British to US units, divide by 1.75 (i.e. 1 British unit=10 ml or 7.9g ethanol = 0.57 US units). The test for trend was conducted by meta-regression.

Rs1229984 A-allele carriage was not associated with carotid intima medial thickness, electrocardiographic measures of left ventricular hypertrophy, fibrinogen, von Willebrand factor, factor VII, fasting glucose, BNP or lipoprotein(a) overall (Table 8.3). For these traits, similar null results were observed when stratified for alcohol consumption or by other exploratory sub-groups (P>0.05 for 47 of 48 comparisons, Figures C.10, C.12, and C.13), with the exception of fasting glucose and lipoprotein(a), where the strength of association was more pronounced in heavy drinkers compared to other alcohol categories (P values for heterogeneity 0.07 and 0.01, respectively, Figures 8.2).

8.2.4 Association of *ADH1B* rs1229984 and lifestyle factors

Carriage of the rs1229984 A-allele was not associated with physical activity. Rs1229984 A-allele carriers did have higher odds of ever smoking (OR 1.06; 95%CI, 1.02 to 1.09). However, the association with ever smoking was in the opposite direction to that seen in observational analysis and no association was observed for other quantitative measures of tobacco exposure such as cigarettes per day, pack years or cotinine levels (Figure 8.4). Rs1229984 A-allele carriers showed higher total years in education (0.04 SD; 95%CI, 0.01 to 0.08). No differential effect of *ADH1B* rs1229984 on any of the life-style factors was identified on stratifying by alcohol intake (making it unlikely that stratifying by alcohol introduced bias) or by other exploratory sub-groups (P>0.05 for all comparisons; Figures 8.4, C.14, C.15 and C.16).

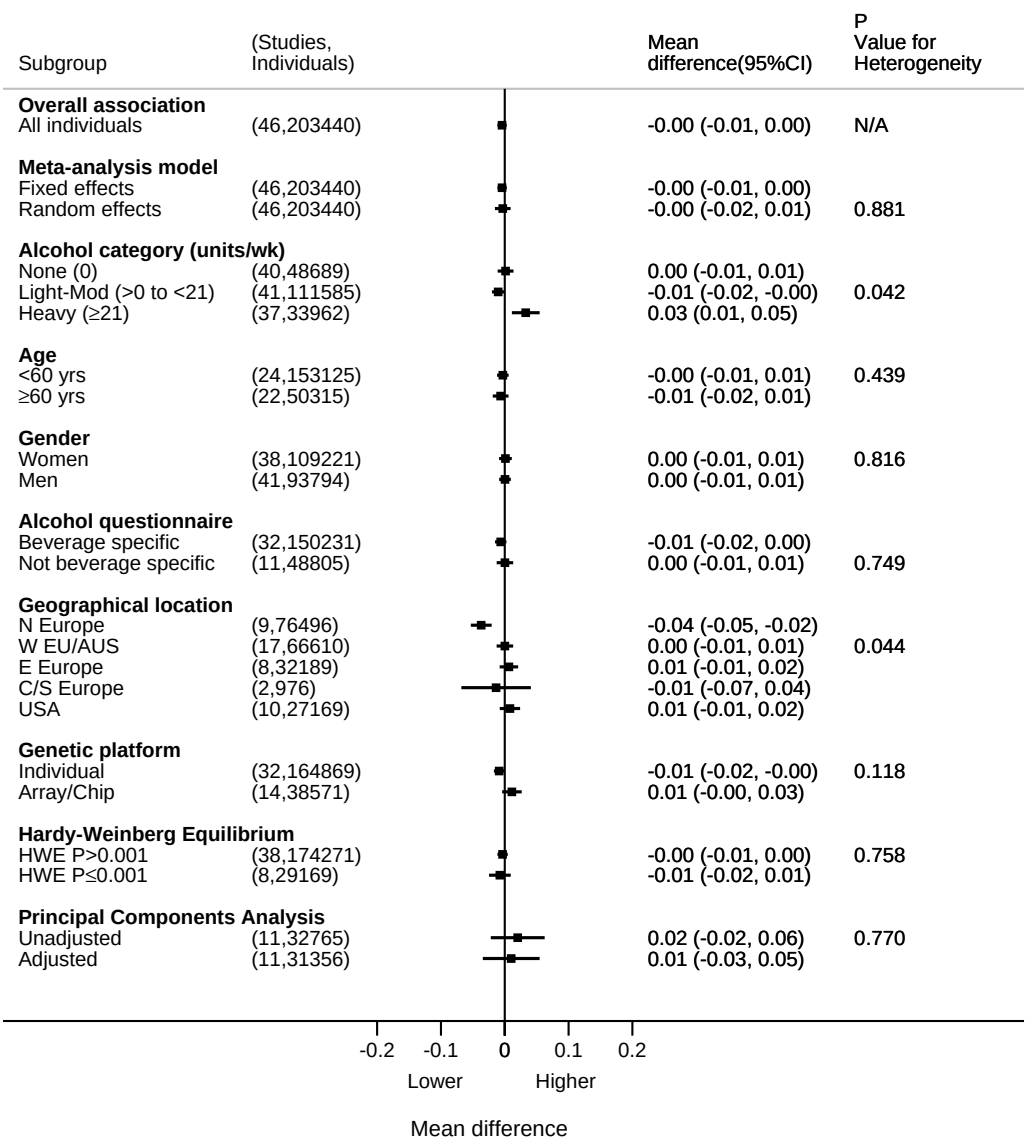


Figure 8.3: Subgroup and sensitivity analysis of the meta-analysis pooled estimate of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) with HDL-C.

Footnote: The mean difference values for the principal components analysis subgroup represent the beta coefficient for one SD difference in HDL by rs1229984 with and without adjustment for PCA. P value for heterogeneity for alcohol represents a test for trend, whereas for other subgroups, it represents a test for heterogeneity (see Methods).

Table 8.3: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular biomarkers, irrespective of alcohol intake, limited to traits not showing association at a nominal P value equal to 0.05.

Trait (units)	Studies, Individuals	Mean difference (95%CI)	P-value	I^2, % (95%CI)
DBP (mmHg)	48/227550	-0.08 (-0.25, 0.10)	0.401	23(0,47)
ln Fibrinogen (g/l)	27/136647	-0.07 (-0.60,0.47)	0.808	41(7,63)
Glucose (fasting, mmol/L)	35/88388	-0.03 (-0.06, 0.00)	0.064	36(4,58)
ln Lipoprotein(a) (mg/dl)	14, 46287	0.75 (-3.11,4.76)	0.709	38(0,67)
Apolipoprotein B (g/)	13, 41865	-0.00 (-0.01, 0.01)	0.357	51(8,74)
Apolipoprotein A-1 (g/l)	12, 39544	0.00 (-0.01, 0.02)	0.57	48(0,73)
ln C-IMT (mm)	18, 30897	-0.09 (-0.95,0.77)	0.839	10(0,47)
Von willebrand factor(IU/dl)	11, 25450	0.07 (-2.43, 2.58)	0.956	41(0,71)
Sokolow-Lyon (mm)∗	4, 21460	22.03 (-19.86, 63.93)	0.303	0(0,84)
QRS Voltage Sum (mm)∗	4, 21445	90.62 (-106.19, 287.42)	0.367	47(0,82)
QRS Voltage Product (mm)∗	4, 21440	8.13 (-17.07, 33.32)	0.527	61(0,87)
Cornell Product (V.S)∗	4, 21408	-2.08 (-5.73, 1.57)	0.264	51(0,84)
ln BNP (ng/l)	8, 20794	-3.02 (-9.79,4.28)	0.407	54(0,79)
Factor VII (U/ml)	10, 20509	0.30 (-1.34, 1.93)	0.721	0(0,62)

Footnote: for log transformed traits, the percentage difference in the geometric mean is reported rather than the mean difference. ∗ denotes indices of left ventricular hypertrophy measured via electrocardiogram. DBP: diastolic blood pressure; BNP: brain-derived natriuretic peptide

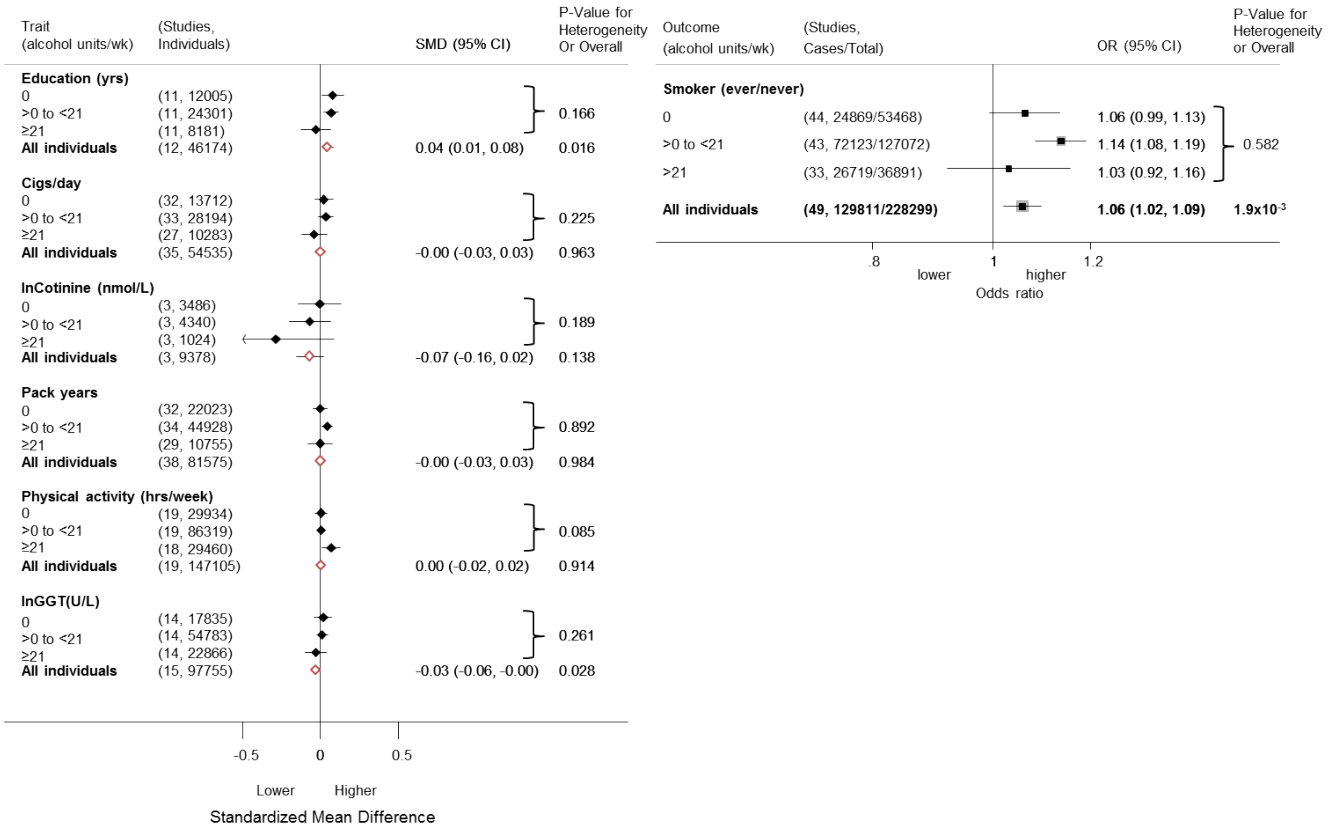


Figure 8.4: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits and liver enzyme overall and stratified by alcohol intake

Footnote: Alcohol units are British; to convert from British to US units, divide by 1.75 (i.e. 1 British unit=10 ml or 7.9g ethanol = 0.57 US units). P value for heterogeneity represents a test for trend (see Methods). The "All individuals" estimates (colored red) also include studies without measures of alcohol.

8.2.5 Association of *ADH1B* rs1229984 and coronary heart disease

Rs1229984 A-allele carriage, associated with a 17.5% reduction in volume of alcohol consumption, showed reduced odds of CHD (20,259 events; OR 0.90; 95%CI, 0.84 to 0.96). When the analysis was restricted to non-drinkers the association was null (OR 0.99; 95%CI, 0.88 to 1.10) whilst among drinkers (>0 units/week alcohol), carriers of the rs1229984 A-allele had reduced odds of CHD (OR 0.85; 95%CI, 0.78 to 0.93). Further subdivision of the drinkers category into >0 to <7, ≥ 7 to <21 and ≥ 21 units/week showed the same protective effect of the variant across all alcohol categories (P value for heterogeneity=0.71; Figure 8.5).

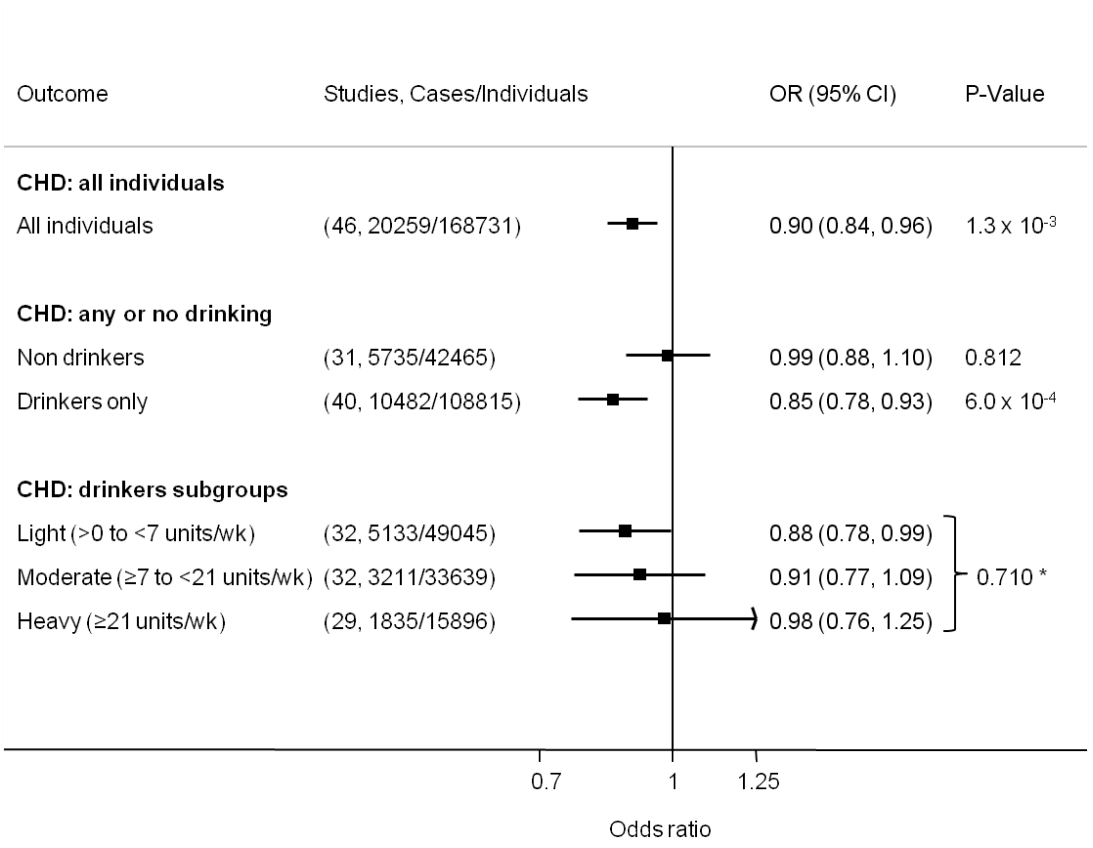


Figure 8.5: Meta-analysis pooled estimate of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and CHD stratified by categories of alcohol intake.
Footnote: Alcohol units are British; to convert from British to US units, divide by 1.75 (i.e. 1 British unit=10 ml or 7.9g ethanol = 0.57 US units).

8.2.6 Association between *ADH1B* rs1229984 with stroke (combined subtypes and ischaemic-only) and diabetes

Analysis of the rs1229984 A-allele did not show association with a combined stroke outcome (including ischaemic and hemorrhagic sub-type) (10,164 cases; OR 0.98; 95%CI,

0.90 to 1.07). When I limited the analysis to studies that reported only ischemic stroke, rs1229984 A-allele carriers had a lower odds of ischaemic stroke (7 studies, 4339 events, OR 0.83; 95%CI, 0.72 to 0.95) than non-carriers (Figure 8.7).

There was no association between the rs1229984 A-allele with type 2 diabetes (14,549 cases; OR 1.02; 95%CI, 0.95 to 1.09; Figure 8.6).

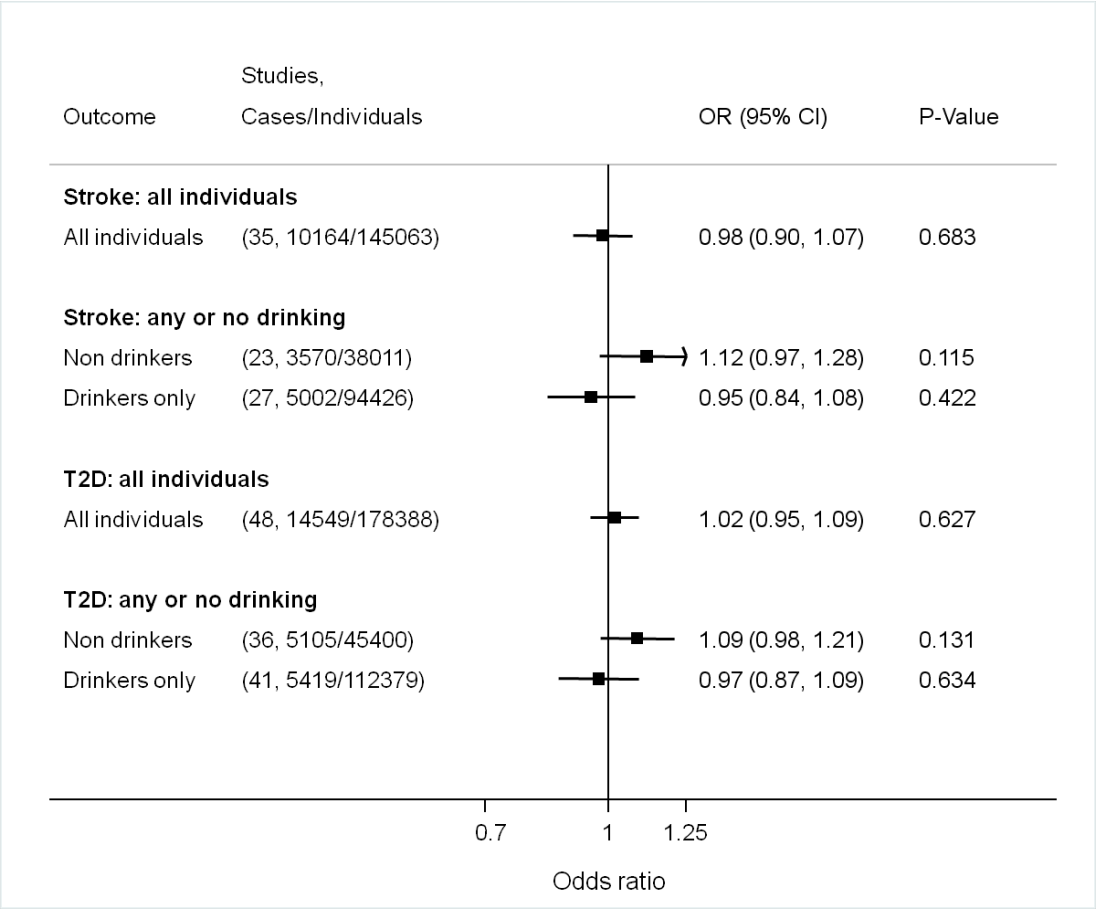


Figure 8.6: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and stroke and type 2 diabetes overall, and stratified by alcohol intake.

Footnote: to convert from British to US units, divide by 1.75 (i.e. 1 British unit=10 ml or 7.9g ethanol = 0.57 US units). The "All individuals" estimate includes studies without measures of alcohol. The test for trend was conducted using meta-regression. Box shading is proportional to the number of cases in each stratum.

8.2.7 Stability of summary estimates to choice of meta-analysis model

Despite the high I^2 values for some traits,[a] random effect estimates for associations of *ADH1B* rs1229984 with all outcomes were similar to those from fixed effect models (Figures C.2 to C.18), meaning that presence of important statistical heterogeneity was unlikely.[66]

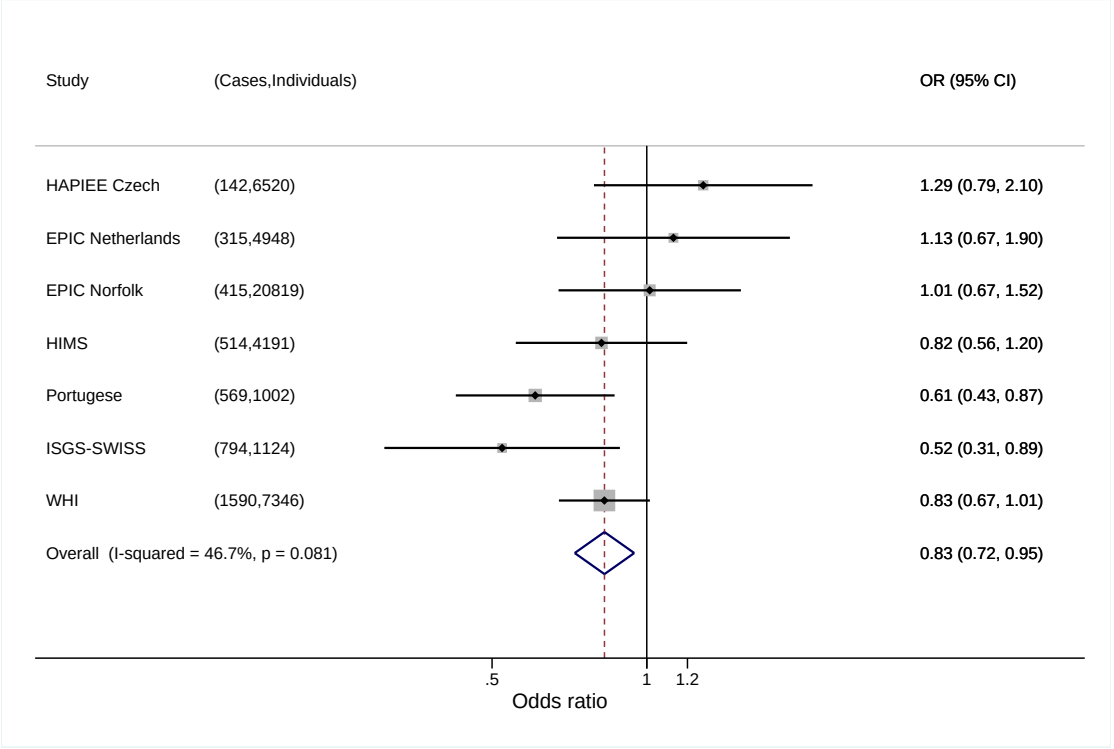


Figure 8.7: Meta-analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and ischemic stroke

8.2.8 Stability of summary estimates to adjustment for population structure

I repeated the analyses for continuous and binary traits with adjustment for principal component traits (Tables 8.1, 8.2 and 8.3), to investigate for evidence of residual population stratification. I did not identify evidence of attenuation of the effect estimates between rs1229984 A-allele and each of the traits after adjusting for principal components traits (Figures 8.9 and 8.8).

[a] A high I^2, indicative of presence of between-study heterogeneity, can mean that summary effect estimates from random and fixed effects models may differ, as the random effects model will assign relatively more weight to small studies compared to the fixed effects model

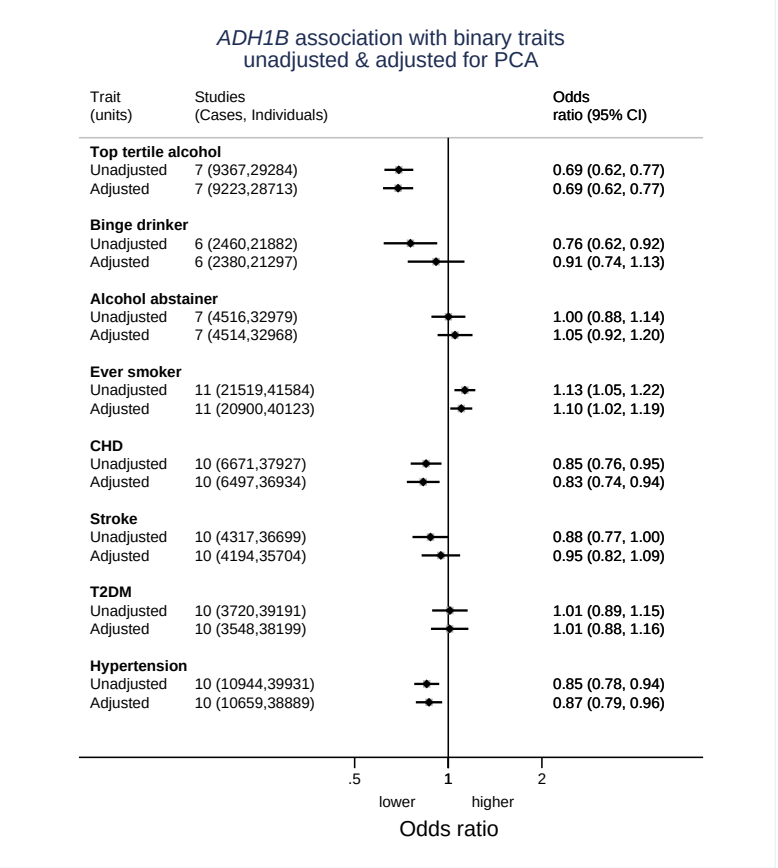


Figure 8.8: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and binary traits adjusted for principal components analysis

8.2.9 *In silico* analysis of potential for confounding between *ADH1B* rs1229984 with other SNPs identified from prior GWAs

The *ADH1B* rs1229984 gene variant was not in linkage disequilibrium with previously-reported GWAs loci for any cardiovascular trait (Table C.10).

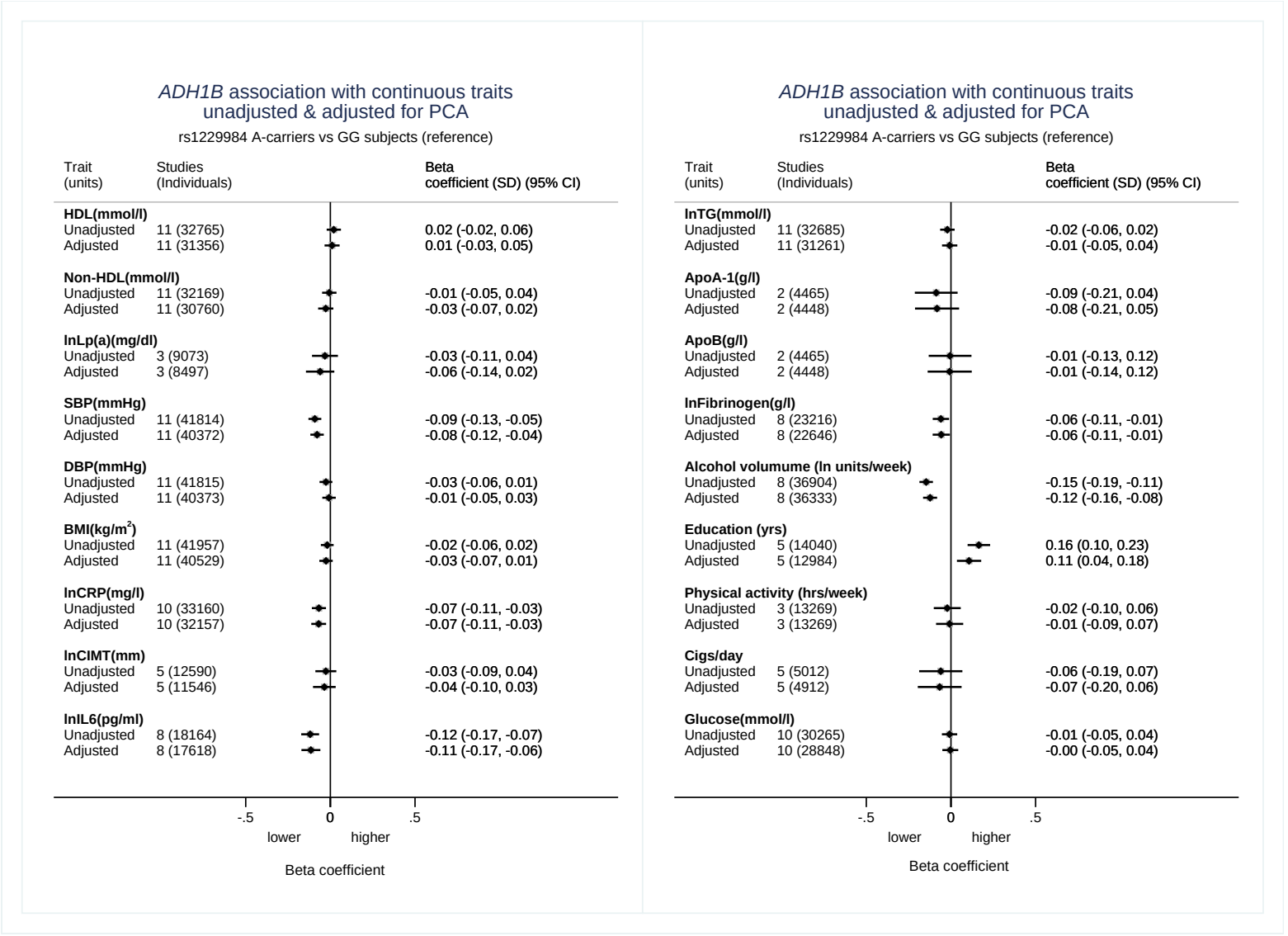


Figure 8.9: Meta-analysis pooled estimates of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and continuous traits adjusted for principal components analysis.

Footnote: Beta coefficient (SD) represents the beta coefficient per standard deviation of each trait

8.2.10 Causal analysis of alcohol on cardiovascular traits and events

The F-statistic for the first-stage gene-alcohol association in the pooled individual participant dataset was 244, indicating that *ADH1B* rs1229984 was an adequate instrument for instrumental variable analysis.[84]

With data on the *ADH1B*-alcohol association and *ADH1B*-traits, the next step was to "triangulate" the true (causal) association between alcohol with those traits (Figure 8.10).

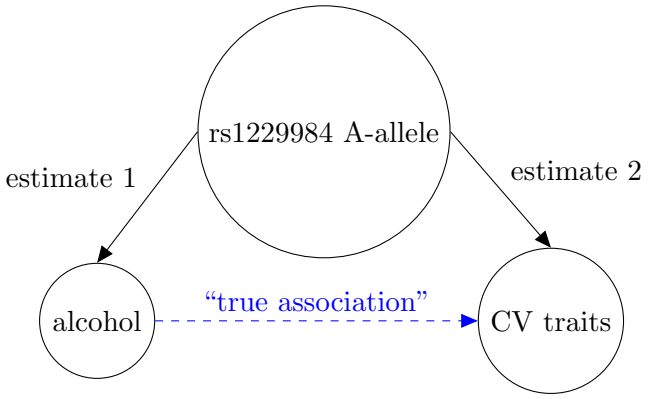


Figure 8.10: Triangulation of the causal association between alcohol and cardiovascular traits.
The true estimate is obtained by dividing estimate 2 by estimate 1, whilst incorporating the uncertainty in both estimates.

The standard instrumental variable approach assumes a linear (or log-linear) relationship between the intermediate phenotype and outcome of interest.[84] This may be problematic when investigating alcohol, as much of the observational associations between alcohol with cardiovascular risk factors and outcomes are J-shaped.[542]

The instrumental variable estimates for alcohol consumption (log units/wk) derived from a linear instrumental variable analysis using 51 studies that included 232,570 participants are reported in Table 8.4. For each log unit increase in alcohol volume (units/week), SBP increased by 4.37 mmHg (95%CI: 2.75, 6.00), non-HDL-C increased by 0.15 mmol/L (95%CI: 0.04, 0.25). A one log unit increase in weekly unit of alcohol increased BMI by 0.81 kg/m^2 (95%CI: 0.44, 1.17) and waist circumference by 1.59 cm (95%CI: 0.34, 2.84). IL-6 increased by 0.27% (95%CI, 0.12, 0.42) and TG reduced by -0.08% (95%CI, -0.13, -0.04). Estimates were comparable using a random effects model, except for triglycerides, where the estimates derived from random effects model yielded a weaker association (Table 8.4).

Table 8.4: Meta-analysis pooled instrumental variable estimates of the effect of a one log unit increase in alcohol volume consumption (units/wk) on selected cardiovascular traits

Trait (units) or Outcome	Studies	Individuals/ Cases	Meta-analysis model	IV mean difference	Lower 95%CI	Upper 95%CI	P-value
SBP (mmHg)	48	227559	Fixed	4.37	2.75	6.00	1.3E-07
	48	227559	Random	4.55	2.46	6.65	2.04E-05
Non-HDL-C (mmol/l)	46	202794	Fixed	0.15	0.04	0.25	5.4E-03
	46	202794	Random	0.17	0.04	0.30	0.01
BMI (mg/kg^2)	51	232570	Fixed	0.81	0.44	1.17	1.69E-05
	51	232570	Random	0.95	0.36	1.53	1.6E-03
Waist circumference (cm)	42	140923	Fixed	1.59	0.34	2.84	0.01
	42	140923	Random	1.74	-0.07	3.56	0.06
Ln CRP (% difference)	42	124498	Fixed	0.18	0.06	0.31	0.00364
	42	124498	Random	0.19	0.06	0.31	4.5E-03
HDL-C (mmol/l)	46	203440	Fixed	0.02	-0.01	0.06	0.23
	46	203440	Random	0.02	-0.04	0.08	0.55
Ln IL-6 (% difference)	17	30950	Fixed	0.27	0.12	0.42	3.6E-4
	17	30950	Random	0.27	0.06	0.48	0.01
Ln-TG (% difference)	46	205824	Fixed	-0.08	-0.13	-0.04	7.1E-04
	46	205824	Random	-0.05	-0.12	0.01	0.12

Footnotes: The IV estimate was obtained using two regressions: (i) a pooled *ADH1B*-mean difference in log units/wk of alcohol (where 1 log unit = 2.7 units/wk, 2 log units = 7.4 units/wk; 3 log units = 20.1 units/wk, 4 log units = 54.6 units/wk), obtained from a meta-analysis of *ADH1B* rs1229984 (A-allele carriers vs. GG homozygotes) on log weekly alcohol units using all studies; (ii) individual study-level *ADH1B*-trait estimates of individual studies. The IV analysis divides the individual study-level *ADH1B*-trait association by the pooled *ADH1B*-log units/wk alcohol, incorporating errors in both estimates.

8.3 Discussion

The purpose of this Mendelian randomization analysis was to use a gene variant (*ADH1B* rs1229984) to investigate the relationship between alcohol and cardiovascular disease. I helped to assemble a large international collaboration of over 250,000 participants. This was in order to yield a sample size so that a Mendelian randomization analysis would be sufficiently powered to have meaningful conclusions.

In Chapter 7, I identified that the datasets amassed were valid and consistent with published observational associations between alcohol and multiple cardiovascular traits.

8.3.1 Validation of *ADH1B* rs1229984 as a proxy for alcohol intake

In this Chapter, I first showed that *ADH1B* rs1229984 A-allele carriage was suitable for use as a genetic instrument for alcohol intake. I showed that rs1229984 A-allele carriers had reduced exposure to all alcohol-related phenotypes, including those that were self-reported (e.g. volume, abstention and binge drinking) and objective (using the liver enzyme GGT as a marker of heavy alcohol consumption). Compared to non-carriers, carriers of the rs1229984 A-allele consumed lower volumes of alcohol, were less likely to be in the top third of drinkers, less likely to report drinking patterns consistent with binge drinking and more likely to be abstainers. A-allele carriers also had lower levels of circulating GGT. This confirmed the validity of this genetic variant as a proxy for various measures of alcohol consumption.

8.3.2 Genetic association between rs1229984 and cardiovascular traits

I then investigated the association of the gene variant with cardiovascular traits and events. Rs1229984 A-allele carriers had a reduced odds of hypertension and lower levels of systolic blood pressure, CRP, IL-6, BMI, waist circumference, and non-HDL-C than non-carriers. Thus, individuals that consumed less alcohol by virtue of their *ADH1B* genotype had lower levels of traits that are established or emerging risk factors for cardiovascular disease (obesity[543], blood pressure[544] and inflammation[83]).

I investigated these genetic associations within subgroups of alcohol consumption (0, $\geq$0 to $<$21 and $\geq$21 units/week) to investigate whether the effect of the gene variant on these traits differed according to alcohol consumption, indicating a dose-response relationship. This also allowed investigation of whether the genetic associations were consistent for those consuming low amounts of alcohol (the part of the dose-response curve associating with a protective cardiovascular profile on observational association). A close inspection

of the genetic associations according to alcohol categories revealed differential effects with null or substantially reduced associations in non-drinkers, as expected under the assumption that the effect of this allele is only explained by exposure to alcohol, and a more pronounced association in heavy drinkers when compared to light-to-moderate drinkers.

8.3.3 Expected effect of alcohol on risk of CHD

From the reported J-shape in observational studies, I would expect that for drinkers below the nadir (12-25 units/wk), a 17.5% reduction in alcohol consumption, corresponding to carriage of the rs1229984 A-allele, would lead to a small increase in the risk of CHD, while for those with alcohol consumption above the nadir, a similar reduction in alcohol consumption would lead to a decrease in CHD risk (Figure 6.6). In contrast, the findings presented in this Chapter reveal that individuals with a genetic predisposition to consume less alcohol had lower, not higher, odds of developing CHD, and this association was constant across all alcohol categories including those located below the minimum risk point estimated from the observational association (>0 to 21 units/wk, Figure 8.5). This argues against presence of a J-shape relationship and suggests that there is no safe amount or cardioprotective threshold for alcohol consumption. As with cardiovascular traits, the association of the rs1229984 A-allele with CHD was null for non-drinkers.

8.3.4 Potential explanations for the absence of association of *ADH1B* rs1229984 with HDL-C and other traits

The lack of association of the *ADH1B* rs1229984 A-allele with HDL-C, coagulation markers, diabetes and combined subtypes of stroke was unexpected. Failure to detect an association with HDL-C could have arisen due to lack of power; however rs1229984 was associated with traits (e.g. CRP, IL-6) for which alcohol volume, on observational analysis, had a less powerful effect and where sample size for genetic analysis was several times smaller than for HDL-C (Figure 7.1). Sub-group analyses also suggest it was unlikely that laboratory technique or type of alcohol questionnaire could be explanations. The association of HDL-C with rs1229984 A-allele carriers restricted to Northern European studies, concordant with observational studies, suggested that population stratification may be an explanation for the lack of association in non-Northern European studies. However, adjustment for population structure using principal components analysis in these studies also identified no association between rs1229984 A-allele and HDL-C. It is possible that confounding by linkage disequilibrium with an HDL-C raising allele could

obscure an association with rs1229984, but the *in-silico* analysis found no support for this hypothesis (Table C.10). It is also possible that other substrates metabolized by the *ADH1B* enzyme may dilute the HDL-C association,[545] but it is unlikely that they would account for the differential effect of the rs1229984 variant by alcohol categories observed with several traits. More broadly, the cardioprotective effect ascribed to HDL-C in light-to-moderate drinkers has recently been brought into question by the halting of a randomized trial of a drug designed to increased HDL-C,[409] while another Mendelian randomization study found no association of an HDL-C gene score with cardiovascular events.[408]

The genetic analysis did not show rs1229984 A-allele carriers to have lower levels of coagulation markers. These results appeared more robust for fibrinogen, but for factor VII and von Willebrand factor the reduced sample size limited my ability to refute a small effect. Although I observed an overall null association of the rs1229984 A-allele with type 2 diabetes and glucose, a stratified analysis by alcohol consumption showed that among heavy drinkers, carriers of the rs1229984 A-allele had lower levels of glucose.

The lower number of stroke events is an important limitation, as well as the fact that the combined stroke outcome could have obscured some differential associations of alcohol by pathological subtype of stroke (ischemic and hemorrhagic) and aetiological subtype of ischemic stroke (large artery, small artery, cardio-embolic, other) as suggested by recent overviews from observational studies.[546] In this regard, a subset of studies that reported ischemic stroke did show an association between the rs1229984 A-allele with a reduced risk of ischaemic stroke, however this requires further replication.

8.3.5 Association of *ADH1B* with lifestyle factors

One of the advantages of a Mendelian randomization study is to reduce the bias in observational studies. In contrast to the observational analyses that showed associations of alcohol with physical activity and different measures of exposure to smoking (as I reported in Chapter 7), *ADH1B* rs1229984 was not associated with physical activity, or any of the more precise measures of exposure to smoking (i.e. cigarettes/day, pack years or cotinine). However, an association was observed with the binary ever/never smoking trait but this was in the opposite direction to the association with CHD and therefore unlikely to explain it. There was also some evidence for a difference in years of education, however given the diversity of measures used across studies and the small magnitude of effect, this association requires further investigation and replication.

8.3.6 Instrumental variable analysis

The instrumental variable estimates reported in Table 8.4 allowed the quantification of the causal effects for each log unit increase in alcohol, however this technique is limited as it assumes a linear relationship between alcohol and each of the traits. Given that many of the observational associations show a curvilinear relationship (as shown in Chapter 7), these instrumental variable estimates must be interpreted with caution.

The limitation of this linear instrumental variable approach highlights the need to develop novel techniques to enable the investigation of non-linear instrumental variable analysis.

8.3.7 Contrast with other Mendelian randomization studies

The use of *ADH1B* genotypes to investigate the effect of alcohol on CHD represents a natural extension of previous Mendelian randomization studies that used genetic variation in alcohol metabolizing enzymes to investigate the association between alcohol and various traits. For example, studies have shown associations between *ALDH2* rs671 with blood pressure, BMI, non-HDL-C, HDL-C, coronary artery disease,[507, 515, 525, 547] liver disease and various cancers.[511] However, in Europeans the *ALDH2* rs671 SNP is monomorphic[548] and cannot be used for Mendelian randomization.

8.3.8 Contrast with existing data and implications for interpretation

The data I present in this chapter run contrary to a large body of observational evidence that suggests low dose alcohol is cardioprotective.[395] However, these are the first reported data between alcohol and CVD to be *free from confounding*. The lack of consistency with observational data is not entirely surprising or novel to this scenario. Just as with vitamin C, vitamin A and hormone replacement therapies (and indeed $sPLA_2$-IIA as reported in Chapters 3 to 5), observational associations are prone to error from confounding and bias, and to reveal the "true" association, a randomized design is needed.

One question is how these findings will be interpreted by the Scientific and lay community. When communicating such findings, it is important to emphasize that the purpose of this experiment was not to identify a genetic test that could predict individuals that consume more (or less) alcohol or that might be at increased risk of CHD on exposure to alcohol, but rather the motivation was as a substitute for a randomized trial. Critics

may argue that it is not reasonable to inform public health policy on data from a single SNP. However, interpreted in the light of the context (i.e. an estimate of the association between alcohol with CHD that is free from confounding), policy-makers and the general public should be encouraged to read these findings as providing evidence against the cardioprotective hypothesis of low-dose alcohol.

8.4 Conclusions

In this Chapter, I presented findings from use of a genetic variant to make unbiased estimates of the nature of the association between alcohol and CVD traits and events. These data are the first of their kind to be synthesized to generate large-scale, reliable evidence. The data do not support a protective effect of alcohol at any dose of alcohol consumption. Therefore, there is no evidence to support drinking alcohol to reduce cardiovascular risk. In light of these findings and the recognized harm that alcohol causes, governmental policy should be revised to advise individuals to minimize alcohol consumption.

Chapter 9

Discussion and Conclusions

9.1 Introduction

The purpose of this thesis was to use practical examples to illustrate how human genetic variation can be used for translation into improvements in human health. This encompassed a number of complementary applications, including: (i) use of genetic variants to stratify drug prescribing in an attempt to target treatments to individuals that are predicted to respond more favourably to drug treatment ("stratified medicine"); (ii) use of a genetic variant as a proxy for a drug target to predict the results of a phase III clinical trial, and; (iii) use of a genetic variant to answer a question on causal relevance of an environmental exposure that would be challenging to obtain from a conventional randomized clinical trial.

9.2 Reaping the rewards of genomic research

Although initial genetic studies have focussed on genomic discovery yielding many thousands of GWAs articles that have identified genetic loci using an agnostic (hypothesis-free) approach, the challenge now lies in how to translate these discoveries into improvements in clinical health.

9.2.1 Pharmacogenetics

Pharmacogenetics is one such translational approach that promises to revolutionize the way treatments are prescribed to patients, by harnessing genetic variants to create subgroups of the population. This research methodology is thought to be one of the most

'proximal to use', as a detailed understanding of how the genetic variant influences treatment response is not a pre-requisite for clinical use. I.e. once a gene that associates with drug response is identified, assuming the required metrics are satisfied (magnitude of effect, cost, clinical utility), it can be rapidly adopted in the clinical setting. One such example are HLA SNPs associated with adverse drug reactions to carbamazepine,[549] recommended to guide treatment prescribing by the UK MHRA.[550]

This is in contrast to loci discovered that associate with biomarkers or disease traits for which the function is not known. In these examples, further studies are often required to elucidate the mechanism by which the genetic variant influences disease risk prior to being able to advance along the translational pipeline from discovery to clinical use.

My chapter on pharmacogenetics investigated the potential use of a genetic variant (*CYP2C19*) to identify individuals who are likely to respond to a widely-prescribed drug, clopidogrel, for the prevention of CVD. Despite what was hoped to be the most promising cardiovascular pharmacogenetic biomarker available[169], the evidence base for the use of *CYP2C19* was heavily undermined by small-study bias and lack of evidence of a gene-by-drug treatment interaction. This undermines the validity of the use of this genetic variant for identifying those who are likely to benefit from clopidogrel. As such, my conclusion is that the available evidence does not support the use of *CYP2C19* in clopidogrel prescribing.

These findings add further doubt to a field of research that is hampered by small study bias, lack of focus on particular gene-drug pairs; and, publication bias.[110] In general, genomic information is more likely to be informative when the association between a gene variant with an outcome has an effect estimate of large magnitude. This arises from well-established doctrines of screening - i.e. to have a sufficient detection rate to be clinically useful, the odds ratios need to be high - typically values in excess of 10.[551] Values beneath this mean that the overlap in treatment response between the genetic groups may render the genetic variant to be of insufficient discrimination to be clinically useful.

Estimates of large magnitude in pharmacogenetics studies have tended to arise from studies that have investigated adverse drug reactions.[118] Therefore, to increase the possibility of yielding pharmacogenetic variants that can be quickly translated into clinical use, investigators ought to focus on adverse drug reactions for which genetic variants

may have qualitative interactions (differences in kind) with treatment response, in contrast to intended drug reactions (which are more likely to yield quantitative, differences in magnitude, drug interactions). Furthermore, use of a hypothesis-free approach (such as a GWAs) using datasets enriched for adverse drug reactions (e.g. case control collections) will not constrain the Scientist to pursuing a limited number of SNPs in candidate genes thought to play a biological role in drug metabolism, but will instead open the possibility for new discoveries that can have immediate translational benefit.

For intended drug effects, more focus should be placed on the underlying epidemiological approach, and in particular harnessing the power of randomized trials, where a pharmacogenetic study is akin to a factorial design trial (as both drug and gene are randomly allocated, the former by man, the latter by Nature).

9.2.2 Mendelian randomization

A second example of post-GWAs translation that is gaining increasing acknowledgement as a "natural randomized trial" is the use of genetic variants to make causal inference on disease. These Mendelian randomization experiments exploit the random allocation of genetic variants for instrumental variable analysis. The two contrasting examples I present in this thesis ($sPLA_2$ and alcohol) illustrate the flexibility of Mendelian randomization applications: one for an *endo*genous trait ($sPLA_2$) encoded by a specific gene, and the other an *exo*genous trait (alcohol) that is influenced by multiple behavioural and social factors.

9.2.3 Mendelian randomization for drug target validation

In Chapters 4 and 5, I investigated the role of $sPLA_2$-IIA as a therapeutic target for CHD. The translational implications of the work I report go hand-in-hand with the results of a phase III clinical trial. Both the Mendelian randomization study and the phase III clinical trial provide bias-free evidence that $sPLA_2$-IIA is very unlikely to represent a valid drug target for CHD prevention.

Although $sPLA_2$-IIA may be considered a "niche" enzyme, the findings of my work have wider implications for the drug development pipe-line, which in its present form costs billions of dollars, has a high attrition rate and is said to be unsustainable (Figure 9.1).[552, 553] Relatively inexpensive Mendelian randomization projects could be

conducted as part of the drug discovery pipeline, which could revolutionize the process by which drug targets are prioritized for clinical trials.

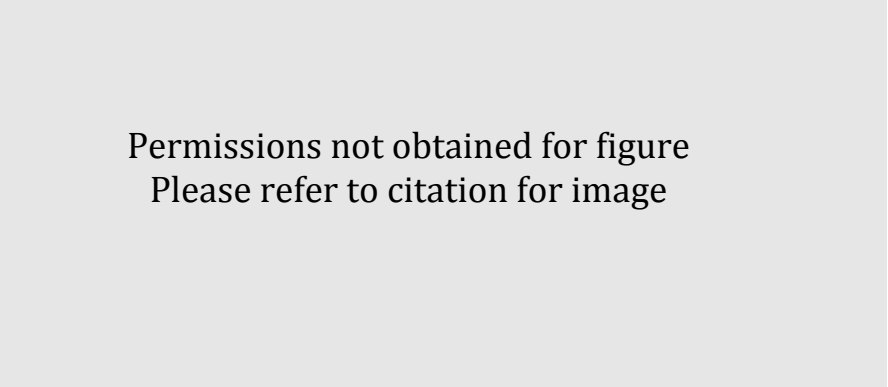


Figure 9.1: Probability of success to market at different stages of drug development pipeline
Reproduced from Arrowsmith[552]

The Mendelian randomization investigation into $sPLA_2$-IIA shows the critical importance of which drug targets to prioritize for phase III clinical trials. This is highlighted by the example of the drug company, Anthera, which conducted the phase III clinical trial VISTA-16. At the time that VISTA-16 was terminated due to lack of efficacy,[381, 382] the stock price of the shares fell by over 50% (Figure 9.2). This led to the company having to lay off half of its staff,[554] illustrating the devastating effect of having a phase III clinical trial fail. And, notably, it could have been averted had a Mendelian randomization study such as the one reported in this thesis been conducted prior to embarking upon the phase III trial. The findings that I present in this thesis would provide strong evidence against pursuing a phase III randomized trial for a drug that targeted $sPLA_2$-IIA, and would suggest that drug development focus on alternative biomarkers to target.

Finally, this study design complements previous work that validated the IL-6 receptor as a therapeutic target in CVD[83] (providing strong evidence for the re-purposing of an existing drug in clinical use for rheumatoid arthritis). These two examples ($sPLA_2$ and IL6R) provide contrasting examples of the use of Mendelian randomization study design to prioritize therapeutic targets.

9.3 Mendelian randomization for casual analysis of environmental exposures

In Chapters 7 and 8, I described the use of a genetic variant to investigate the role of alcohol in cardiovascular disease. This is an epidemiological question that has important

Figure 9.2: Stock price of Anthera Pharmaceuticals prior to and after announcement of VISTA-16 termination
The vertical arrow shows the time the VISTA-16 was terminated.[381] Stock prices derived from http://www.nasdaq.com

public health questions (as alcohol consumption is so widely prevalent within the population and worldwide) but also a question that would be otherwise difficult to answer through use of traditional means (such as a more conventional clinical trial). The next best option is to conduct a Mendelian randomization analysis using a gene variant that associates with alcohol intake, and this is the first-in-kind scaled analysis to address this important topic.

Using this approach, I found that a gene variant that acts as a proxy for several alcohol phenotypes yielded evidence to indicate that alcohol consumption showed increased risk of CHD at all levels of intake, making it unlikely that alcohol has cardioprotective properties. These findings are of critical importance given that the UK government has used observational data (suggesting a protective effect of alcohol on CVD at low volumes) to inform, and what's more relax, public guidance on safe drinking levels.

The Mendelian randomization project of alcohol is, to my knowledge, the largest Mendelian randomization project conducted to date and the one that has the greatest potential for public health translation. For this reason, the data I present in Chapters 7 and 8 can be considered not only the first deconfounded findings of their kind, but also the best evidence that is available. Importantly, they suggest that the hitherto reported cardioprotective effects of alcohol are most likely the result of bias/confounding. This would

place alcohol in the same domain as vitamin A, C and hormone replacement therapy: i.e. that observational evidence suggesting a beneficial effect was all overturned by randomized evidence which were contrary to the observations.

In the absence of clinical trials powered for cardiovascular events, the findings I present suggest that alcohol intake should be minimized for all individuals.

9.4 Future aims for Mendelian randomization

The next frontiers of Mendelian randomization are two-fold. First, using multiple SNPs in combination, and second, investigation of non-linear effects.

9.4.1 Multiple SNPs

In both the *PLA2G2A*-sPLA$_2$ and *ADH1B*-alcohol example, I used a single SNP for Mendelian randomization analysis. However, it is possible to combine multiple SNPs together to improve specificity for the index trait, particularly important when the trait is not a protein, such as a circulating lipid. Furthermore, as a genetic instrument consisting of multiple SNPs would be expected to explain a greater proportion of variance of the index trait (compared to a genetic instrument consisting of a single SNP), using multiple SNPs in combination also increases statistical power, meaning that smaller sample sizes may be required to show similar results.[82]

Working with colleagues at University of Warwick and LSHTM, I have developed a series of Stata scripts that conduct Mendelian randomization experiments combining multiple SNPs together. I am using these scripts to investigate the role of blood lipids and glycaemic traits in CVD, and these will form my next major research outputs.

9.4.1.1 Pushing the frontier: multiple SNPs to investigate putative epidemiological interactions

A natural extension of using allele scores may be to conduct factorial design studies with allele scores for two traits that are thought to interact with one another. An example of this could be to investigate the hypothesis of the 'metabolically healthy but obese' individual. The theory is that although obesity associates with an adverse

metabolic profile (such as increased fasting glucose, insulin and risk of type 2 diabetes), there may be a subgroup of individuals with obesity that do not suffer the typical metabolic sequelae, termed "metabolically-healthy obesity".[555] A recent hypothesis is that inflammation may mediate the risk between obesity and metabolic consequences, thus there may be an interaction between obesity and inflammation.[556]
One way to formally investigate whether this could be possible would be to conduct a factorial design analysis using allele scores for obesity and inflammation and investigate glycaemic traits and risk of type 2 diabetes as outcomes. If there is a true interaction, we should expect a differential risk of type 2 diabetes in individuals with alleles that predispose to obesity that harbor fewer genetic variants associated with inflammation to those that harbor more variants associated with inflammation. This type of 'blue-sky thinking' represents a genetic take on a traditional factorial design conducted in a randomized trial - but as with the conventional factorial trial, the randomized properties of the allele scores should benefit from the same alleviation from confounding.

9.4.2 Non-linear Mendelian randomization

Conventional instrumental variable analyses rely upon the assumption that the association between the biomarker of interest and trait/outcome is linear.[84] Sometimes, as in the example of $sPLA_2$ where I investigated the nature of the association between $sPLA_2$ and CVD, this assumption is valid. However, as evidenced in the example of alcohol, the best fit model may suggest that the underlying relationship is non-linear. In the latter circumstance, it may be desirable, rather than imposing a linear model for instrumental variable analysis, to conduct non-linear Mendelian randomization.

In the example of alcohol, we are interested in whether a causal association between alcohol and CVD could have a J-shaped relationship. Novel methodologies are under development using local average treatment effects (LATEs) that allow for the investigation of non-linear effects. This represents on-going work at the LSHTM/UCL Genetic Epidemiology Group.[557]

9.5 Conclusions

In this thesis, I have used genomic information to investigate the role of genes as pharmacogenetic markers, and I exploited the unique properties of genotype (according to Mendel's second law) to make causal inference on a potential drug target ($sPLA_2$-IIA mass) and a ubiquitous exposure of critical importance to public health policy (alcohol)

on risk of cardiovascular disease.

These serve as contrasting examples to highlight the potential for translational genomic research to make advances in public health. As Mendelian randomization techniques develop, they will undoubtedly become a routine part of the drug discovery pipeline. Furthermore, there will be other opportunities to investigate environmental exposures that, like alcohol, are challenging to answer via conventional means. Taken together, genetic epidemiology will undoubtedly play an important role in future Scientific research, as we strive to answer important questions on human disease aetiopathogenesis, with the ultimate goal of improving public health.

Appendix A

Supplemental Information for *CYP2C19* and clopidogrel response

A.1 Supplementary Methods

A.2 Search strategy for identifying studies in the systematic review

A.2.1 PubMed Search String

A.2.1.1 MeSH Search

(1) clopidogrel [Substance Name]; (2) 2-oxo-clopidogrel [Substance Name]; (3) clopidogrel carboxylic acid [Substance Name]; (4) clopidogrel resinate [Substance Name]; (5) platelet aggregation inhibitors [MeSH]; (6) 1 OR 2 OR 3 OR 4 OR 5; (7) "Cytochrome P-450 Enzyme System"[Mesh]; (8) "Cytochromes"[Mesh]; (9) "CYP2C9 protein, human" [Substance Name]; (10) ("P-Glycoprotein"[Mesh] OR "ABCB1 protein, human "[Substance Name] OR "ABCB1 protein, mouse "[Substance Name] OR "multidrug resistance protein 3 "[Substance Name]); (11) ("Cytochrome P-450 CYP1A2"[Mesh] OR "CYP1A2 protein, human "[Substance Name]); (12) "S-mephenytoin N-demethylase "[Substance Name]; (13) "CYP3A4 protein, human "[Substance Name]; (14) "CYP3A5 protein, human "[Substance Name]; (15) ("Cytochrome P-450 CYP3A"[Mesh] OR "CYP3A protein, human "[Substance Name]); (16) CYP2C19 protein, human [Substance Name]; (17)

"Esterases"[Mesh] ; (18) purinoceptor P2Y12 [Substance Name]; (19) ITGB3 protein, human [Substance Name]; (20)7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19; (21) Genotype[MeSH]; (22) "Genetic Association Studies"[Mesh]; (23) Genes[MeSH]; (24) Alleles[MeSH]; (25) Polymorphism, Genetic[MeSH]; (26) 21 OR 22 OR 23 OR 24 OR 25; (27) 6 AND 20 (28) 6 AND 26; (29) 27 OR 28

A.2.1.2 Text Word search

(1) clopidogrel; (2) 2-oxo-clopidogrel; (3) Plavix; (4) Clopilet; (5) Ceruvin; (6) Clavix; (7) Clopigrel; (8) Clasprin; (9) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8; (10) cytochrome*; (11) cytochrome-P450; (12) cytochrome P-450; (13) ABCB1*; (14) CYP1A2*; (15) CYP2B6*; (16) CYP3A4*; (17) CYP3A5*; (18) CYP2C19*; (19) CYP2C9*; (20) esterase*; (21) P2RY12*; (22) ITGB3*; (23) 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22; (24) genotype*; (25) gene; (26) allele*; (27) polymorphism*; (28) 23 OR 24 OR 25 OR 26; (29) 9 AND 23; (30) 9 AND 28; (31) 29 OR 30

A.2.2 EMBASE Search String

A.2.2.1 Expanded (exp) headings search

(1) clopidogrel/exp; (2) 'antiplatelet drug'/exp; (3) plavix/exp; (4) 1 OR 2 OR 3; (5) 'cytochrome'/exp; (6) 'p450'/exp; (7) 'cytochrome p450 2c9'/exp; (8) 'cytochrome p450 2c19'/exp; (9) 'multidrug resistance protein 3'/exp; (10) cytochrome p450 1a2'/exp ; (11) 'cytochrome p450 2b6'/exp; (12) 'cytochrome p450 3a4'/exp; (13) 'cytochrome p450 3a5'/exp; (14) 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13; (15) 'genotype phenotype correlation'/exp; (16) genotype'/exp; (17) 'association'/exp; (18) 'genes'/exp; (19) 'alleles'/exp; (20) 'polymorphism genetic'/exp; (21) 15 OR 16 OR 17 OR 18 OR 19 OR 20; (22) 4 AND 14 AND 21

A.2.2.2 Text word search

(1) clopidogrel$; (2) 2 oxo clopidogrel; (3) clopidogrel carboxylic acid; (4) clopidogrel resinate; (5) 'antiplatelet drug'; (6) plavix; (7) clopilet; (8) ceruvin; (9) clavix; (10) clavix; (11) clopigrel; (12) clasprin; (13) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12; (14) Cytochrome$; (15) Cytochrome P450; (16) 'cytochrome p450 2c9' OR CYP2C9; (17) 'multidrug resistance protein 3' OR MDR1 OR ABCB1 OR P-glycoprotein OR P glycoprotein; (18) 'cytochrome p450 1a2' OR CYP1A2; (19)

'cytochrome p450 2b6' OR CYP2B6; (20) 'cytochrome p450 3a4' OR CYP3A4; (21) cytochrome p450 3a5 OR CYP3A5; (22) cytochrome p450 2c19 OR CYP2C19; (23) esterase; (24) 'p2y12' OR p2ry12; (25) itgb3 gene' OR itgb3; (26) 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25; (27) genotype'; (28) 'association'; (29) 'genes'; (30) 'alleles'; (31) 'polymorphism genetic'; (32) 27 OR 28 OR 29 OR 30 OR 31; (33) 13 AND 26 AND 32

A.3 Supplementary Tables

Table A.1: Characteristics of the studies included in the systematic review: risk of bias

First Author, Ref/ Study Name (Year)	Comparator drug	PPI at baseline, %	Aspirin at baseline, %	Outcome ascertainment blinded to genotype	Genotype ascertained with blinding to outcome	Study authors received fees from pharmaceutical industry	Genotype call rate provided, (%)	Hardy-Weinberg Equilibrium, P value	Reporting of outcome ascertainment
Anderson *et al* [152]/ IHCS (2009)	NA	NR	NR	NR	NR	NR	No	0.0008	NA
Bouman *et al* [153]/ MAPCAT (2010)	NA	50.2	91.3	Yes	Yes	No	Yes (>95)	0.5336	clear
Campo *et al* [154]/ NA (2011)	NA	53	99	NR	NR	Yes	No	0.8134	clear
Collet *et al* [155]/ AFIJI (2009)	NA	32	97.3	Yes	NR	Yes	No	0.2432	clear
Giusti *et al* [156]/ RECLOSE (2009)	NA	94.8	100	Yes	NR	No	No	0.6218	clear
Harmsze *et al* [159]/ NA (2010)	NA	22.7	100	NR	NR	Yes	No	0.1571	clear
Harmsze *et al* [157]/ POPular (2011)	NA	19.9	100	NR	NR	Yes	NR	0.0637	clear
Jeong *et al* [158]/ACCEL (2011)	NA	1.5	100	NR	NR	Yes	NR	0.7544	NA
Komarov *et al* [160]/NA (2011)	NA	18	NR	NR	NR	NR	NR	0.8384	NA
Malek *et al* [138]/ NA (2008)	NA	NR	100	NR	NR	No	No	1	unclear

Continued on next page

Table A.1 – *Continued from previous page*

First Author, Ref/ Study Name (Year)	Comparator drug	PPI at baseline, %	Aspirin at baseline, %	Outcome ascertainment blinded to genotype	Genotype ascertained with blinding to outcome	Study authors received fees from pharmaceutical industry	Genotype call rate provided, (%)	Hardy-Weinberg Equilibrium, P value	Reporting of outcome ascertainment
Malek *et al* [161]/ NA (2010)	NA	NR	96.9	NR	NR	No	No	0.2273	clear
Oh *et al* [162]/ Sky Registry (2011)	NA	4.4	100	Yes	NR	No	No	NA	clear
Ono *et al* [163]/NA (2011)	NA	28.4	100	NR	NR	No	NR	0.6006	unclear
Sawada *et al* [165]/ NA (2010)	NA	50	100	NR	NR	NR	No	NA	clear
Shuldiner *et al* [140]/ Sinai Hospital of Baltimore Study (2009)	NA	NR	100	Yes	NR	Yes	Yes (98.7)	>0.05 ***	clear
Sibbing *et al* [168]/ ISAR-REACT, ISAR-REACT 2, ISAR-SMART 2, ISAR-SWEET (2009)	NA	NR	96.8	Yes	NR	Yes	No	0.3765	clear
Sibbing *et al* [166]/ NA (2010)	NA	18.1	75.8	Yes	Yes	Yes	No	NA	clear
Sibbing *et al* [167]/NA (2011)	NA	18.1	100	NR	Yes	Yes	100	0.3205	clear
Simon *et al* [169]/ FAST-MI (2009)	NA	72.7	98	Yes	NR	Yes	No	0.8049	unclear

Continued on next page

Table A.1 – *Continued from previous page*

First Author, Ref/ Study Name (Year)	Comparator drug	PPI at baseline, %	Aspirin at baseline, %	Outcome ascertainment blinded to genotype	Genotype ascertained with blinding to outcome	Study authors received fees from pharmaceutical industry	Genotype call rate provided, (%)	Hardy-Weinberg Equilibrium, P value	Reporting of outcome ascertainment
Tello-Montoliu *et al* [170]/NA (2011)	NA	NR	NR	NR	NR	NR	NA	NA	NA
Tiroch *et al* [171]/ NA (2010)	NA	NR	97.7	Yes	Yes	No	No	0.1868	clear
Trenk *et al* [172]/ EXCELSIOR (2008)	NA	NR	100	NR	NR	No	No	0.3015	clear
Worrall *et al* [174]/ NA (2009)	NA	NR	NR	NR	NR	NR	NA	NA	NA
Yamomoto *et al* [175]/NA (2011)	NA	25.8	100	NR	NR	No	NR	0.3755	unclear
Yan *et al* [176]/ NA (2011)	NA	22.3	98	Yes	NR	No	NR	0.389	unclear
Yuan *et al* [177]/ NA (2011)	NA	NR	NR	NR	NR	NR	NR	0.773	NA
Mega *et al* [146]/ TRITON-TIMI 38 (2009)	Prasugrel	NR	99	Yes	NR	Yes	No	NA	clear
Wallentin *et al* [173] / PLATO (2010)	Ticagrelor	41	96.5	Yes	NR	Yes	Yes (>98.8)	0.2356	clear
Bhatt *et al* [151]/ CHARISMA (2009)	Placebo	NR	99.7	Yes	NR	Yes	NA	0.5319	clear

Continued on next page

Table A.1 – *Continued from previous page*

First Author, Ref/ Study Name (Year)	Comparator drug	PPI at baseline, %	Aspirin at baseline, %	Outcome ascertainment blinded to genotype	Genotype ascertained with blinding to outcome	Study authors received fees from pharmaceutical industry	Genotype call rate provided, (%)	Hardy-Weinberg Equilibrium, P value	Reporting of outcome ascertainment
Mega *et al* [145]/ CLARITY-TIMI 28 (2008)	Placebo	NR	98.6	Yes	NR	NR	NA	NA	clear
Pare *et al* [164]/ ACTIVE-A (2010)	Placebo	NR	82.7$	Yes	NR	Yes	Yes (>98)	>0.05	clear$
Pare *et al* [164]/ CURE (2010)	Placebo	NR	66.1	Yes	NR	Yes	Yes (>98)	>0.05	clear

Table A.2: Components of the composite primary cardiovascular outcomes reported by studies contributing to the meta-analysis.

Outcome	**Subtype**	**Proportion (%) of all participants contributing to treatment-only analysis (n=31,076)**		
		Not measured	Measured but not reported in composite CVD outcome	Measured and reported in composite CVD outcome
Mortality	All-cause	19.18	49.95	30.88
	Cardiovascular	42.55	0	57.45
Myocardial infarction	Not specified	33.64	17.81	48.55
	Nonfatal	51.69	17.42	30.89
	ST-elevation	89.52	2.48	8
	Non-ST-elevation	92	0	8
ACS	Hospitalisation for ACS	74.09	23.59	2.32
	Unstable angina	99.35	0	0.65
Stroke	Not specified	50.96	13.66	35.38
	Nonfatal	69.09	14.06	16.85
	Ischaemic	56.74	38.3	4.97
Stent thrombosis	Not specified	61.25	28.65	10.1
	Definite	58.99	31.79	9.22
	Probable	75.87	20.68	3.45
	Sub-acute	99.66	0	0.34
Other	Urgent revascularization	59.68	20	20.32

Table A.3: Outcomes reported individually by studies contributing to the meta-analysis.

Outcome	**Subtype**	**Proportion (%) of all treatment-only participants (n=31,076)**			
		Not measured	Measured but not reported individually	Measured but reported only as part of composite	Measured and reported individually
Mortality	All-cause	17.68	47.42	12.86	22.04
	Cardiovascular	40.22	7.81	36.26	15.71
Myocardial infarction	Not specified	54.34	9.67	22.92	13.07
	Nonfatal	48.7	11.84	27.07	12.39
	ST-elevation	89.52	2.48	0	8
	Non-ST-elevation	92	0	0	8
ACS	Hospitalisation for ACS	79.4	10.15	10.46	0
	Unstable angina	99.35	0	0.65	0
Stroke	Not specified	63.52	13.66	19.83	2.99
	Nonfatal	70.59	7.81	16.85	4.75
	Ischaemic	56.74	30.3	3.92	9.04
Stent thrombosis	Not specified	54.73	16.51	5.76	23
	Definite	54.23	0	11.35	34.41
	Probable	71.11	15.78	10.62	2.48
	Sub-acute	99.66	0	0	0.34
Bleeding	All	66.94	14.63	0	18.44
	Minor	51.28	30.28	5.72	12.72
	Major	41.61	14.23	5.72	38.44
Other	Urgent revascularization	54.85	27.81	9.27	8.06

Table A.4: Genome-wide associations of polymorhpisms in CYP450 enzymes with traits

Gene(s)	Disease/Trait	OR or Beta coefficient [95%CI or SE]	P value(s)	Reference (PMID)
CYP1A1	Caffeine consumption	0.12 [0.08-0.16] mg/day; 0.31 [0.17-0.44] cups per day	5 x 10-14	21490707, 21357676
CYP1A1	Diastolic blood pressure	0.43 [0.35-0.51] mm Hg	1 x 10-23	19430483
CYP1A2	Caffeine consumption	0.08 [0.06-0.10] mg/day; 0.31 [0.17-0.44] cups per day	3 x 10-7; 5 x 10-14	21490707, 21357676
CYP1A2	Diastolic blood pressure	0.43 [0.35-0.51] mm Hg	1 x 10-23	19430483
CYP2A6	Smoking behaviour	0.33 [0.22-0.44]; 0.39 [0.27-0.51] cigs/day	1 x 10-8; 2 x 10-12	20418890, 20418888
CYP2B6	Smoking behaviour	0.2 [0.12-0.28] cigs/day	6 x 10-6	204818888
CYP2C8, CYP2C9, CYP2C19	Clopidogrel response	NR	2 x 10-13	19706858
CYP2C18	Acenocoumarol maintenance dose	NR	8 x 10-12	19578179
CYP2R1	Vitamin D levels/insufficiency	0.25 [0.15-0.35] unit	3 x 10-17	20541252, 20418485
CYP3A43	Serum dehydroepiandrosterone sulphate levels	0.11 [0.07-0.15] umol/	2 x 10-11	21533175
CYP4F2	Alpha-tocopherol (vitamin E)	0.03 (0.01) mg/L	1.4 x 10-8	21729881
CYP17A1	Coronary heart disease	1.12 [1.08-1.16]	1 x 10-9	21378990
CYP17A1	Parkinson's disease	1.25	7 x 10-8	19915575
CYP17A1	Systolic blood pressure	1.05 [0.74-1.36] mm Hg ; 1.16 [0.92-1.40] mm Hg	1 x 10-10; 7 x 10-24	19430479, 19430483
CYP19A1	Alzheimer's disease CSF protein	NR	2 x 10-9	20932310
CYP19A1	Height	0.06 [0.04-0.07] cm	7 x 10-7	20189936
CYP20A1	Height	1.05 [0.62-1.48] cm	2 x 10-6	20966902
CYP27B1	Multiple sclerosis	1.23	5 x 10-11	19525955
CYP27C1	Self-related health	0.03	2 x 10-6	20707712

Table A.5: Details of the studies included in the analysis of *CYP2C19* genotype and platelet response

First Author (ref)	Study participants at baseline	No.	Sex (% female)	Age (mean, SD)	Clopido-grel dose (mg/-day)	Platelet Aggregometer	Units of platelet assay	ADP dose (μmol/L)	Time from clopidogrel to platelet measure
Giusti [182]	ACS admitted to CCU	1419	27	69 (11)	600	4-channel (APACT 4, Labor Biochemical Tech)	MPA	(2), 10	24hr after loading
Trenk [172]	Non-ACS CAD or risk factors for CAD	797	22	66 (10)	600	4-channel FACSCalibur (Becton Dickinson)	RPA	5	After loading
Frere [181]	non-STEMI ACS	601	24	64 (12)	600	PAP4 Aggregomenter (Biodata Corp)	ADP-Ag	10	12hr after loading
Sibbing [167]	CAD and planned PCI	1524	22.8	67.2 (10.7)	600	Multiplate analyzer (Dynabyte)	AU$x$min	6.4	$\geq$2hr after loading

Table A.6: Association of *CYP2C19* ⋆2-⋆8 vs. ⋆1 or ⋆17 on risk of clinical events in treatment-only analysis using random-effects meta-analysis.

Outcome	RR (95%CI)
All-cause mortality	1.56 (0.92, 2.64)
Myocardial infarction fatal/nonfatal	1.39 (1.10, 1.74)
Myocardial infarction nonfatal	1.45 (1.03, 2.03)
Stent thrombosis	1.88 (1.46, 2.41)
Stroke fatal/nonfatal	2.69 (0.55, 13.29)
Bleeding all	0.84 (0.75, 0.94)
Bleeding - severe	1.13 (0.82, 1.55)

Table A.7: Analysis of *CYP2C19* genotype on composite cardiovascular end-points and major bleeding in randomized trials using random-effects meta-analysis.

Outcome	Stratum	RR (95% CI) of clopidogrel vs. placebo	P value (Z test)
CVD composite	Original RCT	0.83 (0.74, 0.93)	0.73
	Genetic substudy	0.79 (0.62, 1.01)	
	CYP2C19 ⋆2 or ⋆3	0.84 (0.54, 1.31)	0.77
	CYP2C19 ⋆1 or ⋆17	0.78 (0.66, 0.92)	
Major bleeding	Original RCT	1.41 (1.25, 1.59)	0.89
	Genetic substudy	1.44 (1.18, 1.75)	
	CYP2C19 ⋆2 or ⋆3	1.99 (1.31, 3.02)	0.07
	CYP2C19 ⋆1 or ⋆17	1.28 (1.02, 1.61)	

A.4 Supplementary Figures

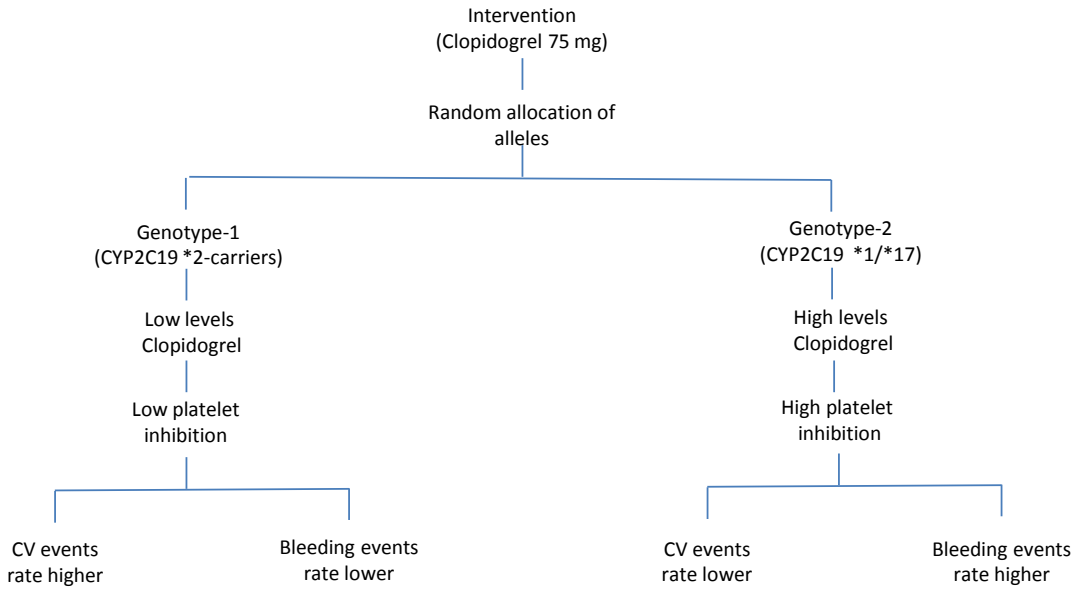


Figure A.1: Schemata for random allocation of *CYP2C19* alleles akin to the random allocation of an intervention in a RCT

Permissions not obtained for figure
Please refer to citation for image

Figure A.2: Schemata for metabolism and activation of clopidogrel by *CYP2C19*. Reproduced from ten Berg and Deneer.[219]

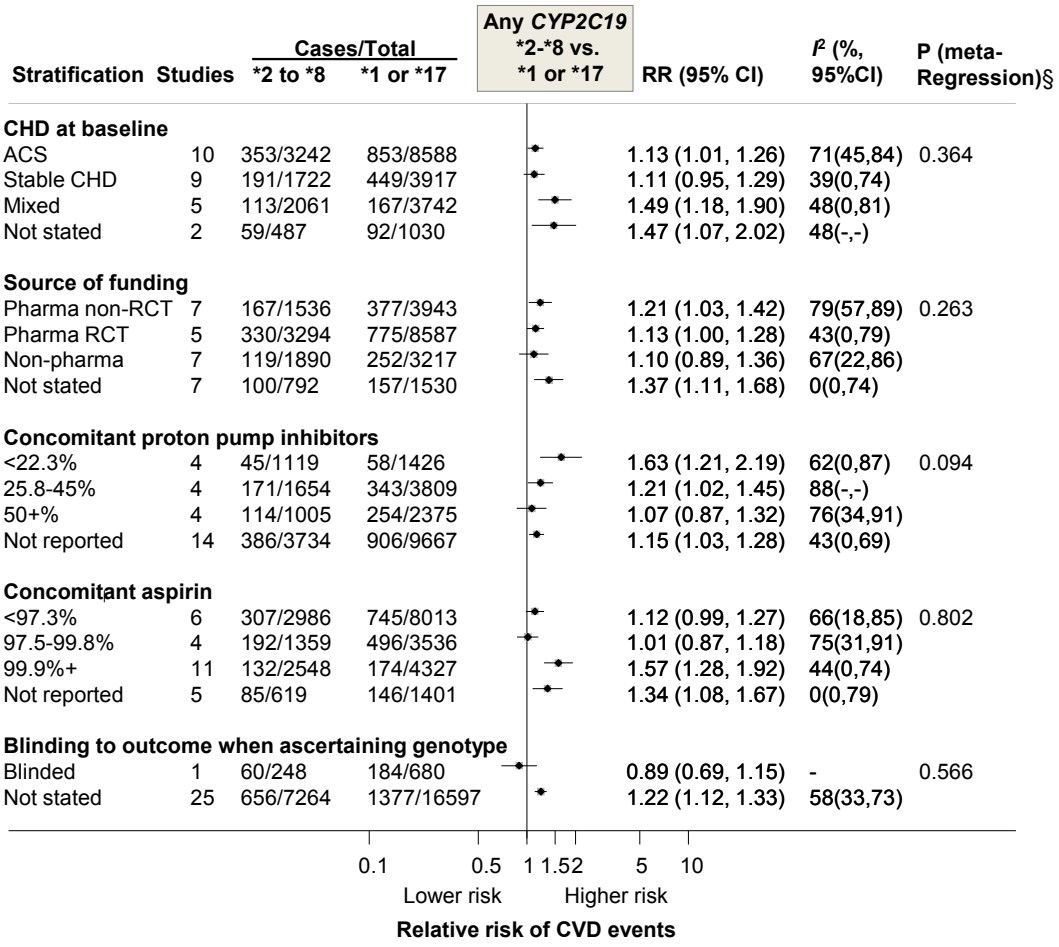


Figure A.3: Subgroup analysis of association between *CYP2C19* and CVD in "treatment-only" analysis.

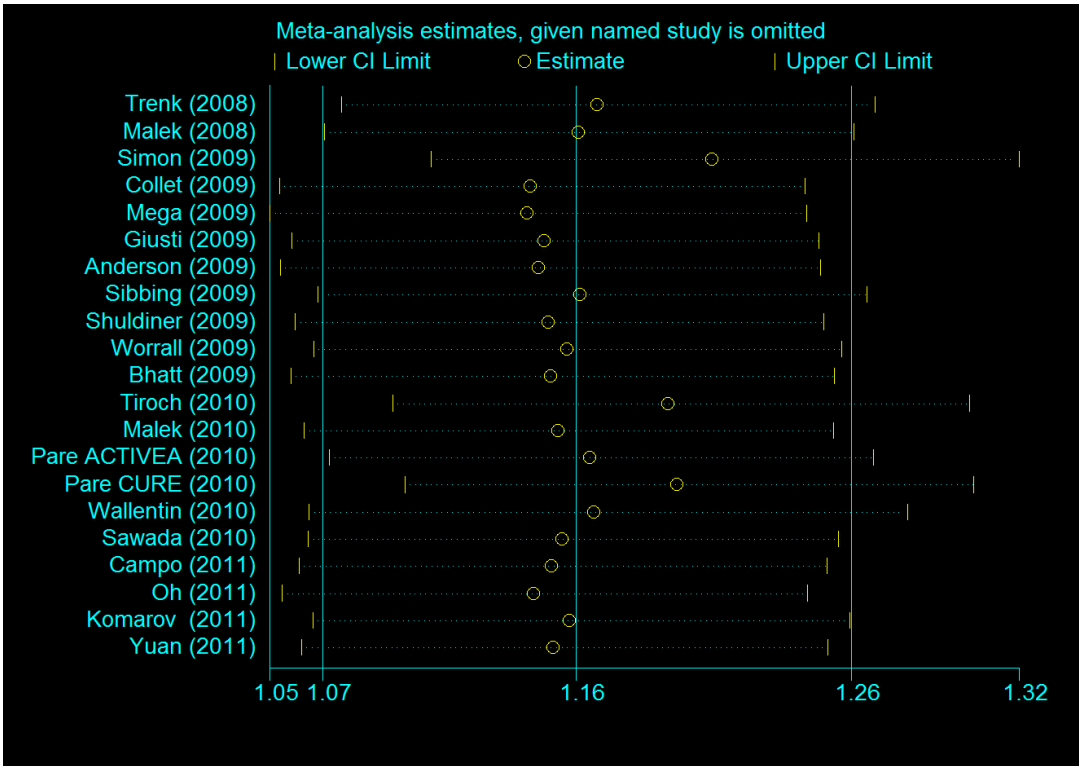


Figure A.4: Influence of removing one study at a time on the meta-analysis summary estimate. Meta-analysis of the risk of CVD events in individuals with any loss-of-function CYP2C19 alleles (*2 to *8) compared to normal/increased (*1 or *17) alleles.
Analysis only permits inclusion of studies that reported counts (of cases/total) per genotype, therefore the summary estimate of 1.16 differs slightly from the summary of 1.18 reported in Figure 2.6, which also includes studies that only report summary estimates.

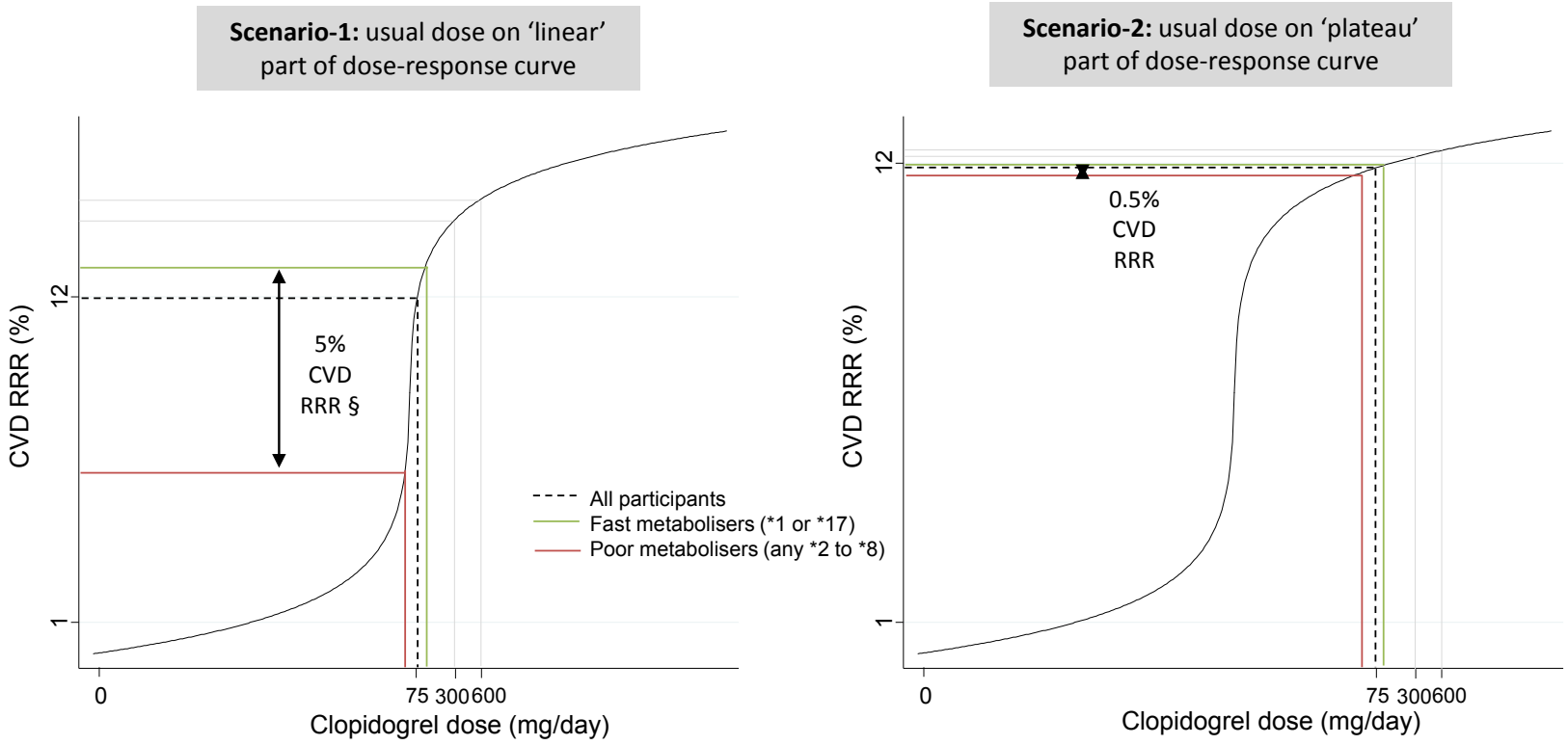


Figure A.5: Potential scenarios for more versus less clopidogrel.

In Scenario 1, 75mg/day clopidogrel lies on the linear part of the dose-response curve, therefore for a given change in drug exposure (e.g. as instrumented by CYP2C19), the relative risk reduction (RRR) for CVD will be considerable (5%). Scenario 2 illustrates the situation in which 75mg/day clopidogrel lies on the plateau: here the same proportional change in clopidogrel exposure conferred by *CYP2C19* will yield a smaller change in CVD RRR.

Appendix B

Supplemental Information for $sPLA_2$ and CVD

B.1 Definition of myocardial infarction and stroke in the collaborating studies

B.1.1 General Population Studies

BHF-FHS MI: Direct assessment of hospital records or confirmation by a general practitioner (GP) of a documented MI

BRHS MI: Events were identified from GP records and confirmed with the GP using WHO criteria. Stroke: Events were identified from GP records and confirmed with GP (acute disturbance of cerebral function of presumed vascular origin lasting 24 hours).

BWHHS MI/stroke: Events were obtained from self-report, GP records and confirmed with the GP using WHO criteria. Fatal events were defined using the following ICD-10 codes: ICD10: I20-I25 and I60-I65.

CCHS MI: ICD8: 410; ICD10: I21-I22; **Stroke**: ICD8: 432-434, ICD10: I63

CYPRUS MI: based on clinical history and electrocardiogram (ECG) findings (which were centrally reported by a cardiologist and vascular internist); **Stroke**: Hospital records and self-report

EAS MI: AHA criteria or death certification recording ICD-10 codes: I20-I25. **Stroke**: Symptom onset $\leq$48 hours previously and lasting $\geq$24 hours, CT evidence of cerebral infarction or haemorrhage, or hospital discharge diagnosis or death certification recording ICD10: I60-I65.

EPIC-Netherlands MI: ICD-9:410; ICD-10: I21, I22; **Stroke**: ICD-9:430-434, 436; ICD-10: I60-I66

EPIC-Norfolk MI: ICD9 codes 410-414

IMPROVE MI/stroke: Medical records and death certificates

NPHSII MI: ICD-9: 410; **Stroke**: ICD-9: 430-436

PREVEND MI: Cardiac events were reviewed by a clinical event committee and divided into ST-segment elevation myocardial infarctions (STEMI) or non-ST-elevation acute coronary syndromes (ACS). ST-segment elevation MI was defined as chest pain and ST elevation >1 mm in at least two contiguous leads. Non-ST-elevation acute coronary syndrome was defined as chest pain with positive cardiac markers (troponin or creatinine kinase) and/or dynamic ST-segment changes (ICD-9 410, 411); **Fatal MI/stroke**: based on ICD-10 codes: I01-99

PROCARDIS MI: documentation of two or more of (a) typical ischaemic chest pain, pulmonary oedema, syncope or shock; (b) development of pathological Q-waves and/or appearance or disappearance of localized ST elevation followed by T-wave inversion in two or more standard electrocardiograph leads; (c) increase in concentration of serum enzymes consistent with MI (e.g. creatine kinase more than twice the upper limit of normal). **Symptomatic ACS**: documentation of hospitalization for one of the following indications: (a) unstable angina diagnosed by typical ischemic chest pain at rest associated with reversible ST-depression in two or more standard electrocardiograph leads; (b) thrombolysis for suspected MI (as indicated by localized ST-elevation in two or more standard electrocardiograph leads) even without later development of T-wave inversion, Q-waves, or a significant enzyme rise; or (c) emergency revascularization (i.e. during same admission) following presentation with typical ischemic chest pain at rest.

PROSPER MI/stroke: participants were monitored every 3 months for clinical events

Rotterdam Information was obtained from general practitioners and discharge reports/letters from medical specialists. Two research physicians independently coded all reported **MI** and **stroke** events according to ICD-10 and a medical expert reviewed all events. Fatal events were defined as deaths related until 28 days after MI or stroke using ICD-10 codes: I20-I25, I46, R96 and I50.

TPT For both **MI** and **Stroke**, diagnosis was by WHO criteria, and verified by an independent reviewer

Whitehall II **MI/stroke** events were obtained from ICD-9 codes 390.0458.9 and ICD-10 codes I00I99.

B.1.2 Acute Coronary Syndrome studies

CURE **MI**: recurrent MI was defined by the presence of at least two of the following: ischemic chest pain; elevation of serum levels of cardiac markers or enzymes (troponin, creatine kinase, creatine kinase MB isoenzyme, or other cardiac enzymes) to at least twice the upper limit of normal reference range or three times the upper limit of normal within 48 hours after percutaneous coronary intervention (or to a level 20% higher than the previous value if the level had already been elevated because of an early myocardial infarction); and electrocardiographic changes compatible with infarction. **Stroke** was defined as a new focal neurologic deficit of vascular origin lasting more than 24 hours. **Death** from cardiovascular causes was defined as any death for which there was no clearly documented nonvascular cause.

FAST-MI **MI**: Recurrent MI was defined as recurrent symptoms with a new rise in cardiac markers. Follow-up data were collected through contacts with the attending physicians, patients, or family. Vital status of missing participants was assessed from the registries of the patients birthplaces.

GENDEMIP **Fatal MI/stroke**: mortality data were obtained from death certificates (ICD-10 codes I00-I99) by the Institute of Health Information and Statistics of the Czech Republic, which includes from the date of admission (2006-2009) to November 2011.

GRACE Scotland/France **MI**: STEMI diagnosis was based on new ST-segment elevation >1 mm in any location, or if a new left-bundle-branch block was identified on ECG, with at least one positive cardiac biochemical marker of necrosis raised above the diagnostic threshold for infarction. Non-STEMI was diagnosed if the marker of necrosis was raised without ST-segment elevation on index or a subsequent ECG. Deaths were recorded during the index period (06 days) and subsequently (>6 days).

IHCS **MI/stroke**: information was obtained from physician report and hospital readmission for MI or stroke using ICD-9 codes.

KAROLA **MI/stroke**: Information was obtained from the primary care physician using a standardized questionnaire. For fatal events, information was obtained

from the death certificate, and the main cause of death was coded according to the ICD-9. Secondary cardiovascular events were defined either as CVD as the main cause of death (as stated in the death certificate), nonfatal myocardial infarction (MI), or ischemic stroke.

MERLIN-TIMI36 MI: criteria adapted from American College of Cardiology (including symptoms suggestive of ischemia/infarction associated with ECG, cardiac biomarker or pathological evidence of infarction. Outcomes were adjudicated by a clinical events committee.

MIRACL MI: cardiac enzyme and ECG data using NOVOCODE classification.[558]

PROVE-IT TIMI 22 MI: Myocardial infarction was defined by the presence of symptoms suggestive of ischemia or infarction, with either electrocardiographic evidence (new Q waves in two or more leads) or cardiac-marker evidence of infarction, according to the standard TIMI and American College of Cardiology definition.

B.1.3 Other studies

AMC-PAS MI/CAD: MI, surgical or percutaneous revascularisation, coronary angiograph with $\geq$70% stenosis in a major epicardial artery

GENDER Restenosis: defined as clinical restenosis (comprising death, MI and target vessel revascularisation) within 9 months. All outcomes were evaluated by an independent committee.

LIFE Heart MI/CAD: AMI or 50% stenosis on coronary angiography

MedStar MI/CAD: 50% stenosis on coronary angiography

PennCath MI/CAD: AMI or 50% stenosis on coronary angiography

SMART MI/Stroke information from hospitalizations and outpatient clinic visits was obtained from participants by 6-monthly questionnaire. If a possible event was reported by participants, all available relevant data were collected. Death was reported by relatives, the GP or the specialist who treated the participant. All events were classified independently by committee, comprising physicians from different departments.

B.2 Supplementary Tables

Table B.1: Characteristics of the studies included in the sPLA$_2$ collaboration

Study	Study design	Geographical location	Sampling frame	Participants included	Baseline year(s)	Proportion Female, %	Age, mean (SD)
Studies in General Population							
BHF-FHS [559]	C-C	UK	Direct media campaign	4521	1998-2003	36.67	50.53(13.90)
BRHS [560]	Cohort	UK	General practice	3835	1998-2000	0	68.74(5.49)
BWHHS [561]	Cohort	UK	General practice	3405	1999-2001	100	68.91(5.49)
CCHS [562]	Cohort	Denmark	General population	10375	1991-1994	55.68	56.57(16.26)
CYPRUS [563]	Cohort	Cyprus	Mayors list	734	2003-2008	52.97	61.27(10.26)
EAS [564]	Cohort	UK	General practices	857	1987	50.64	64.38(5.82)
EPIC-Netherlands [565]	Nested C-C	Netherlands	Existing cohorts	5194	1993-1997	78.11	54.03(10.22)
EPIC-Norfolk [341]	Nested C-C	UK	General practices	3039	1993-1997	34.65	64.68(7.88)
GRAPHIC [566]	Cohort	UK	Nuclear families	2024	2003-2005	49.56	39.19(14.48)
IMPROVE [567]	Cohort	Europe	Clinic	3236	2004-2005	52.83	64.35(5.17)
NPHS-II [568]	Cohort	UK	General practices	2693	1989-1994	0	56.11(3.33)
PREVEND [569]	Cohort	Netherlands	Community	8114	1997	50.86	49.05(12.76)
PROCARDIS [570]	C-C	Sweden, UK, Germany, Italy	Hospital	5463	1998-2002	41.06	60.72(9.04)
PROSPER [571]	RCT	UK, Ireland, Netherlands	General practices	3991	1997-1999	51.79	75.28 (3.35)
Rotterdam [572]	Cohort	Netherlands	District participants	5974	1989-1993	59.37	69.38(8.91)
TPT [573]	RCT	UK	General practice	4014	1984-1989	0	56.06(6.74)
UCP [574]	Nested C-C	Netherlands	National drug registry	1632	1985-2005	25.49	62.76 (9.64)

Continued on next page

Table B.1 – *Continued from previous page*

Study	Study design	Geographical location	Sampling frame	Participants included	Baseline year(s)	Proportion Female, %	Age, mean (SD)
UDACS [342]	Cohort	UK	Clinic	564	2001-2002	41.13	66.73(11.09)
Whitehall II [85]	Cohort	UK	Workplace	5018	1985-1988	26.44	43.90(5.93)
Studies in Acute Coronary Syndrome Patients							
CURE [575]	RCT	28 countries	Hospitals	4334	1998-2000	40.90	64.20(11.00)
FAST-MI [343]	Cohort	France	Nationwide ACS registry	973	2005-ongoing	29.19	66.09(13.65)
GENDEMIP [576]	Cohort	Czech Republic	5 Coronary Units	1432	2006-2009	25.98	57.08(8.62)
GRACE-France [344]	Cohort	France	Hospitals	274	2000-2002	20.44	60.13(12.67)
GRACE-Scotland [345]	Cohort	Scotland	Hospitals	1488	1999-2009	30.04	64.84 (12.04)
IHCS [577]	Nested C-C	USA	Single hospital	2382	1994-ongoing	29.14	62.99 (12.29)
KAROLA [347]	Cohort	Germany	Rehabilitation clinics	1019	1999-2000	15	58.93 (7.96)
MERLIN-TIMI 36 [578]	RCT	17 countries	Hospitals	1606	2004-2007	34.1	63.43 (10.81)
MIRACL [363]	RCT	19 countries	Hospital	2587	1997-1999	34.80	65.7 (11.8)
PROVE-IT TIMI 22 [579]	RCT	8 countries	Hospitals	2260	2000-2003	22.51	57.48 (11.06)
Other Studies							
AMC-PAS [580]	C-C	Netherlands	Hospital clinic	740	1990-2000	19	44.05(3.91)
ASAP[362]	Cohort	Sweden	Hospital clinic	272	2006-present	32.83	62.86 (11.52)
GENDER [361]	C-C	Netherlands	Hospital clinic	866	1999-2001	26	62.01 (11.09)

Continued on next page

Table B.1 – *Continued from previous page*

Study	Study design	Geographical location	Sampling frame	Participants included	Baseline year(s)	Proportion Female, %	Age, mean (SD)
LIFE Heart [581]	C-C	Germany	Hospital clinic	3128	2006-present	32.83	62.86 (11.52)
MedStar [582]	Cohort	USA	Single-centre hospital	1322	2004-2007	54.59	59.76(8.86)
PennCath [582]	C-C	USA	Single-centre hospital	1516	1998-2003	51.92	59.29(9.69)
SMART [360]	Cohort	Netherlands	Hospital clinic	8297	1996-1998	32.24	56.51(12.42)

Footnotes Abbreviations: C-C: case control; ICU: intensive care unit; RCT: randomized clinical trial; X-S: cross sectional. All cohorts were prospective in design. AMC-PAS: Premature Atherosclerosis Patients and Sanquin Blood Bank Controls; ASAP: Advanced Study of Aortic Pathology; BHF-FHS: British Heart Foundation Family Heart Study; BRHS: British Regional Heart Study; BWHHS: British Womens Health and Heat Study; CCHS: Copenhagen City heart Study; CURE: The Clopidogrel in Unstable angina to prevent Recurrent Events; Cyprus: Cyprus Study; EAS: Edinburgh Artery Study; EPIC: European Prospective Investigation into Cancer and Nutrition; GENDEMIP: GENetic DEterminant of Myocardial Infarction in Prague; GRACE: Global Registry of Acute Coronary Events; GRAPHIC: Genetic Regulation of Arterial Pressure of Humans in the Community; IHCS: Intermountain Heart Collaborative Study; IMPROVE: IMPROVE Study; KAROLA: Langzeiterfolge der KARdiOLogischen Anschlussheilbehandlung; LIFE Heart: Leipzig Heart Study; Med-Star: Med-Star Study; MERLIN-TIMI 36: Metabolic Efficiency With Ranolazine for Less Ischemia in NonST-Elevation Acute Coronary Syndromes ; MIRACL: Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering; NPHS-II: Northwick park Heart Study; PennCATH: University of Pennsylvania Catheterization study program; PREVEND: Prevention of Renal and Vascular End-stage Disease; PROCARDIS: PRecOcious Coronary ARtery DISease; PROSPER: PROspective Study of Pravastatin in the Elderly at Risk; PROVE-IT TIMI 22: Pravastatin Or atorVastatin Evaluation and Infection Trial; Rotterdam: Rotterdam Study; SMART: Second Manifestations of ARTerial disease; TPT: Thrombosis Prevention Trial; UDACS: University College London Diabetes And Cardiovascular Disease Study; UCP: Utrecht Cardiovascular Pharmacogenetics; Whitehall II: Whitehall II Study; FAST-MI: French Registry of Acute ST-Elevation or NonST-elevation Myocardial Infarction; GRACE: Global Registry of Acute Coronary Events.

Table B.2: Associations of *PLA2G2A* gene variants with sPLA$_2$-IIA mass in EPIC-Norfolk and GRACE-France.

SNP	Genotype group	EPIC-Norfolk N	EPIC-Norfolk sPLA$_2$-IIa mass (ng/ml), median (IQR)	GRACE-France N	GRACE-France sPLA$_2$-IIa mass (ng/ml), median (IQR)
rs3767221	11	1210	10.10 (6.80, 16.10)	101	3.62 (2.15, 8.67)
(5128T>G)	12	1444	8.60 (5.90, 12.90)	127	3.42 (1.66, 5.71)
	22	490	6.90 (5.00, 9.20)	48	1.84 (1.13, 3.18)
	P value[1]		1.31×10^{-39}		0.0003
	P value[2]		1.32×10^{-30}		0.0017
rs876018	11	2295	8.86 (6.10, 13.72)	199	2.90 (1.62, 6.78)
(4982T>A)	12	820	8.15 (5.80, 12.60)	68	3.12 (1.61, 8.27)
	22	86	7.67 (5.7, 11.8)	10	3.14 (1.94, 5.55)
	P value[1]		0.002		0.96
	P value[2]		0.002		0.8
rs955587	11	2557	8.97 (6.23, 13.95)	202	3.47 (1.73, 8.02)
(3758G>A)	12	589	7.70 (5.41, 10.9)	66	2.35 (1.53, 4.30)
	22	41	5.7 (4.71, 8.15)	5	1.32 (7.90, 1.77)
	P value[1]		1.07×10^{-14}		0.002
	P value[2]		4.64×10^{-10}		0.003
rs3753827	11	935	10.42 (7.20, 15.80)	85	3.68 (1.84, 7.33)
(1022G>T)	12	1606	8.61 (5.90, 13.00)	136	2.79 (1.62, 6.52)
	22	701	7.10 (5.27, 9.76)	55	2.58 (1.36, 5.76)
	P value[1]		2.55×10^{-43}		0.09
	P value[2]		6.43×10^{-40}		0.15
rs11573156	11	1834	6.80 (5.08, 9.0)	178	2.26 (1.37, 4.24)
(763C>G)	12	1046	12.00 (8.74, 16.47)	88	4.35 (2.61, 1.25)
	22	159	16.10 (10.94, 23.73)	9	7.30 (4.66, 18.84)
	P value[1]		1.08×10^{-170}		2.27×10^{-8}
	P value[2]		7.82×10^{-193}		2.48×10^{-6}
rs1774131	11	1433	7.22 (5.30, 10.40)	141	2.35 (1.53, 5.55)
(655T>C)	12	1312	9.6 (6.70, 14.71)	116	3.47 (1.80, 7.08)
	22	341	11.9 (8.40, 18.00)	20	9.88 (3.54, 19.19)
	P value[1]		1.05×10^{-63}		0.0007
	P value[2]		1.01×10^{-54}		0.002

Footnotes: Genotype groups 11: homozygotes for the common allele (CC); 12: heterozygotes (CG); 22: homozygotes for the rare allele (GG). P values represent test for trend: 1 univariate; 2 adjusted for age, gender and CHD

Table B.3: Associations of *PLA2G2A* gene variants with sPLA$_2$ enzyme activity in EPIC-Norfolk and GRACE-France.

SNP	**Genotype group**	**EPIC-Norfolk**		**GRACE-France**	
		N	**sPLA$_2$ activity (nmol/min/mL), median (IQR)**	**N**	**sPLA$_2$ activity (nmol/min/mL), median (IQR)**
rs3767221	11	1192	4.45 (3.85, 5.22)	101	2.00 (1.50, 2.70)
(5128T>G)	12	1418	4.40 (3.85, 5.15)	127	2.00 (1.40, 2.90)
	22	486	4.33 (3.81, 4.97)	48	1.80 (1.20, 3.30)
	P value[1]		0.08		0.5
	P value[2]		0.17		0.36
rs876018	11	2261	4.42 (3.85, 5.16)	199	2.00 (1.30, 3.00)
(4982T>A)	12	807	4.37 (3.83, 5.09)	68	2.00 (1.50, 2.90)
	22	86	4.37 (3.84, 5.15)	10	1.65 (1.20, 1.90)
	P value[1]		0.64		0.57
	P value[2]		0.84		0.5
rs955587	11	2519	4.41 (3.84, 5.15)	202	2.00 (1.40, 2.90)
(3758G>A)	12	579	4.42 (3.85, 5.18)	66	1.60 (1.20, 2.80)
	22	41	4.58 (3.87, 4.92)	5	1.80 (1.50, 1.80)
	P value[1]		0.7		0.04
	P value[2]		0.9		0.12
rs3753827	11	935	4.48 (3.90, 5.21)	85	1.80 (1.50, 2.90)
(1022G>T)	12	1606	4.36 (3.81, 5.13)	136	2.00 (1.50, 2.95)
	22	701	4.40 (3.83, 5.12)	55	1.70 (1.00, 2.50)
	P value[1]		0.13		0.09
	P value[2]		0.14		0.03
rs11573156	11	1834	4.33 (3.78, 5.04)	178	1.85 (1.20, 2.80)
(763C>G)	12	1046	4.47 (3.90, 5.23)	88	2.00 (1.50, 3.20)
	22	159	4.56 (3.91, 5.39)	9	2.80 (1.50, 2.80)
	P value[1]		0.00004		0.03
	P value[2]		0.0002		0.07
rs1774131	11	1415	4.35 (3.78, 5.10)	141	1.80 (1.20, 2.90)
(655T>C)	12	1287	4.45 (3.87, 5.16)	116	2.00 (1.50, 2.95)
	22	336	4.37 (3.89, 5.14)	20	2.15 (1.60, 2.80)
	P value[1]		0.05		0.13
	P value[2]		0.08		0.17

Footnotes: Genotype groups 11: homozygotes for the common allele (CC); 12: heterozygotes (CG); 22: homozygotes for the rare allele (GG). P values represent test for trend: 1 univariate; 2 adjusted for age, gender and CHD

Table B.4: Genotyping characteristics in the collaborating studies

Study	Genotyping platform	*PLA2G2A* rs11573156					Hardy-Weinberg statistics	
		Call rate (%)	Proxy SNP used (LD r^2)	CC	CG	GG	Estimated disequilibrium coefficient	Exact significant probability
Studies in General Population								
BHF-FHS	IBC CardioChip	100	N/A	2720	1552	249	0.004	0.16
BRHS	KASPar	97.2	N/A	2379	1267	186	0.003	0.31
BWHHS	IBC CardioChip	99.8	N/A	2066	1150	189	0.005	0.08
CCHS	ABI TaqMan	99.8	N/A	5482	4089	804	0.002	0.28
CYPRUS	ABI TaqMan	94.3	N/A	456	236	42	0.01	0.13
EAS	ABI TaqMan	96.4	N/A	547	257	53	0.017	0.004
EPIC-Netherlands	IBC CardioChip	100	N/A	2961	1927	306	-0.001	0.76
EPIC-Norfolk	ABI TaqMan	91.5	N/A	1834	1046	159	0.002	0.53
GRAPHIC	IBC CardioChip	100	N/A	1201	728	95	-0.004	0.28
IMPROVE	ABI TaqMan	91.6	N/A	1941	1109	186	0.005	0.1
LIFE Heart	Homogeneous fluorescence-based melting curve†	95.5	N/A	1738	1196	194	-0.007	0.2
MedStar	Affymetrix 6.0	98.3	Multiple SNPs (0.77)	726	511	85	-0.006	0.62
NPHS-II	ABI TaqMan	97	N/A	1617	936	140	0.001	0.78
PennCath	IBC CardioChip	98.6	N/A	916	540	60	-0.017	0.03
PREVEND	KASPar	98	N/A	4635	2974	505	0.002	0.34
PROSPER	Illumina 660K chip	97.5	N/A	2571	1246	174	0.004	0.15
Rotterdam	Illumina Infinium II HumanHap550 (v3)	100	Multiple SNPs (0.79)	3290	2322	362	-0.004	0.08

Continued on next page

Table B.4 – *Continued from previous page*

Study	Genotyping platform	*PLA2G2A* rs11573156					Hardy-Weinberg statistics	
		Call rate (%)	Proxy SNP used (LD r2)	CC	CG	GG	Estimated disequilibrium coefficient	Exact significant probability
TPT	ABI TaqMan	97.5	N/A	2367	1423	224	0.001	0.6
UCP	IBC CardioChip	100	N/A	939	595	98	0.001	0.79
UDACS	ABI TaqMan	94.2	N/A	337	195	32	0.004	0.63
Whitehall II	IBC CardioChip	91.7	N/A	2992	1774	252	-0.001	0.63
Studies in Acute Coronary Syndrome Patients								
CURE	ABI TaqMan	98.9	N/A	2475	1578	281	0.0039	0.18
FAST-MI	SNPlex	98.3	N/A	623	301	49	0.008	0.12
GENDEMIP	PCR-RFLP	96.7	N/A	750	559	123	0.007	0.19
GRACE France	ABI TaqMan	97.8	N/A	178	87	9	-0.004	0.85
GRACE Scotland	ABI TaqMan	97.8	N/A	901	506	81	0.004	0.37
IHCS	ABI TaqMan	92.4	N/A	1475	792	115	0.002	0.5
KAROLA	ABI TaqMan	98.9	N/A	560	399	49	-0.012	0.04
MERLIN-TIMI 36	IBC CardioChip	99.9	N/A	935	578	93	0.001	0.78
PROVE-IT TIMI 22	IBC CardioChip	99.7	N/A	1392	756	112	0.003	0.5
Other Studies								
AMC-PAS	IBC CardioChip	99.6	N/A	117	64	10	0.004	0.83
GENDER	Illumina Human 610-Quad	100	rs10732279 (0.91)	503	313	50	0.001	0.93
SMART	KASPar	98.2	N/A	4649	2882	465	0.001	0.52

Footnote: Abbreviations: LD: linkage disequilibrium; N/A (not applicable) in column titled "Proxy SNP used" indicates rs11573156 directly genotyped; † methodology described in Holdt *et al*[583]

Table B.5: Data availability for sPLA$_2$ assays, cardiovascular biomarkers and measures of atherosclerosis in the collaborating studies

Study	**sPLA$_2$ assays**		**Circulating biomarkers/anthropometric traits**								
	IIA mass	**Activity**	**SBP**	**LDL-C**	**HDL-C**	**TG**	**IL6**	**CRP**	**Glucose**	**BMI**	**C-IMT**
STUDIES IN GENERAL POPULATION											
AMC-PAS			•	•	•	•		•	•	•	
BFH-FHS											
BRHS			•	•	•	•	•	•	•	•	
BWHHS			•	•	•	•	•	•	•	•	
CCHS			•	•	•	•		•	•	•	
CYPRUS			•	•	•	•		•		•	•
EAS			•	•	•	•	•	•	•	•	•
EPIC-Netherlands			•	•	•	•		•	•	•	
EPIC-Norfolk	•	•	•	•	•	•		•		•	
GRAPHIC			•	•	•	•		•	•	•	
IMPROVE			•	•	•	•		•	•	•	•
LIFE Heart				•	•	•		•	•	•	•
MedStar			•	•	•					•	
NPHS-II			•	•	•	•		•		•	
PennCath			•	•	•					•	
PREVEND			•	•	•	•		•	•	•	•
PROSPER			•	•	•	•	•			•	
Rotterdam			•		•		•	•		•	•
TPT			•							•	
UCP										•	
UDACS	•		•	•	•	•	•	•	•	•	
Whitehall II			•	•	•	•	•	•	•	•	

Continued on next page

Table B.5 – *Continued from previous page*

Study	sPLA$_2$ assays		Circulating biomarkers/anthropometric traits								
	IIA mass	Activity	SBP	LDL-C	HDL-C	TG	IL6	CRP	Glucose	BMI	C-IMT
STUDIES IN ACUTE CORONARY SYNDROME PATIENTS											
CURE											
FAST-MI	•	•	•				•	•	•	•	
GENDEMIP			•	•	•	•		•	•	•	
GRACE-France	•	•	•	•				•		•	
GRACE-Scotland	•	•	•	•	•	•		•	•	•	
IHCS			•	•	•	•		•	•	•	
KAROLA	•†	•	•	•	•	•	•	•	•	•	
MERLIN-TIMI 36											
MIRACL	•	•	•							•	
PROVE-IT TIMI 22											
OTHER STUDIES											
GENDER											
SMART											•

† Not used for analyses as measured >4 weeks after ACS event

Table B.6: Contribution of each study to individual outcomes and primary outcome for the Mendelian randomization analysis

Study	Prevalent			Incident/Recurrent				Contributes to primary outcome (MVE)
	MI	Stroke	Coronary Stenosis	Non-fatal MI	Non-fatal Stroke	Fatal MI or stroke	All-cause mortality	
STUDIES IN GENERAL POPULATION								
BHF-FHS	•		-	-	-	-	-	•
BRHS	•	•	-	•	•	•	-	•
BWHHS	•	•	-	•	•	•	-	•
CCHS	•	•	-	•	•	•	-	•
CYPRUS	•	•	-	-	-	-	-	•
EAS	•	•	-	•	•	•	-	•
EPIC-Netherlands	-	-	-	•	•	•	-	•
EPIC-Norfolk	-	-	-	•	-	•	-	•
GRAPHIC	-	-	-	-	-	-	-	-
IMPROVE	•	-	-	•	•	•	-	•
NPHS-II	-	-	-	•	•	•	-	•
PREVEND	•	•	-	•	•	•	-	•
PROCARDIS	•	-	-	-	-	-	-	•
PROSPER	•	•	-	•	•	•	-	•
Rotterdam	•	•	-	•	•	•	-	•
TPT	-	-	-	•	•	•	-	•
UCP	-	-	-	•	-	-	-	•
UDACS	-	-	-	-	-	-	-	-

Continued on next page

Table B.6 – *Continued from previous page*

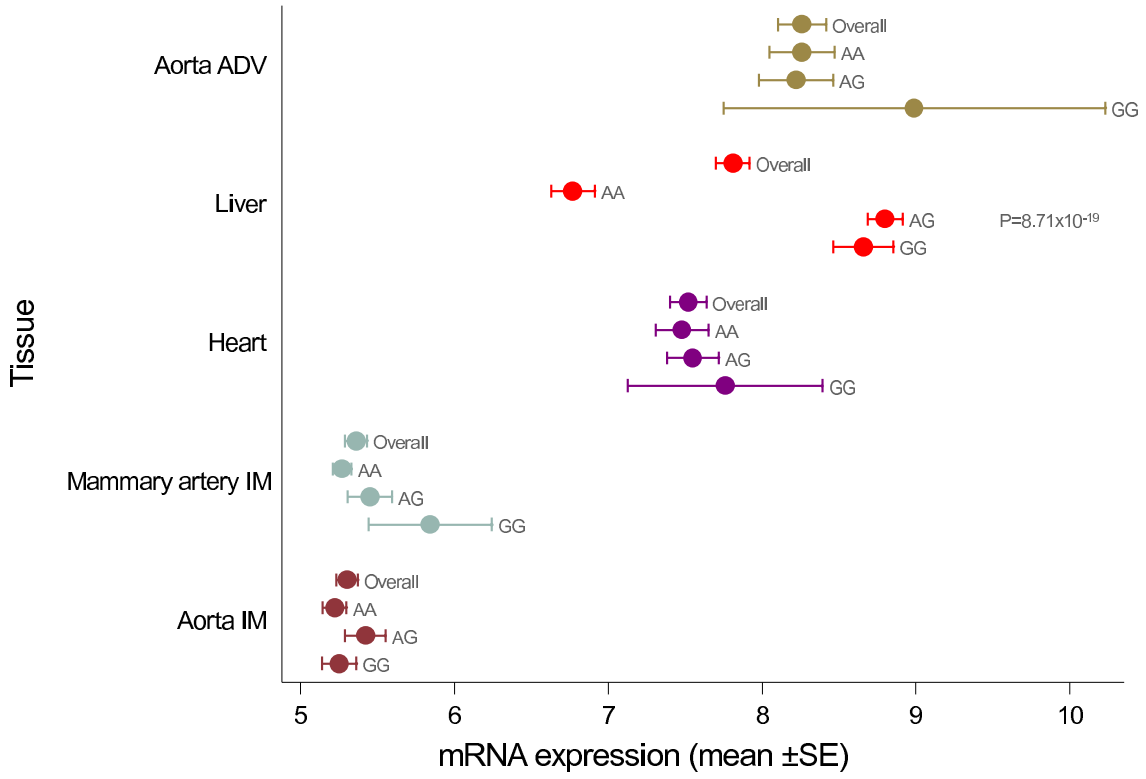
Study	Prevalent			Incident/Recurrent				Contributes to primary outcome (MVE)
	MI	Stroke	Coronary Stenosis	Non-fatal MI	Non-fatal Stroke	Fatal MI or stroke	All-cause mortality	
Whitehall II	•	-	-	-	-	-	-	•
STUDIES IN ACUTE CORONARY SYNDROME								
CURE	-	-	-	•	•	•	-	•
FAST-MI	-	-	-	•	•	-	•	•
GENDEMIP	-	-	-	-	-	•	-	•
GRACE France	-	-	-	•	-	-	•	•
GRACE Scotland	-	-	-	•	•	-	•	•
IHCS	-	-	-	•	•	•	-	•
KAROLA	-	-	-	•	•	•	-	•
MERLIN-TIMI 36	-	-	-	•	-	•	-	•
MIRACL	-	-	-	-	-	-	-	-
PROVE-IT TIMI 22	-	-	-	•	•	•	-	•
OTHER STUDIES								
AMC-PAS	•	-	•	-	-	-	-	-
GENDER	-	-	-	-	-	-	-	-
LIFE Heart	•	-	•	-	-	-	-	-
MedStar	-	-	•	-	-	-	-	-
PennCath	•	-	•	-	-	-	-	-
SMART	-	-	-	•	•	•	-	-

Table B.7: Meta-analysis pooled estimates of the per C allele association between *PLA2G2A* rs11573156 and major vascular events (including individual components) stratified by clinical setting using **random** effects modelling.

Outcome	Studies (Events/Total)	Odds Ratio (95%CI)	I^2,% (95%CI)
General Population: Incident			
Major vascular events	13 (8021/56359)	1.03 (0.98, 1.08)	26 (0, 51)
Nonfatal MI	13 (4208/51016)	1.05 (0.98, 1.13)	23 (0, 60)
Nonfatal Stroke	11 (2304/46790)	1.00 (0.92, 1.09)	19 (0, 59)
Fatal MI/Stroke	12 (1509/48118)	1.02 (0.90, 1.15)	41 (0, 70)
General Population: Prevalent			
Major vascular events	12 (7513/55523)	1.00 (0.94, 1.06)	38 (0, 64)
MI	12 (6411/54884)	0.98 (0.91, 1.07)	52 (8, 75)
Stroke	8 (1102/37280)	1.03 (0.93, 1.15)	0 (0, 68)
Acute Coronary Syndrome: Recurrent			
Major vascular events	9 (2520/15768)	0.96 (0.90, 1.03)	0 (0, 45)
Nonfatal MI	8 (1158/14152)	0.97 (0.85, 1.10)	28 (0, 68)
Nonfatal Stroke	6 (223/12283)	0.85 (0.69, 1.06)	0 (0, 75)
Fatal MI/Stroke	9 (1139/15724)	0.96 (0.87, 1.06)	0 (0, 65)

Footnotes Fatal MI/stroke includes death for some ACS studies (see Table B.6 for further details).

B.3 Supplemental Figures



Footnotes: ADV: adventitia; IM: intima media

26

Figure B.1: Differential expression of *PLA2G2A* mRNA by rs10732279 genotype. P-value corresponds to the differential expression by genotype.

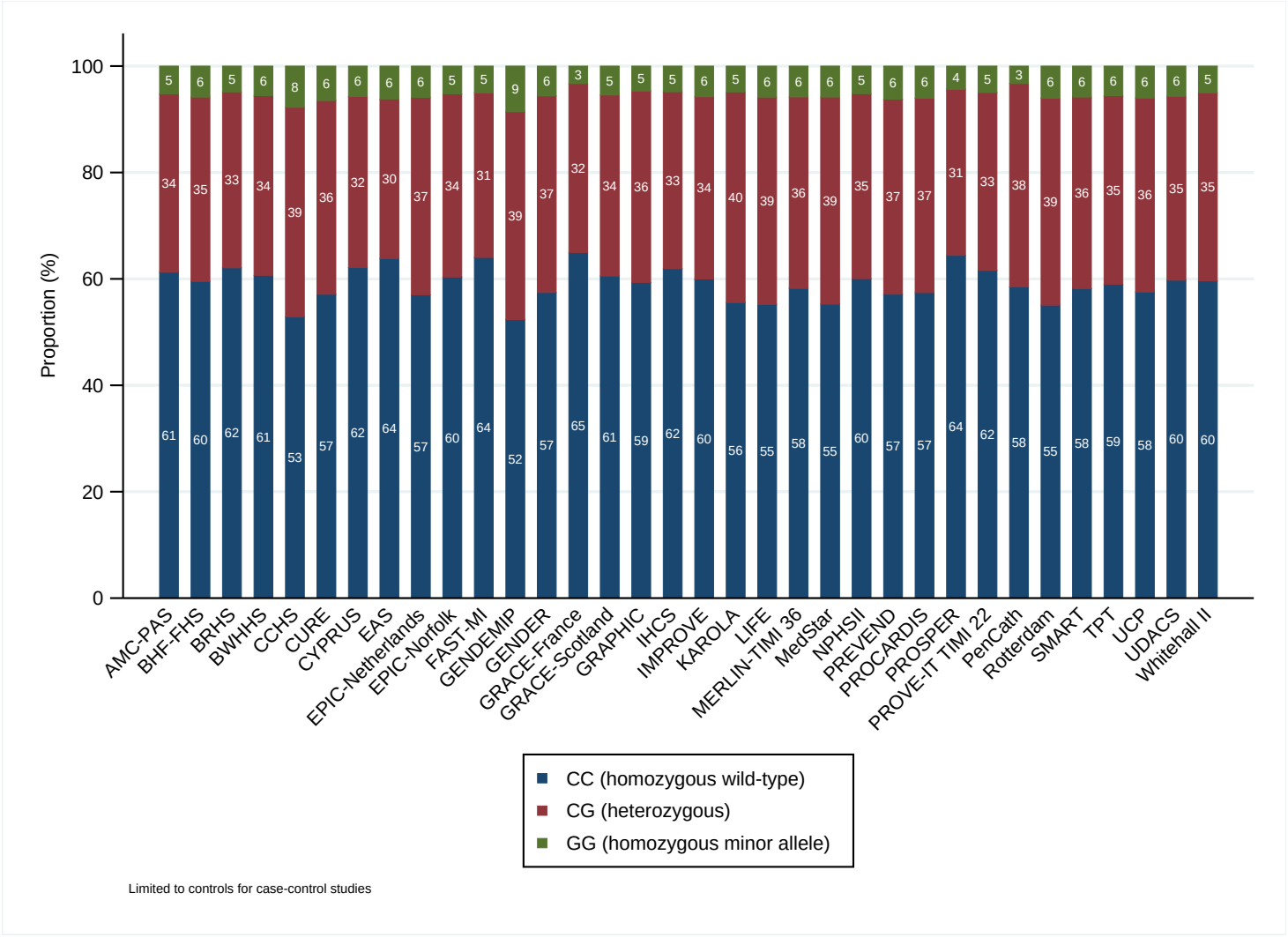


Figure B.2: Genotype frequency of *PLA2G2A* rs11573156 in the 34 studies.

B.4 VISTA-16 Summary from Anthera Pharmaceuticals

Name of Sponsor/Company: Anthera Pharmaceuticals, Inc.	**Individual Study Table Referring to Part of the Dossier** **Volume:** **Page:**	(For National Authority Use only)
Name of Finished Product: A-002 (oral) Varespladib Methyl		
Name of Active Ingredient: Varespladib		

SYNOPSIS
Clinical Study Report for Study AN-CVD2233
ABBRIVIATED REPORT

TITLE OF STUDY: Evaluation of the Safety and Efficacy of Short-term A-002 Treatment in Subjects with Acute Coronary Syndromes

INVESTIGATORS/STUDY CENTERS: 375 sites in 17 countries

PUBLICATION: not applicable

Study Period: 1 June 2010 to 9 March 2012 **Phase of Development:** Phase 3

INTRODUCTION: Inflammation is associated with early recurrent cardiovascular events in subjects with an acute coronary syndrome. The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16, NCT01130246) study tested the hypothesis that varespladib methyl, an inhibitor of $sPLA_2$ would reduce cardiovascular risk among patients with acute coronary syndromes.

OBJECTIVES:

- Primary:
  To determine whether 16 weeks of treatment with A-002 plus atorvastatin and standard of care is superior to placebo plus atorvastatin and standard of care for reducing the hazard of the first occurrence of the combined endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke or documented unstable angina with objective evidence of ischemia requiring hospitalization.
- Secondary:
  To determine whether A-002 plus atorvastatin and standard of care is superior to placebo plus atorvastatin and standard of care for reducing the occurrence of the hazard of the combined endpoint of all-cause mortality, non-fatal myocardial infarction, non-fatal stroke, or documented unstable angina with objective evidence of ischemia requiring hospitalization, or multiple occurrences of the non-fatal components of the composite primary endpoint.
- Exploratory:

To determine if A-002 plus atorvastatin and standard of care is superior to placebo plus atorvastatin and standard of care on the change (or % change) from baseline in biomarkers of cardiovascular risk (e.g., LDL-C, hs-CRP, IL-6, $sPLA_2$).

METHODOLOGY: This was a double-blind, randomized, parallel group, placebo controlled study in subjects presenting with an ACS. Up to 8500 subjects were to be randomized to receive either A-002 500 mg once daily (QD) or placebo tablets in addition to atorvastatin QD and standard of care. Treatment was 16 weeks in duration. The dose of atorvastatin could be adjusted after 8 weeks if subject's LDL-C is ≥100 mg/dL, but otherwise was to remain stable throughout the 16-week duration of study. The survival status for all enrolled subjects was to be ascertained 6 months after they completed the study; however, due to early termination of the study, this information was not collected for all subjects.

Randomization occurred within ≤96 hours of hospitalization for the index ACS event, or if already hospitalized, within ≤96 hours of index event diagnosis**.** Follow-up visits were to occur at Hours 24, 48, 72, and 96 and Weeks 1, 2, 4, 8, and 16. Due to the early termination of the study, some subjects did not complete the 16 weeks of therapy.

Randomization was stratified by the presence or absence of lipid-altering therapy prior to the index event as well as the type of index event (admission diagnosis of unstable angina, NSTEMI or STEMI). The number of subjects who underwent PCI following the index event and prior to randomization was limited to no more than 55% of the total study population.

NUMBER OF SUBJECTS (Planned and Analyzed): Planned: up to 8500. Analyzed: 5189

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:

Men and women ≥40 years of age

A diagnosis of unstable angina, non-ST-segment elevation myocardial infarction (NSTEMI), or ST-segment elevation myocardial infarction (STEMI)

All subjects (unstable angina, NSTEMI, or STEMI) must have had the presence of at least one of the following risk factors:

i. Diabetes Mellitus* or
ii. Presence of any 3 of the following characteristics of metabolic syndrome
- Waist circumference >102 cm in males, >88 cm in females
- Serum triglycerides ≥150 mg/dL (≥1.7 mmol/L)
- HDL-C <40 mg/dL (<1 mmol/L) in males, <50 mg/dL (<1.3 mmol/L) in females
- Blood pressure ≥130/85 mmHg
- Plasma glucose ≥110 mg/dL (≥6.1 mmol/L) or

iii. history of cerebrovascular disease (stroke or TIA) or
iv. history of peripheral vascular disease or
v. previous CABG or
vi. previous documented myocardial infarction or
vii. previous coronary revascularization

Subjects must be randomized within ≤96 hours of hospital admission for the index event, or if already hospitalized, within ≤96 hours of index event diagnosis

Revascularization, if required or planned, must occur prior to randomization

* Defined as previously documented fasting plasma glucose level of at least 126 mg/dL (7.0 mmol/L) or a 2-hour plasma glucose level of at least 200 mg/dL (11.1 mmol/L) during a 75 g oral glucose tolerance test.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER: Oral A-002: 500 mg QD, as two 250 mg tablets. Batch Numbers: 1004001, 1011001, 1101001, 1108001, 1109001

DURATION OF TREATMENT: All subjects were followed on treatment for 16 weeks. Survival status was to be ascertained 6 months after the subject withdrew from, or completed, the study. Due to early termination of the study, this information was not collected for all subjects.

REFERENCE THERAPY, DOSE, AND MODE OF ADMINISTRATION, BATCH NUMBER:

Comparator: placebo
In addition to study drug (A-002 or placebo) atorvastatin ≥20 mg/day was to be given to all study subjects.
Placebo Batch Numbers: 1004001, 1011001, 1101001, 1108001, 1109001

CRITERIA FOR EVALUATION:

Efficacy: Efficacy was assessed in the intent-to-treat (ITT) population defined as all randomized subjects.

Safety: Safety was assessed in all subjects who receive any amount of study therapy by monitoring for the occurrence of treatment-emergent adverse events (TEAEs), including possible allergic reactions and the collection of conventional laboratory data (chemistry panel and complete blood count [CBC] with differential).

Six months following their participation in the study, the survival status for all subjects who had not withdrawn consent was to be ascertained; however, due to early termination of the study, this information was not collected for all subjects.

STATISTICAL METHODS: The primary efficacy endpoint was the time to first occurrence of the combined endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, or documented unstable angina with objective evidence of ischemia requiring hospitalization. All events that were suspected components of the primary efficacy endpoint were adjudicated in a blinded fashion by an independent event committee.

An interim analysis for determining early stopping for efficacy or futility was performed by the DSMB on 9 March 2012 when approximately 50% of the primary endpoint events had occurred. Monitoring for early stopping at the 50% point was based on analysis of the primary endpoint. Specifically, this analysis compared the incidence of the combined endpoint of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke, or documented unstable angina with objective evidence of ischemia requiring hospitalization between the treatment groups. A total of 383 potential endpoint events were reviewed and 247 were determined to be positive endpoints as defined by the protocol; 212 were the first occurrence of an endpoint and thus, primary efficacy endpoints. The Kaplan Meier (KM) method was used to estimate event rates. The hazard ratios and 95% confidence intervals were estimated by a Cox proportional hazards regression model with factors for treatment, use of a lipid-altering therapy prior to the index event (yes/no) and type of index event (unstable angina, NSTEMI, STEMI).

SUMMARY OF RESULTS:

Efficacy Results: At the time of study termination the hazard ratio for the primary endpoint was 1.244 (p=0.155). The hazard ratio for the combination of cardiovascular death, non-fatal myocardial infarction and stroke was statistically significant: HR 1.436 p=0.025. This is primarily driven by the increased occurrence of non-fatal myocardial infarction: HR 1.686 p=0.009.

PK Results: Due to the early termination of the study, PK analysis was not performed.

Safety Results: Adverse event data are available from 5102 subjects. Cardiac disorders (17.5%) was the most frequently reported organ system affected, followed by gastrointestinal disorders (11.2%), general disorders (7.3%), investigations (9.5%), nervous system disorders (7.2%), respiratory disorders (7.0%) and vascular disorders (4.4%).

A total of 528 (10.3%) subjects reported a treatment-emergent serious adverse event. The majority were cardiac disorders and also related to the primary efficacy endpoint as reported by 328 (6.4%) subjects (Table 14.3.12). Amongst the cardiac disorders, unstable angina was reported by 109 subjects (2.1%), and acute myocardial infarction combined with myocardial infarction by 56 subjects. All other SAEs were reported at frequency of ≤0.5%.

No new or otherwise unexpected trends were observed in the AE, SAE, laboratory, hemodynamic or ECG data.

CONCLUSIONS:

The study was prematurely terminated by the DSMB because of the inability of VISTA-16 to detect a statistically significant benefit of the drug on the prespecified primary and secondary endpoints even if the trial continued to its scheduled termination with the proposed expanded sample size.

No obvious clinical or scientific reason has been found for the increased hazard for non-fatal myocardial infarction amongst subjects treated with A-002 despite positive treatment-related changes in LDL-C and CRP.

Appendix C

Supplemental Information for *ADH1B*, alcohol and cardiovascular disease

C.1 Studies included in the *ADH1B* collaboration

Atherosclerosis Risk In Communities Study The Atherosclerosis Risk In Communities (ARIC) Study is a population-based prospective cohort study of cardiovascular disease sponsored by the National Heart, Lung, and Blood Institute (NHLBI). ARIC originally included 15,792 individuals aged 45-64 years at baseline (1987-89), chosen by probability sampling from four US communities. Cohort members completed four clinic examinations each spread over about three years, conducted approximately three years apart between 1987 and 1998. The data used in this study are from the first visit in 1987-1989. A detailed study protocol is available on the ARIC study website (http://www.cscc.unc.edu/aric). For this study the sample was restricted to individuals of European descent by self-report and principal component analysis using genome-wide genotypes.

Avon Longitudinal Study of Parents and Children The Avon Longitudinal Study of Parents and Children (ALSPAC) was established to understand how genetic and environmental characteristics influence health and development in parents and children (http://www.bristol.ac.uk/alspac/researchers/resources-available).1,2 All pregnant women resident in a defined area in the South West of England, with an expected date of delivery between 1st April 1991 and 31st December 1992, were eligible and 13 761women (contributing 13 867 pregnancies) were recruited. These women have been followed over the last 1922 years and have completed up

to 20 questionnaires, including those providing information about their alcohol consumption before and during the index pregnancy, which was used in this study to define abstainers, binge drinkers and drinking levels (before pregnancy). Abstainers were defined as women not drinking at either of the following time points: before pregnancy, during pregnancy, and at 5 time points postnatally (when child is 8, 21, 33, 61 months old). Binge drinkers were women consuming 4+ alcoholic drinks per occasion at least once either around 18 weeks or around 32 weeks of gestation. Weekly alcohol units derived from the questionnaire at 18 weeks gestation were used for the genotype-alcohol analysis, and so were the variables defining abstainers and binge drinkers. Baseline questionnaires completed around the time of the index pregnancy also provided information on education, social class, and smoking habits. A follow-up assessment was completed 1718 years postnatal at which anthropometry (weight, height, waist circumference), blood pressure (from both arms, then averaged to derive SBP and DBP used in analyses), carotid intima media thickness were assessed, and a fasting blood sample taken, from which the following were assayed: total and HDL cholesterol, triglycerides, CRP, insulin and glucose. DNA has been extracted from saliva or blood samples collected at various time points. The sample used for this study included only women of self-reported white ethnic origin, or, where this information was missing, those predicted to be of European origin based on 5 ancestry-informative markers. Additionally, women of self-declared Jewish faith were excluded (n=4) because the prevalence of the rare allele is much higher in most Jewish populations. Ten principal components variables derived from GWA panel data were available for sensitivity analyses.

British Womens Heart and Health Study British Womens Heart and Health Study is a study comprising 4,286 women aged 60-79 years who were randomly selected from 23 British towns between 1999 and 2001. Of the 4,278 participants who gave consent for genetic testing, 15 were defined by the examining nurse as being non-white and were excluded from further analysis. Of the remaining 4,263 women, 3,800 (89%) had DNA available for genotyping.

British Regional Heart Study From 1978 to 1980, 7735 men aged 40-59 were recruited from general practices across the UK. A wide range of phenotypic measures is available for established risk markers such as lipids, blood pressure and inflammatory and haemostatic markers. Most of these measures were taken both at recruitment and re-examination, which occurred in 1998-2000 when men were aged 60-79. At this re-examination 4252 participants attended and DNA was extracted for 3945. Data on important behavioural variables such as cigarette and alcohol consumption, as well as physical activity, have been regularly collected through

follow up. Well validated outcome variables including major coronary heart disease and stroke, as well as cause-specific mortality, continue to be collected from medical records 30 years after recruitment.

Caerphilly Prospective Study The Caerphilly Prospective Study (CAPS) to examine the importance of lipids, haemostatic factors, and hormones such as testosterone, cortisol and insulin (Lichtenstein et al 1987) in the development of ischemic heart disease (IHD). The initial design attempted to contact all men aged 45 to 59 years from the town of Caerphilly and adjoining villages. 2512 subjects (response rate 89%) identified from the electoral register and general practice lists were examined between July 1979 until September 1983 (phase I). Men were initially seen at an evening clinic, where they completed a questionnaire, had anthropometric measures and an ECG taken. They also completed a food frequency questionnaire at home. They subsequently re-attended an early morning clinic to have fasting blood samples for a wide variety of tests. Quality control was examined by the use of both "blind" split samples as well as a second repeat measure on a random sub-sample to examine intra-individual variation.

Cardiovascular Health Study The Cardiovascular Health Study (CHS) is a population-based cohort study of risk factors for cardiovascular disease in adults 65 years of age or older conducted across four field centres. The original predominantly white cohort of 5,201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists and an additional 687 African-Americans were enrolled in 1992-93 for a total sample of 5,888.

Cleveland Family Study The Cleveland Family Study (CFS) is the largest family-based study of sleep apnea world-wide, consisting of 2284 individuals (46% African American) from 361 families studied on up to 4 occasions over a period of 16 years. NIH renewals provided expansion of the original cohort (including increased minority recruitment) and longitudinal follow-up, with the last exam occurring in February 2006. Index probands (n=275) were recruited from 3 area hospital sleep labs if they had a confirmed diagnosis of sleep apnea and at least 2 first-degree relatives available to be studied. In the first 5 years of the study, neighbourhood control probands (n=87) with at least 2 living relatives available for study were selected at random from a list provided by the index family and also studied. All available first degree relatives and spouses of the case and control probands also were recruited. Second-degree relatives, including half-sibs, aunts, uncles and grandparents, were also included if they lived near the first degree relatives (cases or controls), or if the family had been found to have two or more relatives with sleep apnea. Blood was sampled and DNA isolated for participants seen in the last

two exam cycles (n=1447). The sample, which is enriched with individuals with sleep apnea, also contains a high prevalence of individuals with sleep apnea-related traits, including: obesity, impaired glucose tolerance, and hypertension.

Copenhagen City Heart Study The Copenhagen City Heart Study (CCHS) is a prospective study of 10 388 individuals randomly selected from the population of Copenhagen, followed from blood sampling in 19911994 through 2007. Individuals were invited based on their Central Person Registration number, the participation rate was 55% and follow-up was 100% complete, that is, no individual was lost to follow-up. Data on all-cause mortality were from the national Danish Civil Registration System, whereas information on cause-specific mortality was from the national Danish Causes of Death Registry.

Copenhagen General Population Study The Copenhagen General Population Study (CGPS) is a large general population cohort study that aims to eventually recruit 100,000 participants and collect genotypic and phenotypic data of relevance to a wide range of health related problems. Individuals are randomly selected from the national Danish Civil Registration System and have to be aged 20 years or older and resident in greater Copenhagen; they also have to be white and of Danish decent. Recruitment began in 2003 and is still on-going.

Coronary Artery Risk Development in Young Adults The Coronary Artery Risk Development in Young Adults (CARDIA) Study is a study examining the development and determinants of clinical and subclinical cardiovascular disease and its risk factors. It began in 1985 with a group of 5115 black and white men and women aged 18-30 years. The participants were selected so that there would be approximately the same number of people in subgroups of race, gender, education (high school or less and more than high school) and age (18-24 and 25-30) in each of 4 centers: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. These same participants were asked to participate in follow-up examinations during 1987-1988 (Year 2), 1990-1991 (Year 5), 1992-1993 (Year 7), 1995-1996 (Year 10), 2000-2001 (Year 15), and 2005-2006 (Year 20). A majority of the group has been examined at each of the follow-up examinations (90%, 86%, 81%, 79%, 74%, and 72%, respectively).

Cyprus Study The Cyprus Study is a population-based cohort study of cardiovascular disease in 1106 individuals aged 40 years or more from two areas in Cyprus. Baseline data have been collected from Pedoulas, a village in the Troodos Mountains of Cyprus their relatives who live in any one of the main towns and from a section of Nissou, a village in the Mesaoria plain 10 km south of the capital, Nicosia, between 2003-2008. These sites were randomly selected by having a blindfolded

person throw darts at a map of Cyprus. All inhabitants were identified through the population list held at the Mayors office and all those over the age of 40 years were invited to participate. This was done by setting up an open public meeting as arranged through the districts Mayor and Local Council Committee and the local Greek Orthodox Priest. The overall participation rate of those invited was 95%.

Danish Cancer and Health The Danish Diet, Cancer, and Health (DCH) Study is a prospective cohort study with the primary aim of studying the role of diet in cancer risk but with a potential for studying other diseases as well. From December 1993 through May 1997, 80 996 men and 79 729 women aged 50 to 64 y were invited to participate in the study; 27 177 men and 29 876 women accepted the invitation. Eligible cohort members were born in Denmark, living in the Copenhagen and Aarhus areas, and had no previous cancer diagnosis in the Danish Cancer Registry. The baseline data were linked to the Danish Cancer Registry and other population-based registries, including the Danish National Registry of Patients, and the Danish Civil Registration System, using the civil registry number, which is a unique number given to everyone with an address living in Denmark since 1968. The Civil Registration System has electronic records of all changes in vital status for the Danish population since 1968, including date of death. The Danish National Registry of Patients was established in 1977, and has records for 99.4% of all discharges from non-psychiatric hospitals in Denmark. The Danish Diet, Cancer, and Health Study and the present study were approved by the Regional Ethics Committees in Copenhagen and Aarhus and by The Danish Data Protection Agency.

Edinburgh Artery Study The Edinburgh Artery Study (EAS) is an age-stratified random sample of men and women, aged 55-74 years, which was selected between August 1987 and September 1988 from the age-sex registers of ten general practices with a geographical and socio-economical catchment population spread throughout the city of Edinburgh, UK. Subjects were excluded if they were unfit to participate (e.g., due to severe mental illness or terminal disease); excluded individuals were replaced by other randomly sampled subjects.

English Longitudinal Study of Ageing The English Longitudinal Study of Ageing (ELSA) is a national cohort of participants (48% men) aged over 50 years recruited from the Health Surveys for England in 1998, 1999, and 2001. Genetic data were collected at wave 2 of the study (2004/5); the phenotype measurements taken at wave 2 were used for this study.

The EPIC-InterAct Case-Cohort Study Individuals with T2D in European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts between 1991 and 2007 from eight of the ten countries participating in EPIC (26 centres) were identified. Prevalent diabetes was identified on the basis of baseline self-report of a history of diabetes, doctor-diagnosed diabetes, diabetes drug use, or evidence of diabetes after baseline with a date of diagnosis earlier than the baseline recruitment date. All ascertained cases with any evidence of diabetes at baseline were excluded. Ascertainment of incident T2D involved a review of the existing EPIC datasets at each centre using multiple sources of evidence including self-report, linkage to primary-care registers, secondary-care registers, medication use (drug registers), hospital admissions and mortality data. Information from any follow-up visit or external evidence with a date later than the baseline visit was used. To increase the specificity of the case definition, further evidence for all cases with information on incident T2D was sought from fewer than two independent sources at a minimum, which included individual medical records review in some centres. Cases in Denmark and Sweden were not ascertained by self-report, but identified via local and national diabetes and pharmaceutical registers, and hence all ascertained cases were considered to be verified. Follow-up was censored at the date of diagnosis, 31 December 2007, or the date of death, whichever occurred first.

European Prospective Investigation of Cancer: Netherlands The European Prospective Investigation of Cancer (EPIC) study in The Netherlands is based in two centres, Bilthoven and Utrecht. The population in the two cohorts has been recruited from two regions, from the general population (Bilthoven) and from those attending for breast cancer screening (Utrecht). Recruitment was carried out between 1993 and 1997. In 2006-2007, the two Dutch cohorts have been merged into one cohort (www.epicnl.eu) to gain efficiency and sample size and to optimise the use of the data locally. The separate cohorts, however, will co-exist besides the merged cohort.

European Prospective Investigation of Cancer: Norfolk The European Prospective Investigation of Cancer (EPIC) Norfolk is a population-based cohort study of 25,663 European men and women aged 39-79 years recruited in Norfolk, UK between 1993 and 1997. 2,100 randomly selected control subjects were chosen from a BMI study in which genome-wide genotyping data had been obtained.

European Prospective Investigation of Cancer: Potsdam The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study is part of the large-scale EPIC cohort and includes 10,904 male and 16,644 female participants recruited from the general population of Potsdam and surrounding areas. The

preferred age range for recruitment was 3565 years. Baseline examination was conducted from 1994 through 1998 and included blood sampling, measurements of blood pressure and anthropometric parameters, self-administered questionnaires on diet and lifestyle, and personal computer-assisted interviews.

European Prospective Investigation of Cancer: Turin The European Prospective Investigation of Cancer (EPIC) Turin study, part of the large-scale EPIC cohort, and includes a longitudinal cohort of 10,603 volunteers, aged 35-64 years at baseline, from the Turin area, Italy.

Framingham Heart Study The Framingham Heart Study began in 1948 with the recruitment of an original cohort of 5,209 men and women (mean age 44 years; 55 percent women). In 1971 a second generation of study participants was enrolled; this cohort consisted of 5,124 children and spouses of children of the original cohort. The mean age of the offspring cohort was 37 years; 52 percent were women. A third generation cohort of 4,095 children of offspring cohort participants (mean age 40 years; 53 percent women) was enrolled beginning in 2002. Details of study designs for the three cohorts are summarized elsewhere25-27. At each clinic visit, a medical history was obtained with a focus on cardiovascular content.

Health, Alcohol and Psychosocial factors In Eastern Europe The multi-centre study Health, Alcohol and Psychosocial factors In Eastern Europe (HAPIEE) study assessing the effects of dietary factors, alcohol consumption and psychosocial factors on health is being conducted in random samples of men and women aged 45-64 selected in Russia (city of Novosibirsk), Poland (2 districts of Krakow), Lithuania (city of Kaunas) and the Czech Republic (2 cities)

Health In Men Study The Health In Men Study (HIMS) arose out of a population-based randomized trial of screening for abdominal aortic aneurysms (AAAs) conducted in Perth, Western Australia in 199699. Only men aged 65 years and over were recruited into the trial as AAAs are uncommon below this age and are six times more common in men than women. The aim of the trial was to assess whether screening reduced mortality from AAA. Secondary outcomes included assessments of the impact of screening on all-cause mortality and quality of life3 and a study of the rates of expansion of screen-detected AAAs

Health Professionals Follow-up Study The Health Professionals Follow-up Study (HPFS) is a prospective cohort study of 51,529 US male health professionals aged 4075 years in 1986, who completed detailed questionnaires assessing dietary intake, lifestyle factors and medical history at baseline. Follow-up questionnaires were mailed to participants every 2 years to update baseline information and to ascertain

newly diagnosed disease. Participants included in this collaboration were from a nested case-control study of MI.

IMPROVE study The IMPROVE study is a multicentre, longitudinal, observational study, which involves seven recruiting centres in five European countries: Finland, France, Italy, the Netherlands, and Sweden. Recruitment of a total of 3598 patients (514 per centre) was targeted. Men and women, aged from 55 to 79 years, with at least three vascular risk factors, asymptomatic for cardiovascular diseases and free of any conditions that might limit longevity or IMT visualization were considered as eligible for the study. The primary objective of the IMPROVE study was to evaluate the association between C-IMT progression at 15 months and future vascular events (myocardial infarction, cardiovascular death, stroke, or any intervention in the carotid, coronary, or peripheral arterial districts occurring from the 15th to the 36th month of follow-up).

Inter99 study The Inter99 study is a population-based randomized controlled trial, investigating the effect of lifestyle intervention (smoking cessation, increased physical activity, and healthier dietary habits) on CVD. Data were collected with self-administered questionnaires, a physical examination, a 2 hour oral glucose tolerance test and various blood tests. The Inter99 study population were residents in the southern part of the former Copenhagen County. An age- and sex-stratified random sample of 13,016 men and women born in 193940, 194445, 194950, 195455, 195960, 196465, and 196970 was drawn from the Danish Civil Registration System and invited to participate in a health examination during 19992001, so that they were aged 30, 35, 40, 45, 50, 55, 60, and 65 years on the day of the examination. A total of 12,934 were eligible for invitation. The baseline participation rate was 52.5% (n = 6,784).

Ischemic Stroke Genetic Study/Siblings with Ischemic Stroke Study The Ischemic Stroke Genetic Study (ISGS) is a multicenter cohort study. Cases were recruited from inpatient stroke services at five United States academic medical centers. Cases are adult men and women over the age of 18 years diagnosed with first-ever ischemic stroke confirmed by a study neurologist on the basis of history, physical examination and CT or MR imaging of the brain. Cases had to be enrolled within 30 days of onset of stroke symptoms. The Siblings with Ischemic Stroke Study (SWISS) is a multicenter affected sibling pair study. Probands with ischemic stroke were enrolled at 66 US medical centers and 4 Canadian medical centers. Probands are adult men and women over the age of 18 years diagnosed

with ischemic stroke confirmed by a study neurologist on the basis of history, physical examination and CT or MR imaging of the brain. Probands were required to have a history of at least one living sibling with a history of stroke.

Izhevsk Family study The Izhevsk Family study is a population-based case-control study, conducted between 2003-2005 to investigate the causes of working age male mortality. The case-control study used proxy informants (usually wife or partner) to find out about the circumstances and behaviours of the deceased men. The participants contributing towards the *ADH1B* project are controls who were followed-up as a cohort.

Malmo Diet and Cancer The Malmo Diet and Cancer (MDC) study is set in Malm, Sweden's third largest city. The background population consisted of all men born between 1923 and 1945 and all women born between 1923 and 1950 who were living in Malm during the screening period 1991 to 1996 (n = 74,138). This population was identified through the Swedish national population registries. The final cohort consisted of 28,098 individuals (participation rate 40.8%). The subjects were recruited through advertisements in local media and through invitation by mail. The only exclusion criteria were inadequate Swedish language skills and mental incapacity. The Ethics Committee at Lund University approved the design of the MDC study (LU 5190). Written informed consent was obtained from the participants.

Medical Research Council 1958 Birth Cohort The 1958 birth cohort or the National Child Development Study (NCDS) was designed to examine how developmental, lifestyle, and environmental factors act throughout the lifespan to influence current ill health, and physiological and psychological function in early middle age. Participants are survivors from an original sample of over 17 000 births, all born in England, Wales, and Scotland, during 1 week in 1958, and followed-up by parental interview and examination at ages 7, 11, and 16 yr and by cohort member interview at 23, 33, and 42 yr. The first biomedical assessment in adulthood was conducted by a research nurse visiting the home at 4445 yr. During childhood, cohort members were traced through schools and immigrants born in the reference week were added to the sample. The cohort is flagged for mortality and cancer registration.

Medical Research Council National Survey of Health and Development The Medical Research Council (MRC) National Survey of Health and Development (NSHD) is an ongoing prospective birth cohort study consisting of a sample of all singleton births, born to married mothers, in England, Scotland and Wales in one week in March 1946. The sample includes all births whose fathers were in

non-manual or agricultural occupations and a randomly selected one in four of all others, whose fathers were in manual occupations. The original cohort comprised 2,547 women and 2,815 men who have been followed up over 20 times since their birth. The data collected to date include cognitive function, physical, lifestyle and anthropomorphic measures as well as blood analytes and other measures. Through MRC Unit funding, a particularly intensive clinical assessment, with biological sampling, blood and urine sampling and analysis, and cardiac and vascular imaging has recently been completed when the cohort were aged 60-64 years.

Multi-Ethnic Study of Atherosclerosis The Multi-Ethnic Study of Atherosclerosis (MESA) investigation is a population-based study of 6,814 men and women age 45 to 85 years, without clinical cardiovascular disease, recruited from six United States communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and St. Paul, MN). The main objective of MESA is to determine the characteristics of subclinical cardiovascular disease and its progression. Sampling and recruitment procedures have been previously described in detail57. Adults with symptoms or history of medical or surgical treatment for cardiovascular disease were excluded. During the recruitment process, potential participants were asked about their race/ethnicity. Self-reported ethnicity was used to classify participants into groups58.Additional individuals were derived from the MESA Family Study, an ancillary study to MESA whose goal is to identify genes contributing to the risk for cardiovascular disease, by looking at the early manifestations of atherosclerosis within families, mainly siblings. MESA Family studied siblings of index subjects from the MESA study and sib-pairs in new families ascertained through index subjects meeting MESA enrollment criteria. In a small proportion of subjects, parents of MESA index subjects participating in MESA Family were studied but only to have blood drawn for genotyping. The MESA Family cohort was recruited from the six MESA Field Centers during May 2004 - May 2007. The number of non-classic MESA family members recruited was 1,633 (950 African-Americans and 683 Hispanic-Americans) from 594 families, yielding 3,026 sib-pairs. Participants underwent the same examination as MESA participants.

Multinational monitoring of trends and determinants in cardiovascular diseases: Czech The Multinational monitoring of trends and determinants in cardiovascular diseases (MONICA) was established in the early 1980s in many Centres around the world to monitor trends in cardiovascular diseases, and to relate these to risk factor changes in the population over a ten year period. All suspected coronary events in the study populations were monitored continuously from mid 1980s to mid 1990s. MONICA Czech represents the component of MONICA set in Czech Republic.

The cohort used for analysis continued after the official international MONICA collaboration, and is termed "Czech post-MONICA".

National Health and Nutrition Examination Survey III The National Health and Nutrition Examination Survey (NHANES) is an ongoing series of surveys that have been conducted by the National Center for Health Statistics since the early 1960s to assess the health and nutritional status of the US civilian non-institutionalized population using a complex, stratified, multistage survey design. NHANES has been reviewed and approved by the Institutional Review Board at the National Center for Health Statistics. DNA Specimens were available for 7,159 individuals who participated in the second phase of NHANES III (1991-1994), were 12 years of age or older, and who consented to having specimens of their blood stored for future research. Household interview data provided information on age, sex, race/ethnicity, alcohol intake, smoking status, educational attainment, physical activity, and history of heart attack and diabetes. Physical examination data provided information on body mass index, waist circumference, and blood pressure. Serum samples provided information on cotinine, cholesterol, triglycerides, and glucose. Individuals who self-reported their race/ethnicity as non-Hispanic white were eligible to be included in the current analysis. Binge drinking was defined as drinking five or more drinks of alcohol on one or more days in the past year. Hypertension was defined as SBP 140 mmHg and/or diastolic blood 90. Fasting glucose levels were available in a subset of participants (n=1108) who had fasting blood samples drawn in the morning.

Nordic Diltiazem Study The Nordic Diltiazem intervention study (NORDIL) was started in September 1992. This trial was a prospective randomized open blinded-endpoint multicenter, parallel-group study conducted in Norway and Sweden. The study was designed to evaluate the potential preventive effects of diltiazem compared with conventional antihypertensive drug treatment. Primary endpoints were cardiovascular mortality defined as fatal acute myocardial infarction, fatal acute cerebrovascular disease (stroke), sudden death and other fatal cardiovascular disease as well as cardiovascular morbidity defined as myocardial infarction and cerebrovascular disease (stroke). Secondary endpoints are total mortality, the development or deterioration of ischemic heart disease, congestive heart failure, atrial fibrillation, transient ischemic attacks, diabetes mellitus and renal insufficiency. Male and female patients, aged 50-69, with primary hypertension were randomly allocated to therapy starting with either diltiazem (180-360 mg daily) or conventional treatment (diuretics or beta-adrenergic blockers). Add-on therapy in the conventional treatment group excluded all types of calcium antagonists. The goal

of treatment was a target diastolic blood pressure of 90 mmHg or a 10% diastolic blood pressure reduction.

Northwick Park Heart Study II The Northwick Park Heart Study II is a prospective study of 3,012 healthy middle-aged men aged 50-64 years at recruitment, sampled from nine UK general practices between 1989 and 1994. Full details of recruitment, measurements, follow-up and definitions of incident disease have been reported elsewhere64. Exclusion criteria were: history of unstable angina or acute myocardial infarction, a major Q wave on the ECG, regular anti-platelet or anticoagulant therapy, cerebrovascular disease, and life-threatening malignancy. Blood pressure was recorded with a random-zero sphygmomanometer (average of 2 measurements) at baseline and on five following annual visits. Baseline measures were used for these analyses.

Nurses Health Study I The Nurses Health Study I (NHS), established in 1976, is a prospective cohort study of 121,701 US female registered nurses aged 30-55 years at baseline, who completed detailed questionnaires assessing diet, lifestyle and medical history. Follow-up questionnaires were mailed to participants every 2 years to update baseline information and to ascertain newly diagnosed disease. Participants included in this collaboration were from a nested case-control study of MI.

Portuguese stroke study The Portuguese stroke study consisted of five-hundred sixty-five unrelated patients with a clinical diagnosis of ischemic stroke, who were under the age of 65 at stroke onset, recruited through Neurology and Internal Medicine Departments throughout Portugal. Stroke was defined by the presence of a new focal neurological deficit, with an acute onset and with symptoms and signs persisting for more than 24 h. The stroke was confirmed in all patients by a computed tomography scan in 97% of cases and/or magnetic resonance imaging in 25% of patients. All patients were seen, and all neuroradiology tests were reviewed by study neurologists. Trauma, tumors, infection, and other causes of neurological deficit were excluded. Data collection forms were developed for this study that included extensive clinical information such as stroke characteristics, general clinical observation, neurological symptoms and signs, complications and interventions during hospitalization, and situation at discharge. Data were also collected on relevant lifestyle aspects and previous clinical risk factors. Five-hundred seventeen unrelated healthy individuals were included in this study as a control sample population. Control individuals were verified to be free of stroke by direct interview before recruitment, but no brain imaging studies were performed. The interview

also included questions on established clinical and lifestyle risk factors for stroke. All participants were adults of Portuguese Caucasian origin.

Prevention of REnal and Vascular ENd stage Disease The Prevention of REnal and Vascular ENd stage Disease (PREVEND) study is an ongoing prospective study investigating the natural course of increased levels of urinary albumin excretion and its relation to renal and cardiovascular disease65,66. Inhabitants 28 to 75 years of age (N=85,421) in the city of Groningen, The Netherlands, were asked to complete a short questionnaire, 47% responded, and individuals were then selected with a urinary albumin concentration of at least 10 mg/L (N= 7,768) and a randomly selected control group with a urinary albumin concentration less than 10 mg.

PRrecOcious Coronary ARtery DIsease Study The Precocious Coronary Artery Disease study (PROCARDIS) is a European consortium investigating the genetics of coronary artery disease (CAD) in German, Italian, Swedish, and British CAD patients and controls. Controls in this study had no personal history of CAD, hypertension, or diabetes. Ascertainment criteria for PROCARDIS probands were MI or symptomatic ACS (SACS), on the assumption that the latter represents a similar pathological process according to modified World Health Organisation diagnostic criteria before the age of 66 y. Diagnosis of MI required documentation of two or more of: (a) typical ischemic chest pain, pulmonary oedema, syncope or shock; (b) development of pathological Q-waves and/or appearance or disappearance of localized ST-elevation followed by T-wave inversion in two or more standard electrocardiograph leads; (c) increase in concentration of serum enzymes consistent with MI (e.g. creatine kinase more than twice the upper limit of normal). Diagnosis of SACS required documentation of hospitalization for one of the following indications: (a) unstable angina diagnosed by typical ischemic chest pain at rest associated with reversible ST-depression in two or more standard electrocardiograph leads; (b) thrombolysis for suspected MI (as indicated by localized ST-elevation in two or more standard electrocardiograph leads) even without later development of T-wave inversion, Q-waves, or a significant enzyme rise; or (c) emergency revascularization (i.e. during same admission) following presentation with typical ischemic chest pain at rest. Probands completed questionnaires in order to recruit affected siblings with a range of CAD diagnoses at age ¡66 y (MI, SACS, chronic stable angina, or intervention for coronary revascularization), who were then invited to participate in the study if their diagnoses were confirmed. Parents and up to four unaffected siblings per family were recruited wherever possible to augment the recovery of linkage phase information. Informative families were recruited in Germany, Italy, Sweden, and the United Kingdom; 99.5% of

the study participants reported having a white European ancestry. The protocol was approved by the Ethics Committees of the participating institutions and all participants gave written, informed consent.

Prospective Study of Pravastatin in the Elderly at Risk The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trial was designed to determine whether pravastatin 40 mg/day has primary and secondary roles in reducing coronary and cerebral events in older patients with preexisting vascular disease or who are at high risk for vascular disease and stroke. The double-blinded, randomized, controlled trial initially screened 23,770 patients, and the patient population was subsequently narrowed (due to ineligibility or refusal to participate) to 5804 patients who were then randomized to either placebo (n = 2913) or 40 mg of pravastatin (n = 2891). Patients were recruited if they had either preexisting vascular disease (coronary, cerebral, or peripheral) or were at increased risk for vascular disease due to such factors as smoking, hypertension, or diabetes. Inclusion criteria called for men and women between the ages of 70 and 82 years with a total plasma cholesterol of 155-350 mg/dL (4-9/mmol/L) and triglyceride levels ¡ 200 mg/dL (6 mmol/L) . Patients were excluded if they showed signs of cognitive decline, which was assessed by a Mini Mental State Examination and a series of psychometric tests. The study population was distributed evenly between those with existing vascular disease and those with qualifying risk factors. Patients were followed every 3 months for an average of 3.2 years.

Rotterdam Study The Rotterdam Study is an ongoing, prospective, population-based cohort study on determinants of a number of chronic diseases. All inhabitants of Ommoord, a district of Rotterdam in the Netherlands, who were 55 years or over, were invited to participate in this study. Of all 10275 eligible individuals, 7983 agreed to participate (78%). Written informed consent was obtained from all participants and the Medical Ethics Committee of the Erasmus Medical Center approved the study.

Second Manifestations of Arterial disease The Second Manifestations of ARTerial disease (SMART) study is an ongoing, prospective, single-center cohort study in patients with clinically manifest vascular disease or cardiovascular risk factors. The main inclusion criteria are coronary artery disease, cardiovascular disease, peripheral arterial disease, abdominal aortic aneurysm, or any or all of the following risk factors for atherosclerosis: hyperlipidemia, diabetes mellitus (type 1 and 2), or hypertension. Patients with a terminal malignancy, patients not able to live independently (Rankin scale >3), or patients who are not sufficiently fluent in Dutch were excluded.

Thrombosis Prevention Trial The Thrombosis Prevention Trial (TPT) was a factorial -designed trail of aspirin and warfarin in prevention of CHD. The trial was done through 108 General practices representing all parts of the UK in men aged between 45 years and 69 years. Those excluded were current or recent history of possible peptic ulceration, a history of possible or definite MI or stroke, and other medication incompatible with trial treatment. Men in the top 20% of the risk score distribution, or in the top 25% in regions with particularly high IHD mortality rates, were considered to be at increased risk and eligible for the trial (n=10,557). Participants who decided to take part in the trial (n=5499) visited their doctor for a medical examination, including an electrocardiogram (ECG), to confirm eligibility.

Uppsala Longitudinal Study of Adult Men Uppsala Longitudinal Study of Adult Men (ULSAM)is a longitudinal, epidemiologic study based on all available men, born between 1920 and 1924, in Uppsala County, Sweden. The men were investigated at the ages of 50, 60, 70, 77, 82 and 88 years. Full screening and official registry data are available.

Utrecht Cardiovascular Pharmacogenetics study The Utrecht Cardiovascular PHarmacogenetics (UCP) study enrolled participants from the population-based PharmacoMorbidity Record Linkage System (PHARMO, www.pharmo.nl). PHARMO links drug dispensing histories from a representative sample of Dutch community pharmacies to the national registration of hospital discharges (Dutch National Medical Registry). First, patients who received a prescription for an antihypertensive drug, and/or had hypercholesterolemia (prescription for a cholesterol-lowering drug or total cholesterol >5.0mmol/l), were selected from the PHARMO database for pharmacogenetic studies on antihypertensive drugs and statins, respectively. From this cohort, a nested casecontrol study was designed using hospital discharge records. Patients hospitalized for MI [International Classification of Diseases 9 code 410] were included as cases if they were registered in PHARMO for at least 1 year and were older than 18 years. The index date was defined as the date of hospitalization for the first MI. Controls met the same eligibility criteria as the cases, but had not developed MI. Controls were matched with cases on age, sex, and region, and assigned the same index.

Whitehall II The Whitehall II Study recruited 10,308 participants (70% men) between 1985 and 1989 and involved 20 London based civil service departments. In this longitudinal study blood pressure was recorded at phase 1 (1985-1988), phase 3 (1991-1993), phase 5 (1997-1999) and phase 7 (2003-2004). DNA was stored

from phase 7 from over 6,000 participants. The study participants are all highly phenotyped for cardiovascular and other ageing related health outcomes.

Women's Health Initiative The Women's Health Initiative (WHI) was initiated in 1992 as a major disease-prevention research program among postmenopausal women. The program includes a randomized controlled intervention trial involving 68,132 women and four distinct interventions: conjugated equine estrogens, alone or in combination with medroxyprogesterone acetate, for coronary heart disease prevention with breast cancer as an anticipated adverse effect; a low-fat eating pattern for breast and colorectal cancer prevention; and calcium and vitamin D supplementation for hip fracture prevention

C.2 Supplemental Tables

Table C.1: Design and genotyping characteristics of the studies included in the collaboration

Study	Study design	Sampling Frame	N with DNA in this analysis	Year of blood sampling used for DNA extraction	Genotyping method	Country	Contributes to observational analysis	HWE P value (exact significance probability)	Call rate (%)
ALSPAC	Cohort	Pregnant women (Avon County)	2557	1991-2010	KASPar	UK	N	0.41	97.7
ARIC	Cohort	Community	9557	1987-89	IBC 50k CardioChip	USA	Y	0.705	97.8
BRHS	Cohort	General practices	3843	1998-2000	KASPar	UK	Y	0.42	100
BWHHS	Cohort	General practices	3412	1999-2001	Illumina HumanCVD array	UK	Y	0.912	99.7
CaPS	Cohort	Electoral register & General practices	1102	1993-1994	KASPar	UK	Y	0.46	98.4
CARDIA	Cohort	Community	1433	1995-1996	IBC 50k CardioChip	USA	Y	0.0005	97.3
CCHS	Cohort	Population	9081	1991-94	Nanogen	Denmark	Y	0.522	99.6
CFS	Cohort	Family	134	2001-2006	IBC 50k CardioChip	USA	N	0.462	98
CGPS	Cohort	Population	57041	2003-ongoing	TaqMan	Denmark	N	0.473	NA
CHS	Cohort	Community	3936	19921993	IBC 50k CardioChip	USA	Y	0.001	97.9
CYPRUS	Cohort	Community	730	2003-2008	TaqMan	Cyprus	Y	0.081	99.9
Czech post-MONICA	Cohort	Administrative districts	2558	2000-2001	PCR-RFLP	Czech Republic	Y	0.801	97.9
DCH	Case cohort	General population (born in Denmark)	2736	1993-97	TaqMan	Denmark	Y	0.203	91.8
EAS	Cohort	General practices	873	2004	TaqMan	UK	Y	0.693	95.6
ELSA	Cohort	Respondents of HSE	5450	2004	KASPar	UK	Y	0.263	98.8
EPIC InterAct	Nested case control	Population	9427	1991-1998	Metabochip plus	Denmark, France, Germany, Italy, Netherlands, Spain, Sweden, UK	N	0.0013	99.6

Continued on next page

Table C.1 – *Continued from previous page*

Study	Study design	Sampling Frame	N with DNA in this analysis	Year of blood sampling used for DNA extraction	Genotyping method	Country	Contributes to observational analysis	HWE P value (exact significance probability)	Call rate (%)
EPIC Netherlands	Nested case control	Population (Bilthoven & Utrecht)	5186	1993 and 1997	IBC 50k CardioChip	The Netherlands	N	0.095	99.9
EPIC Norfolk	Nested case control	Population (Norwich & E Anglia)	20195	1997-2000	TaqMan	UK	Y	0.091	99
EPIC Potsdam	Case cohort	Population (Potsdam (Germany)	2253	2007	TaqMan	Germany	N	0.454	98.8
EPIC Turin	Cohort	Population (Torino area)	4526	2008	TaqMan	Italy	Y	0.362	99
FHS	Cohort	Community	1082	1948-present	IBC 50k CardioChip	USA	Y	0.002	99
HAPIEE Czech	Cohort	City districts	6678	2003-2005	KASPar	Czech Republic	Y	0.745	98.6
HAPIEE Lithuania	Cohort	City districts	6936	2006-2008	KASPar	Lithuania	Y	0.149	98.6
HAPIEE Poland	Cohort	City districts	8779	2003-2005	KASPar	Poland	Y	0.238	97
HAPIEE Russia	Cohort	City districts (Novosibirsk City)	7083	2003-2005	KASPar	Russia	Y	0.041	98.8
HIMS	Cohort	City population	4191	2001-04	TaqMan	Australia	Y	4.06E-08	98.7
HPFS	Nested case control	Health Professionals	1264	1994	IBC 50k CardioChip	USA	N	0.45	93
IMPROVE Groningen	Cohort	Clinic	421	2004-2005	TaqMan	Netherlands	N	0.109	>99
IMPROVE Kuopio 1	Cohort	Clinic	481	2004-2005	TaqMan	Finland	N	0.927	>99
IMPROVE Kuopio 2	Cohort	Clinic	440	2004-2005	TaqMan	Finland	N	0.943	>99
IMPROVE Milan	Cohort	Clinic	514	2004-2005	TaqMan	Italy	N	0.574	>99
IMPROVE Paris	Cohort	Clinic	436	2004-2005	TaqMan	France	N	0.008	>99
IMPROVE Perugia	Cohort	Clinic	464	2004-2005	TaqMan	Italy	N	0.347	>99
IMPROVE Stockholm	Cohort	Clinic	480	2004-2005	TaqMan	Sweden	N	3.43E-09	>99
Inter99	RCT	Population	6332	1999-2001	KASPar	Denmark	Y	6.16E-27	97.6
ISGS-SWISS	Case control	Clinic	1124	2002-2008	TaqMan	USA	N	1	>99
Izhevsk	Case control	Population-based controls from CC	653	2008-2009	PCR + electrophoresis	Russia	Y	0.192	>99
MDC	Cohort	Population	1937	1991-1996	IBC 50k CardioChip	Sweden	N	0.537	>99
MESA	Cohort	Population	2293	2000-2002	IBC 50k CardioChip	USA	Y	0.012	97

Continued on next page

Table C.1 – *Continued from previous page*

Study	Study design	Sampling Frame	N with DNA in this analysis	Year of blood sampling used for DNA extraction	Genotyping method	Country	Contributes to observational analysis	HWE P value (exact significance probability)	Call rate (%)
MRC NSHD	Cohort	Population (Born 3-9 March 1946)	2696	1999	KASPar	UK	N	0.222	>99
MRC 1958BC	Cohort	Population	2587	2002-2004	Illumina 1.2M	UK	N	0.2609	95.4
NHANES III	Cross-sectional	General population	2388	1991-1994	TaqMan	USA	N	1	98
NHS	Nested case control	Nurses (Boston)	1322	1990	IBC 50k CardioChip	USA	N	<2.2E-16	97
NORDIL	RCT	Clinic	1921	1992-1999	IBC 50k CardioChip	Norway and Sweden	N	1	>99
NPHS II	Cohort	General practices	2659	2000	TaqMan	UK	Y	0.874	96.1
Portuguese Stroke Study	Case control	Clinic	1002	1995 and 1998	TaqMan	Portugal	N	0.006	99.4
PREVEND	Cohort	Mixed population (Groningen City)	7729	1997-1998	KASPar	Netherlands	N	3.05E-09	>95
PROCARDIS	Case control	Hospital	6440	1998-200?	IBC 50k CardioChip	Germany, Italy, Sweden, UK	N	1	>99
PROSPER	RCT	Elderly; cholesterol 4-9 mmol/l	5504	1997 to 1999	TaqMan	Scotland, Ireland, The Netherlands	N	0.008	95.5
Rotterdam	Cohort	Administrative district	5827	1992	TaqMan	Netherlands	Y	0.341	90
SMART	Cohort	Atherosclerosis hospital referrals	7917	1996-2006	KASPAR	Netherlands	N	3.85E-24	97
TPT	RCT	Acute coronary syndrome	3175	1984-1989	TaqMan	UK	N	0.68	86.2
UCP	Nested case control	Hospital patients	1615	2007	IBC 50k CardioChip	Netherlands	N	1	100
ULSAM	Cohort	General population (Uppsala County)	453	2004	Illumina Golden Gate	Sweden	Y	0.775	98.91
Whitehall II	Cohort	Workplace (civil servants)	5029	2002-2004	IBC 50k CardioChip	UK	Y	0.106	99.3
WHI	Nested case control	Community	7882	1993-1998	IBC 50k CardioChip	USA	Y	3.15E-25	99.2

Table C.2: Characteristics of the alcohol questionnaires used in the collaborating studies

STUDY	Questionnaire beverage specific	Binge drinker	Alcohol Abstainer
ALSPAC	Y	≥4 drinks / occasion	Not drinking at 5 waves, before and after pregnancy
ARIC	Y	>70g/ day	Self-declared abstainer at first wave
BRHS	Y	>6 drinks / occasion	Never consumed alcoholic drinks
BWHHS	Y	> 6 drinks/ day	Never drink at baseline
CaPS	Y	≥ 5 drinks/ normal occasion	Not drinking at 5 waves
CARDIA	Y	> 5 drinks on day drank most in past month	Not drinking at 4 waves
CCHS	Y	N/A	N/A
CFS	N/A	N/A	Never drink alcohol
CGPS	Y	N/A	N/A
CHS	Y	≥ 5 drinks per day	N/A
Cyprus	Y	N/A	N/A
Czech post-MONICA	Y	N/A	No drinking in past six months
DCH	Y	N/A	Answering never drink to all beverages
EAS	Y	N/A	N/A
ELSA	Y	> 10 units on heaviest day in last 7	Always an abstainer
EPIC-InterAct	Y	N/A	N/A
EPIC Netherlands	Y	N/A	Never drink alcohol
EPIC Norfolk	Y	N/A	Never drunk alcohol in the past
EPIC-Potsdam	Y	N/A	Never drunk alcohol in the past
EPIC Turin	Y	N/A	Never drunk beer/ wine/ spirits
FHS	Y	≥5 beverage specific drinks at one time	N/A
HAPIEE Czech	N	≥5 drinks/ day	Answering never drink to all beverages

Continued on next page

Table C.2 – *Continued from previous page*

STUDY	Questionnaire beverage specific	Binge drinker	Alcohol Abstainer
HAPIEE Lithuania	N	≥5 drinks/ day	Answering never drink to all beverages
HAPIEE Poland	N	≥5 drinks/ day	Answering never drink to all beverages
HAPIEE Russia	N	≥5 drinks/ day	Answering never drink to all beverages
HIMS	N	≥5 drinks on usual drinking day	Never drunk alcohol
HPFS	N	>6 drinks on largest day in typical month	Self-declared abstainer (in 1994)
IMPROVE	Y	N/A	No beer/wine/spirits (time period not specified
Inter99	Y	≥5 drinks at least once/ week	Self-declared abstainer in past year
ISGS-SWISS	N	N/A	Self-declared abstainer/rare drinker in past year
Izhevsk	Y	≥5 drinks on one occasion	Never drunk in life other than few occasions
MDC	Y	N/A	N/A
MESA	Y	> 5 drinks on day drank most	Not drinking at 4 waves
MRC NHSD	Y	N/A	N/A
MRC 1956BC	N/A	N/A	N/A
NHANES III	N	≥5 drinks on any day in the past 12 months	Less than 12 drinks in entire life
NORDIL	N/A	N/A	N/A
NPHS II	Y	N/A	N/A
NHS	N	N/A	Self-declared abstainer (in 1990)
Portuguese Stroke Study	N/A	N/A	N/A
PREVEND	N	N/A	Almost never drink
PROCARDIS	N/A	N/A	N/A
PROSPER	N	N/A	N/A

Continued on next page

Table C.2 – *Continued from previous page*

STUDY	Questionnaire beverage specific	Binge drinker	Alcohol Abstainer
Rotterdam	Y	>6 alcoholic beverages on one day during the last year	Self-declared abstainer in past year
SMART	N/A	N/A	N/A
TPT	N/A	N/A	N/A
UCP	Y	N/A	Self-declared never used alcohol
ULSAM	Y	N/A	Self-declared abstainer(age 60)
WHITEHALL II	N	>5 beers or wine / spirits in one sitting	Non drinker at 3 waves
WHI	Y	N/A	Less than 12 drinks in entire life

Table C.3: Event definitions in the studies included in the collaboration

	Coronary heart disease								Stroke (combined subtypes)					Diabetes	
	Fatal		Non-fatal			Non-fatal			Fatal			Non-fatal			
Study	Self report	Medical records	Clinical/ laboratory measures	Death certificate	ICD coded	Self report	Medical records	Clinical/lab. /imaging measures	Death certificate	ICD coded	Self report	Medical records	Clinical/lab. measures		
ALSPAC															
ARIC		•		•	•		•		•	•		•	•		
BRHS		•		•	•		•		•	•		•			
BWHHS	•	•		•	•	•	•		•	•	•	•			
CaPS	•	•		•	•	•	•		•	•	•	•	•		
CARDIA		•		•			•		•			•			
CCHS		•		•	•		•		•	•	•				
CFS	•					•						•			
CGPS															
CHS	•	•		•	•	•	•		•	•	•		•		
Cyprus	•	•	•			•	•				•		•		
Czech post-MONICA	•			•		•			•		•		•		
DCH		•		•	•		•		•	•	•				
EAS	•	•	•	•	•	•	•		•	•	•				
ELSA	•				•	•	•			•	•		•		
EPIC InterAct											•	•			

Continued on next page

Table C.3 – *Continued from previous page*

	Non-fatal			Fatal		Non-fatal			Fatal		Non-fatal		
Study	Self report	Medical records	Clinical/ laboratory measures	Death certificate	ICD coded	Self report	Medical records	Clinical/lab. /imaging measures	Death certificate	ICD coded	Self report	Medical records	Clinical/lab. measures
EPIC Norfolk		•		•	•		•		•	•	•	•	
EPIC Netherlands		•		•	•		•		•	•	•	•	
EPIC Potsdam		•		•	•	•						•	
EPIC Turin		•	•	•	•		•	•	•	•		•	•
FHS		•		•			•		•			•	
HAPIEE Czech	•	•	•	•	•	•	•	•	•	•	•		
HAPIEE Lithuania	•	•	•	•	•	•	•	•	•	•	•		
HAPIEE Poland	•	•	•	•	•	•	•	•	•	•	•		
HAPIEE Russia	•	•	•	•	•	•	•	•	•	•	•		
HIMS	•	•		•	•	•	•		•	•	•	•	•
HPFS	•	•	•	•	•	•					•		
IMPROVE	•	•		•		•	•		•		•	•	•
Inter99	•					•					•		•
ISGS-SWISS							•	•	•				
Izhevsk											•		
MDC		•	•	•	•		•	•	•	•	•	•	•
MESA	•					•					•		

Continued on next page

Table C.3 – *Continued from previous page*

	Non-fatal				Fatal		Non-fatal			Fatal		Non-fatal		
Study	Self report	Medical records	Clinical/ laboratory measures	Death certificate	ICD coded	Self report	Medical records	Clinical/lab. /imaging measures	Death certificate	ICD coded	Self report	Medical records	Clinical/lab. measures
MRC 1958BC											•		•
MRC NSHD	•					•					•		
NHANES III	•					•					•		
NHS	•	•	•	•	•	•					•		
NORDIL		•	•	•	•		•	•	•	•	•	•	•
NPHS-II	•	•		•	•	•	•		•	•	•	•	
Portuguese Stroke Study							•	•					
PREVEND		•		•	•		•		•	•		•	
PROCARDIS		•	•										
PROSPER		•	•	•	•		•	•	•	•		•	•
Rotterdam		•	•				•	•				•	•
SMART													
TPT		•		•			•		•			•	
UCP		•											
ULSAM		•		•	•		•		•	•	•	•	•
Whitehall II		•		•			•		•			•	•
WHI	•					•					•		

Table C.4: General and alcohol characteristics of studies included in the collaboration

Study Name	Age (yrs)		Gender (male)	Median Alcohol (units/wk)		Ln units/week		Binge drinking (5 drinks in one setting)	Self-declared non-drinker	Ln GGT (IU/L)	
	N	mean (SD)	Proportion (%)	Men	Women	N	mean (SD)	Proportion (%)	Proportion (%)	N	mean (SD)
ALSPAC	2557	47.75 (4.31)	0	NA	NA	NA	NA	NA	NA	NA	NA
ARIC	9557	54.28 (5.69)	46.46	2	0	9532	0.98 (1.26)	0.62	18.15	NA	NA
BRHS	3843	68.74 (5.51)	100	8	NA	3789	1.94 (1.28)	7.32	3.35	3790	3.35 (0.60)
BWHHS	3412	68.86 (5.51)	0	NA	0	3407	0.99 (1.19)	0.28	16.24	3334	3.12 (0.63)
CaPS	1102	51.71 (4.37)	100	14	NA	1061	2.40 (1.23)	40.2	2.12	NA	NA
CARDIA	1433	25.58 (3.37)	46.34	11	5	1433	1.89 (1.22)	38.03	4.4	1427	1.86 (0.60)
CCHS	9081	58.38 (15.09)	44.49	15	5	8985	2.03 (1.31)	NA	NA	8254	3.59 (0.64)
CFS	134	53.2 (14.75)	57.46	NA	NA	NA	NA	NA	46.97	NA	NA
CGPS	57041	56.10 (13.30)	43.63	18	9	56970	2.37 (1.10)	15.96	9.73	56997	3.45 (0.57)
CHS	3936	72.78 (5.60)	43.83	1	0	3919	0.83 (1.18)	9.16	NA	NA	NA

Continued on next page

Table C.4 – *Continued from previous page*

Study Name	Age (yrs)		Gender (male)	Median Alcohol (units/wk)		Ln units/week		Binge drinking (5 drinks in one setting)	Self-declared non-drinker	Ln GGT (IU/L)	
	N	mean (SD)	Proportion (%)	Men	Women	N	mean (SD)	Proportion (%)	Proportion (%)	N	mean (SD)
Cyprus	730	60.48 (10.21)	46.85	2	0	729	0.64 (0.92)	NA	NA	NA	NA
Czech post-MONICA	2558	48.76 (10.73)	46.44	11	0	2558	1.38(1.34)	NA	38.58	NA	NA
DCH	2736	56.71 (4.47)	62.02	17	7	2735	2.47 (1.11)	NA	2.96	NA	NA
EAS	873	64.34 (5.62)	49.37	7	1	873	1.44 (1.19)	2.87	NA	872	3.21 (0.61)
ELSA	5450	67.51 (9.80)	45.56	10	3	5450	1.76 (1.24)	6.39	3.66	NA	NA
EPIC InterAct	9427	54.00 (9.70)	38	10	2	6090	1.66 (1.26)	NA	NA	NA	NA
EPIC Netherlands	5186	54.06 (10.11)	21.89	11	3	5164	1.58 (1.26)	NA	13.03	4339	3.23 (0.52)
EPIC Norfolk	20195	59.29 (9.23)	47.15	7	3	20005	1.59 (1.03)	NA	85.78	NA	NA
EPIC-Potsdam	2253	50.64(9.01)	40.26	7	4	2253	2.05 (1.11)	NA	NA	2253	2.93(0.84)
EPIC Turin	4526	49.09 (7.62)	62.62	24	3	4314	2.29(1.39)	NA	9.43	NA	NA

Continued on next page

Table C.4 – *Continued from previous page*

Study Name	Age (yrs)		Gender (male)	Median Alcohol (units/wk)		Ln units/week		Binge drinking (5 drinks in one setting)	Self-declared non-drinker	Ln GGT (IU/L)	
	N	mean (SD)	Proportion (%)	Men	Women	N	mean (SD)	Proportion (%)	Proportion (%)	N	mean (SD)
FHS	1082	45.7 (10.10)	49.35	5	0	312	1.25(1.33)	5.05	NA	725	4.87 (0.62)
HAPIEE Czech	6678	58.32 (7.13)	45.91	15	1	6553	1.77 (1.44)	22.15	11.68	900	3.24(0.62)
HAPIEE Lithuania	6936	60.96 (7.58)	45.73	5	1	6899	1.14 (1.15)	25.63	6.68	NA	NA
HAPIEE Poland	8779	57.69 (6.98)	49.09	3	0	8668	1.00 (1.47)	10.62	33.71	906	3.26 (0.59)
HAPIEE Russia	7083	58.85 (7.09)	43.1	3	0	7082	0.69 (1.18)	25.87	16.29	7080	3.30(0.54)
HIMS	4191	71.11 (4.22)	100	6	NA	4191	1.67(1.36)	6.16	5.81	NA	NA
HPFS	1264	64.35 (8.57)	100	5	NA	1263	1.73(1.3)	6.01	14.64	NA	NA
IMPROVE Groeningen	421	63.85 (6.05)	49.64	7	0	421	1.31(1.58)	NA	NA	NA	NA
IMPROVE Kuopio 1	481	63.93 (5.44)	60.91	6	0	481	1.28(1.36)	NA	NA	NA	NA
IMPROVE Kuopio 2	440	64.43 (5.52)	53.86	7	0	440	1.31(1.34)	NA	NA	NA	NA

Continued on next page

Table C.4 – *Continued from previous page*

Study Name	Age (yrs)		Gender (male)	Median Alcohol (units/wk)		Ln units/week		Binge drinking (5 drinks in one setting)	Self-declared non-drinker	Ln GGT (IU/L)	
	N	mean (SD)	Proportion (%)	Men	Women	N	mean (SD)	Proportion (%)	Proportion (%)	N	mean (SD)
IMPROVE Milan	514	65.28 (5.75)	48.44	18	0	514	2.00 (1.72)	NA	NA	NA	NA
IMPROVE Paris	436	64.34 (6.34)	50.23	14	0	436	1.58 (1.70)	NA	NA	NA	NA
IMPROVE Perugia	464	60.63 (4.15)	24.14	27	0	464	1.67 (1.60)	NA	NA	NA	NA
IMPROVE Stockholm	480	66.79 (0.38)	51.46	11	5	480	1.71 (1.42)	NA	NA	NA	NA
Inter99	6332	46.02 (7.91)	48.89	15	4	6025	2.25 (1.14)	36.65	9.83	NA	NA
ISGS-SWISS	780	72.06 (14.99)	28.9	NA	NA	NA	NA	NA	59.57	NA	NA
Izhevsk	653	48.21 (8.20)	100	10	NA	642	2.21 (1.27)	55.44	0.46	653	3.50 (0.76)
MDC	1937	57.78 (5.84)	57.61	10	5	1466	1.80 (1.13)	NA	NA	NA	NA
MESA	2293	62.7 (10.24)	52.25	7	2	2054	1.63 (1.29)	12.09	22.99	NA	NA
MRC 1958BC	2585	NA	51.87	2	1	2580	0.93 (0.39)	NA	NA	NA	NA

Continued on next page

Table C.4 – *Continued from previous page*

Study Name	Age (yrs)		Gender (male)	Median Alcohol (units/wk)		Ln units/week		Binge drinking (5 drinks in one setting)	Self-declared non-drinker	Ln GGT (IU/L)	
	N	mean (SD)	Proportion (%)	Men	Women	N	mean (SD)	Proportion (%)	Proportion (%)	N	mean (SD)
MRC NSHD	2696	53 (NA)	49.89	8	3	2696	1.64 (1.15)	NA	NA	NA	NA
NHANES III	2388	53.10 (20.60)	40	1	0	NA	NA	50	19.98	NA	NA
NHS	1322	59.95 (6.45)	0	NA	1	1322	1.08 (1.11)	3.33	37.9	NA	NA
NORDIL	1921	56.00 (3.98)	51.17	NA	NA	NA	NA	NA	NA	NA	NA
NPHS II	2659	56.10 (3.42)	100	6	NA	2659	1.85 (1.23)	NA	NA	NA	NA
Portuguese Stroke Study	1002	62.9 (6.8)	0	NA	NA	NA	NA	NA	NA	NA	NA
PREVEND	7729	49.56 (12.74)	49.24	5	1	7729	1.49 (1.19)	NA	24.53	NA	NA
PROCARDIS	6440	60.72 (9.04)	58.94	NA	NA	NA	NA	NA	NA	NA	NA
PROSPER	5504	75.33 (3.36)	48.27	4	0	5504	1.08 (1.16)	NA		NA	NA
Rotterdam	5827	69.12 (8.93)	41.07	9	1	4688	1.52 (1.3)	6.59	20.44	4187	3.22 (0.52)

Continued on next page

Table C.4 – *Continued from previous page*

Study Name	Age (yrs)		Gender (male)	Median Alcohol (units/wk)		Ln units/week		Binge drinking (5 drinks in one setting)	Self-declared non-drinker	Ln GGT (IU/L)	
	N	mean (SD)	Proportion (%)	Men	Women	N	mean (SD)	Proportion (%)	Proportion (%)	N	mean (SD)
SMART	8068	56.51 (12.40)	57.61	NA	NA	NA	NA	NA	NA	NA	NA
TPT	3175	57.30 (6.76)	100	NA	NA	NA	NA	NA	NA	NA	NA
UCP	1615	62.78 (9.65)	74.37	10	0	1323	1.75 (1.33)	NA	12.3	NA	NA
ULSAM	453	71.29 (0.44)	100	4	NA	421	1.58 (1.06)	NA	15.65	NA	NA
Whitehall II	5029	43.87(5.94)	73.53	9	4	4990	2.01(1.08)	37.99	2.57	NA	NA
WHI	7882	67.98 (6.58)	0	NA	0.5	7620	0.97 (1.14)	NA	11.33	NA	NA

Table C.5: Number and proportion of outcomes in studies included in the collaboration

Study	Coronary heart disease		Stroke (combined sub-types)		Diabetes		Hypertension	
	N	Proportion (%)	N	Proportion (%)	N	Proportion (%)	N	Proportion (%)
ALSPAC	NA	NA	NA	NA	NA	NA	145	5.81
ARIC	1308	13.77	520	5.44	1182	12.37	1195	12.51
BRHS	532	13.8	307	8	59	1.54	2551	66.38
BWHHS	303	13.7	290	8	338	9.91	2104	61.66
CaPS	193	28.47	NA	NA	10	0.91	685	62.16
CARDIA	10	0.7	4	0.28	99	6.91	26	1.81
CCCHS	993	10.94	NA	NA	303	3.34	4786	52.76
CFS	10	20.83	12	9.09	17	42.5	37	27.61
CGPS	NA	NA	NA	NA	NA	NA	30456	53.48
CHS	110	2.94	547	13.9	573	14.61	1527	38.85
Cyprus	41	5.48	4	0.55	98	13.42	450	61.64
Czech post-MONICA	58	2.27	52	2.03	100	3.97	715	27.95
DCH	135	NA*	66	NA*	105	3.84	1633	59.69
EAS	144	16.53	73	8.38	25	2.86	452	51.78
ELSA	184	3.38	155	2.84	360	6.61	3352	61.5
EPIC InterAct	NA	NA	NA	NA	3535	NA*	NA	NA
EPIC Netherlands	1221	NA*	443	NA*	369	8.26	1906	36.79
EPIC Norfolk	613	NA*	300	NA*	449	2.22	8218	40.77
EPIC Potsdam	224	NA*	30	NA*	106	4.7	812	36.04
EPIC Turin	32	0.74	9	0.21	63	1.46	1920	42.42
FHS	28	2.77	37	3.42	110	14.29	64	53.78

Continued on next page

Table C.5 – *Continued from previous page*

Study	Coronary heart disease		Stroke (combined sub-types)		Diabetes		Hypertension	
	N	Proportion (%)	N	Proportion (%)	N	Proportion (%)	N	Proportion (%)
HAPIEE Czech	401	6.12	293	4.47	350	5.26	3630	54.49
HAPIEE Lithuania	652	9.4	311	4.48	392	5.68	3824	55.35
HAPIEE Poland	673	7.67	207	2.36	656	7.49	4236	48.48
HAPIEE Russia	630	8.89	461	6.51	256	3.61	4038	57.03
HIMS	540	13.47	224	5.59	339	8.09	3410	81.36
HPFS	424	NA*	NA	NA	69	5.46	404	31.96
IMPROVE Groeningen	59	14.05	13	3.09	234	56.52	277	66.27
IMPROVE Kuopio 1	38	7.9	7	1.46	84	17.57	295	61.46
IMPROVE Kuopio 2	67	14.77	11	2.5	201	46.1	364	82.73
IMPROVE Milan	16	3.11	3	0.58	65	11.95	205	39.88
IMPROVE Paris	29	6.65	7	1.61	107	25.12	99	22.71
IMPROVE Perugia	47	10.13	8	1.72	69	15	231	49.78
IMPROVE Stockholm	38	7.92	13	2.71	97	20.77	367	76.46
Inter99	45	0.74	59	0.97	364	6.03	2341	36.97
ISGS-SWISS	NA	NA	794	NA *	NA	NA	NA	NA
Izhevsk	NA	NA	NA	NA	11	1.7	383	58.65
MDC	57	2.94	47	2.43	35	1.81	4	0.21
MESA	47	2.05	32	1.4	220	9.59	483	21.08
MRC 1958BC	NA	NA	NA	NA	78	3.1	609	23.64
MRC NSHD	42	1.56	20	0.74	77	2.86	1194	44.92
NHANES III	239	10.01	NA	NA	239	10.01	716	29.98
NHS	442	NA*	NA	NA	120	9.08	466	35.25

Continued on next page

Table C.5 – *Continued from previous page*

Study	Coronary heart disease		Stroke (combined sub-types)		Diabetes		Hypertension	
	N	Proportion (%)	N	Proportion (%)	N	Proportion (%)	N	Proportion (%)
NORDIL	23	1.2	26	1.35	131	6.82	1921	100
NPHSII	179	6.73	84	3.16	67	2.52	1362	51.22
Portuguese Stroke Study	NA	NA	569	NA*	NA	NA	NA	NA
PREVEND	451	5.95	197	2.59	274	3.56	2111	27.31
PROCARDIS	3116	NA *	NA	NA	NA	NA	NA	NA
PROSPER	731	13.28	617	11.21	592	10.76	4326	78.6
Rotterdam	1094	18.84	835	14.33	608	10.44	2672	47.26
TPT	36	1.13	NA	NA	NA	NA	1604	50.52
UCP	622	NA *	NA	NA	323	20.15	NA	NA
ULSAM	85	18.76	92	20.31	48	10.6	331	73.07
Whitehall II	212	4.22	116	2.31	183	3.64	765	15.21
WHI	2943	NA*	2126	NA*	NA	NA	2736	34.71

Footnote: * for case control, nested case-cohort or nested case-control studies, the proportion was not estimated

Table C.6: Lifestyle characteristics of the studies included in the collaboration

Study	Ever smoker	Smoking frequency (cigs/day)		ln Cotinine (nmol/l)		Pack years		Physical exercise (hours/week)		Education (years)	
	Proportion (%)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
ALSPAC	38.96	522	15.21 (8.72)	NA	NA	919	16.43 (14.00)	NA	NA	NA	NA
ARIC	59.75	1950	20.23 (12.18)	NA	NA	9382	17.02 (21.78)	NA	NA	NA	NA
BRHS	67.45	399	13.87 (9.09)	3666	-0.41 (2.9)	NA	NA	NA	NA	3325	11.35 (2.79)
BWHHS	43.9	353	12.25 (6.59)	3352	0.61 (2.61)	867	18.95 (16.98)	3275	2.74 (5.55)	3183	11.21 (2.60)
CaPS	81.59	447	27.27 (13.84)	NA	NA	NA	NA	1079	0.69 (0.46)	NA	NA
CARDIA	23.7	582	14.92 (10.72)	NA	NA	580	6.19 (6.77)	NA	NA	1433	14.76 (2.29)
CCHS	77.49	9010	8.13 (10.54)	NA	NA	NA	NA	8992	2.26 (0.71)	9011	9.16 (2.15)
CFS	57.5	NA	NA	NA	NA	127	16.83 (22.12)	NA	NA	NA	NA
CGPS	58.03	NA	NA	NA	NA	NA	NA	57041	2.47 (0.72)	NA	NA
CHS	54.18	NA	NA	NA	NA	3830	18.83 (27.59)	NA	NA	3927	14.00 (4.58)
Cyprus	38.63	282	24.21 (17.55)	NA	NA	730	13.56 (27.97)	729	2.67 (1.81)	NA	NA
Czech post-MONICA	47.15	1164	14.23 (9.44)	NA	NA	1206	16.89 (15.03)	NA	NA	2555	12.53 (2.66)
DCH	72.54	NA	NA	NA	NA	NA	NA	2710	1.24 (2.10)	NA	NA
EAS	60.58	518	15.96 (8.02)	NA	NA	502	25.08 (18.05)	873	.59(1.84)	NA	NA
ELSA	70.28	5403	2.67 (7.00)	NA	NA	5395	5.37 (14.34)	NA	NA	NA	NA
EPIC InterAct	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPIC Netherlands	63.85	1487	15.44 (9.03)	NA	NA	1471	25.54 (16.73)	5186	2.81(1.03)	NA	NA
EPIC Norfolk	54.43	2048	14.33 (8.21)	NA	NA	20185	9.88 (15.35)	20195	2.3 (1.09)	20185	1.31 (1.09)
EPIC Potsdam	54.68	2253	3.12 (7.24)	NA	NA	NA	NA	2253	0.99 (1.71)	NA	NA
EPIC Turin	59.7	1049	13.41 (9.07)	NA	NA	1048	19.27 (14.81)	4313	327.89 (275.32)	4305	11.97 (4.53)
FHS	61.15	NA	NA	NA	NA	112	13.16 (19.05)	NA	NA	768	14.35 (2.77)
HAPIEE Czech	55.32	4043	12.21 (9.81)	NA	NA	3462	20.97 (16.88)	6496	4.32 (5.32)	NA	NA
HAPIEE Lithuania	37.3	2487	15.00 (9.81)	NA	NA	2479	22.82 (18.36)	6894	3.25 (5.91)	NA	NA
HAPIEE Poland	59.34	6174	15.27 (12.23)	NA	NA	5069	28.15 (20.14)	8329	5.39 (6.06)	NA	NA
HAPIEE Russia	40	2793	16.06 (9.56)	NA	NA	2792	28.08 (20.44)	7076	2.41 (5.86)	NA	NA
HPFS	41.61	111	2.00 (0.97)	NA	NA	1232	15.18 (20.37)	1264	3.49 (5.01)	NA	NA
HIMS	66.45	4176	14.56 (17.11)	NA	NA	2740	37.75 (34.10)	4186	5.52(5.63)	4189	3.53 (0.99)

Continued on next page

Table C.6 – *Continued from previous page*

Study	Ever smoker	Smoking frequency (cigs/day)		ln Cotinine (nmol/l)		Pack years		Physical exercise (hours/week)		Education (years)	
	Proportion (%)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
IMPROVE Groeningen	69.83	292	14.61 (8.46)	NA	NA	419	15.38 (17.50)	NA	NA	NA	NA
IMPROVE Kuopio 1	53.01	248	14.18 (8.52)	NA	NA	462	9.79 (16.02)	NA	NA	NA	NA
IMPROVE Kuopio 2	47.05	195	15.41 (10.12)	NA	NA	428	8.93(14.58)	NA	NA	NA	NA
IMPROVE Milan	51.75	262	17.53 (13.43)	NA	NA	510	13.91 (23.08)	NA	NA	NA	NA
IMPROVE Paris	47.25	203	16.31 (10.29)	NA	NA	431	9.61 (15.39)	NA	NA	NA	NA
IMPROVE Perugia	40.09	186	16.38 (7.10)	NA	NA	464	8.51 (15.08)	NA	NA	NA	NA
IMPROVE Stockholm	55.83	249	14.1(7.68)	NA	NA	461	11.25 (15.18)	NA	NA	NA	NA
Inter99	64.34	2207	16.81 (8.65)	NA	NA	2418	15.14 (13.80)	5889	4.86 (2.70)	6081	12.16 (3.17)
ISGS-SWISS	N/A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Izhevsk	81.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MDC	66.75	522	13.83 (8.15)	NA	NA	516	24.31 (16.65)	NA	NA	NA	NA
MESA	55.88	NA	NA	NA	NA	2264	15.35 (28.33)	NA	NA	NA	NA
MRC 1958BC	53.64	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MRC NSHD	71.28	597	16.88 (9.76)	NA	NA	2169	11.42 (14.52)	NA	NA	2696	2.86 (2.63)
NHANES III	50	494	21.10 (12.30)	2360	1.58 (3.36)	488	16(19.80)	2383	5.5 (6.10)	2382	12.4(2.90)
NHS	64.52	346	1.95 (0.90)	NA	NA	1304	18.8(22.67)	1322	1.87 (2.89)	NA	NA
NORDIL	25.82	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NPHSII	68.45	1883	18.04 (14.38)	NA	NA	1665	29.65 (23.35)	NA	NA	NA	NA
Portuguese Stroke Study	48.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PREVEND	71.37	NA	NA	NA	NA	NA	NA	NA	NA	7729	0.86 (0.35)
PRO-CARDIS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PROSPER	26.84	NA	NA	NA	NA	NA	NA	NA	NA	5504	15.12 (2.03)
Rotterdam	64.62	1198	14.56 (8.68)	NA	NA	3328	28.03 (24.34)	3893	42.08 (19.15)	5827	3.4 (1.94)
SMART	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TPT	N/A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
UCP	69.36	690	18.15 (11.95)	NA	NA	660	31.85 (26.55)	NA	NA	NA	NA
ULSAM	66.67	NA	NA	NA	NA	300	39.92 (35.54)	NA	NA	NA	NA
Whitehall II	41.63	709	14.88 (10.21)	NA	NA	655	19.62 (14.67)	4899	3.01 (3.38)	3700	14.46 (3.59)
WHI	49.94	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table C.7: Cardiovascular traits characteristics of the studies included in the collaboration (1)

Study	SBP (mmHg)		HDL-C (mmol/l)		non-HDL-C (mmol/l)		ln triglycerides (mmol/l)		Apolipoprotein A (g/l)		Apolipoprotein B (g/l)		ln lipoprotein(a) (mg/dl)		Fasting glucose (mmol/l)	
	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
ALSPAC	2496	117.70 (12.14)	2395	1.47 (0.39)	2395	3.42 (0.89)	2395	-0.08 (0.44)	NA	NA	NA	NA	NA	NA	NA	NA
ARIC	9554	118.35 (17.00)	9540	1.31 (0.43)	9538	4.24 (1.12)	9540	0.29 (0.52)	NA	NA	NA	NA	NA	NA	7717	5.91 (1.69)
BRHS	3827	149.29 (24.22)	3763	1.32 (0.34)	3763	4.67 (1.08)	3788	0.49 (0.48)	NA	NA	NA	NA	NA	NA	3823	5.55 (1.24)
BWHHS	3401	147.03 (25.12)	3367	1.66 (0.45)	3367	4.98 (1.25)	3373	0.51 (0.46)	NA	NA	NA	NA	NA	NA	3357	6.06 (1.64)
CaPS	1076	145.73 (22.4)	1067	1.39 (0.38)	1067	4.50 (1.11)	1059	0.38 (0.57)	1036	1.26 (0.20)	1033	0.96 (0.20)	NA	NA	NA	NA
CARDIA	1433	109.26 (10.81)	1427	1.34 (0.33)	1427	3.22 (0.86)	1426	-0.27 (0.51)	1425	1.36 (0.19)	1425	0.91 (0.23)	1321	1.86 (1.33)	1321	4.74 (0.97)
CCHS	9072	138.89 (22.52)	9066	1.58 (0.50)	9066	4.58 (1.34)	9053	0.47 (0.54)	NA	NA	NA	NA	NA	NA	NA	NA
CFS	134	128.92 (17.05)	40	1.06 (0.34)	40	3.49 (1.09)	40	0.48 (0.48)	NA	NA	NA	NA	NA	NA	40	6.00 (2.24)
CGPS	56948	140.31 (21.32)	57012	1.64 (0.52)	57012	4.03 (1.11)	57010	0.37 (0.55)	NA	NA	NA	NA	NA	NA	NA	NA
CHS	3930	135.48 (21.45)	3922	1.38 (0.40)	3922	4.09 (1.00)	3927	0.38 (0.43)	NA	NA	NA	NA	3921	1.09 (1.24)	3922	6.04 (1.82)
Cyprus	713	138.97 (17.06)	711		711		711	0.41 (0.48)	711	1.44 (0.24)	710	1.20 (0.24)	688	2.01 (0.92)	710	5.77 (1.59)
Czech post-MONICA	2526	127.38 (17.14)	2518	1.39 (0.36)	2518	4.27 (1.22)	2523	0.39 (0.56)	NA	NA	NA	NA	NA	NA	2548	5.30 (1.18)
DCH	2735	143.47 (21.08)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EAS	872	143.24 (23.63)	867	1.45 (0.38)	867	5.63 (1.31)	872	0.32 (0.45)	NA	NA	NA	NA	568	-1.09 (1.40)	871	5.80 (1.49)
ELSA	4027	140.13 (19.22)	2019	1.45 (0.43)	2019		5419	0.45 (0.51)	NA	NA	NA	NA	NA	NA	3235	5.02 (0.93)
EPIC InterAct	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPIC Nether-lands	5164	133.07 (21.17)	3580	1.40 (0.41)	3060	4.23 (1.25)	4321	0.44 (0.56)	NA	NA	NA	NA	NA	NA	3456	5.41 (2.21)
EPIC Norfolk	20158	135.46 (18.19)	18958	1.42 (0.43)	18958	4.73 (1.17)	19635	0.46 (0.53)	15602	1.55 (0.33)	15520	0.97 (0.25)	15782	2.57 (0.97)	NA	NA

Continued on next page

Table C.7 – *Continued from previous page*

Study	SBP (mmHg)		HDL-C (mmol/l)		non-HDL-C (mmol/l)		ln triglycerides (mmol/l)		Apolipoprotein A (g/l)		Apolipoprotein B (g/l)		ln lipoprotein(a) (mg/dl)		Fasting glucose (mmol/l)	
	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
EPIC Potsdam	2253	130.12 (18.04)	2253	1.35 (0.37)	2253	3.19 (0.91)	2253	0.19 (0.59)	NA	NA	NA	NA	NA	NA	325	5.56 (1.54)
EPIC Turin	4526	132.39 (15.74)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
FHS	119	135.61 (20.4)	756	1.32 (0.40)	756	3.8 (1.09)	757	1.05 (0.67)	704	1.44 (0.30)	704	1.00 (0.25)	506	1.93 (1.37)	740	5.60 (0.60)
HAPIEE Czech	6662	139.14 (19.66)	6502	1.39 (0.39)	6501	4.33 (1.07)	6522	0.49 (0.53)	NA	NA	NA	NA	NA	NA	901	5.77 (1.63)
HAPIEE Lithuania	6909	139.65 (21.65)	6776	1.49 (0.38)	6776	4.46 (1.16)	6894	0.26 (0.48)	NA	NA	NA	NA	NA	NA	6764	5.83 (1.25)
HAPIEE Poland	8738	138.27 (21.15)	8772	1.44 (0.38)	8772	4.4 (1.10)	8768	0.38 (0.49)	NA	NA	NA	NA	NA	NA	8761	5.37 (1.40)
HAPIEE Russia	7080	143.1 (25.05)	7081	1.54 (0.41)	7081	4.85 (1.29)	7079	0.34 (0.44)	NA	NA	NA	NA	NA	NA	1045	5.97 (1.97)
HIMS	4191	155.84 (20.42)	3835	1.39 (0.36)	3835	3.51 (0.92)	3834		NA	NA	NA	NA	NA	NA	3835	5.71 (1.45)
HPFS	NA	NA	1264	1.16 (0.32)	1264	4.16 (0.96)	1264	0.37 (0.55)	NA	NA	1264	0.93 (0.22)	750	-0.76 (1.28)	NA	NA
IMPROVE Groeningen	418	147.08 (18.14)	419	1.12 (0.32)	419	3.97 (1.03)	419	0.47 (0.55)	NA	NA	NA	NA	NA	NA	417	6.52 (2.31)
IMPROVE Kuopio 1	480	144.87 (17.58)	479	1.30 (0.34)	479	3.75 (0.92)	479	0.15 (0.48)	NA	NA	NA	NA	NA	NA	480	5.85 (1.06)
IMPROVE Kuopio 2	440	153.10 (17.35)	440	1.27 (0.37)	440	4.03 (1.04)	440	0.33 (0.55)	NA	NA	NA	NA	NA	NA	439	7.08 (2.12)
IMPROVE Milan	514	131.49 (14.38)	512	1.21 (0.34)	512	4.68 (0.98)	512	0.34(0.49)	NA	NA	NA	NA	NA	NA	514	5.41 (0.99)
IMPROVE Paris	436	128.17 (14.35)	436	1.35 (0.40)	436	4.31 (1.16)	436	0.36 (0.60)	NA	NA	NA	NA	NA	NA	436	5.42 (1.18)
IMPROVE Perugia	464	139.48 (13.43)	464	1.29 (0.33)	464		464	0.35 (0.53)	NA	NA	NA	NA	NA	NA	464	5.26 (1.23)
IMPROVE Stockholm	480	149.45 (18.57)	480	1.28 (0.38)	480	4.16 (1.00)	480		NA	NA	NA	NA	NA	NA	480	6.03 (1.45)
Inter99	6331	130.20 (17.50)	6328	1.43 (0.40)	6327	4.09 (1.12)	6328	0.12 (0.54)	629	4.49 (2.84)	715	12.24 (22.59)	NA	NA	6326	5.60 (1.13)
ISGS-SWISS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Izhevsk	652	144.86 (22.39)	623		623	4.02 (1.02)	611	0.28 (0.52)	621	1.47 (0.31)	621	0.90 (0.26)	NA	NA	NA	NA
MDC	1937	115.70 (6.15)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Continued on next page

Table C.7 – *Continued from previous page*

Study	SBP (mmHg)		HDL-C (mmol/l)		non-HDL-C (mmol/l)		ln triglycerides (mmol/l)		Apolipoprotein A (g/l)		Apolipoprotein B (g/l)		ln lipoprotein(a) (mg/dl)		Fasting glucose (mmol/l)	
	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
MESA	2291	123.53 (20.68)	2286	1.35 (0.41)	2286	3.71 (0.93)	2288	0.26(0.54)	NA	NA	NA	NA	NA	NA	2288	5.04 (1.23)
MRC 1958BC	2576	126.70 (16.82)	2544	1.56 (0.40)	2544	4.32 (1.10)	2543	0.54 (0.60)	NA	NA	NA	NA	NA	NA	NA	NA
MRC NSHD	2658	136.11 (19.96)	2333		2333	4.36 (1.13)	2506	0.58 (0.58)	NA	NA	NA	NA	NA	NA	NA	NA
NHANES III	2383	126.80 (20.2)	2364	1.3 (0.40)	2362	4.10 (1.20)	2379	0.37 (0.57)	NA	NA	NA	NA	NA	NA	1108	5.70 (1.7)
NHS	NA	NA	1322	1.48 (0.43)	1321	4.44 (1.07)	1227	0.23 (0.51)	183	1.73 (0.33)	1322	1.09 (0.32)	683	-0.97 (1.27)	NA	NA
NORDIL	1921	177.23 (14.53)	1820	1.37 (0.46)	1819		1889	0.47 (0.51)	NA	NA	NA	NA	NA	NA	1892	5.26 (1.54)
NPHS II	2658	138.54 (19.08)	1771	0.84 (0.25)	1759	4.90 (1.04)	2641	0.59(0.53)	2266	1.63 (0.32)	2266	0.90 (0.26)	2206	-1.07 (1.36)	NA	NA
Portuguese Stroke Study	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PREVEND	7726	129.20(20	7607	1.32 (0.40)	7575	4.33 (1.21)	7280	0.19 (0.52)	7405	1.39 (0.30)	7406	1.03 (0.31)	7405	-2.76 (1.02)	6946	4.90 (1.10)
PRO-CARDIS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PROSPER	5504	154.62 (21.87)	5504	1.28(0.35)	5504	4.41 (0.90)	5504	0.35(0.41)	5444	1.32 (0.24)	5444	1.15 (0.22)	5444	2.61 (1.25)	5482	5.47 (1.45)
Rotterdam	5654	139.32 (22.34)	5738	1.34 (0.36)	5737	5.26 (1.23)	3355	0.33 (0.43)	NA	NA	NA	NA	NA	NA	3400	5.96 (1.57)
SMART	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TPT	3175	138.49 (17.88)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
UCP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
ULSAM	452	149.18(18.88)	452	1.29 (0.34)	452	4.49 (0.95)	453	0.24 (0.46)	313	1.27 (0.23)	315	1.03 (0.22)	314	-0.49 (1.18)	453	5.73 (1.36)
Whitehall II	5022	122.23 (13.93)	826	1.47 (0.40)	826	4.80 (1.18)	857	0.56 (0.56)	3834	1.55 (0.26)	3834	1.02 (0.25)	4737	- 0.3(0.89)	4541	5.22 (0.59)
WHI	7877	132.96 (18.69)	4248		4175	4.54 (1.08)	3314	0.50 (0.48)	NA	NA	NA	NA	NA	NA	NA	NA

Table C.8: Cardiovascular traits characteristics of the studies included in the collaboration (2)

Study	ln Fibrinogen (g/l)		von Willebrand factor (IU/dl)		ln CRP (mg/l)		ln IL6 (pg/ml)		BMI (kg/m2)		Waist circumference (cm)		ln CIMT (mm)		ln BNP (ng/l)	
	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
ALSPAC	NA	NA	NA	NA	2395	0.10 (1.13)	NA	NA	2554	26.54 (5.21)	2554	84.37 (12.19)	2543	-0.59 (0.11)	NA	NA
ARIC	9464	1.07 (0.20)	NA	NA	7559	0.81 (1.08)	297	0.69(0.68)	9549	26.98 (4.85)	9547	96.11 (13.33)	NA	NA	NA	NA
BRHS	3835	1.16 (0.22)	3837	140.08 (46.3)	3811	0.57 (1.11)	3807	0.90(0.67)	3824	26.90 (3.70)	3818	97.17 (10.44)	NA	NA	NA	NA
BWHHS	3341	1.22 (0.20)	3348	148.26(47.55)	3244	0.62 (1.11)	3341	0.85(0.69)	3383	27.56 (4.94)	3368	86.17 (12.09)	NA	NA	1200	5.07 (0.95)
CaPS	1092	1.30 (0.22)	NA	NA	741	0.51 (1.00)	NA	NA	1084	26.3 (3.47)	1077	94.13 (10.31)	NA	NA	NA	NA
CARDIA	675	0.69 (0.22)	675	90.77(33.84)	1308	-0.03 (1.20)	224	0.01(0.65)	1427	23.63 (3.99)	1426	76.96 (10.45)	84	-0.68 (0.18)	NA	NA
CCHS	8818	1.09 (0.27)	NA	NA	8251	0.79 (0.78)	NA	NA	9051	25.62 (4.34)	NA	NA	NA	NA	NA	NA
CFS	37	1.19 (0.22)	NA	NA	40	0.79 (0.94)	40	1.05(0.65)	134	32.35 (8.04)	45	103.36 (19.48)	NA	NA	NA	NA
CGPS	56859	1.32 (0.24)	NA	NA	NA	NA	NA	NA	56742	26.15 (4.29)	NA	NA	NA	NA	NA	NA
CHS	3902	1.14 (0.20)	NA	NA	3908	0.63 (1.01)	3620	0.57(0.62)	3924	26.38 (4.50)	3907	93.81 (12.83)	3918	-0.18 (0.22)	NA	NA
Cyprus	704	0.97 (0.20)	NA	NA	697	0.79 (1.27)	216	0.62(1.09)	716	28.08 (4.59)	65	97.12 (19.91)	730	-0.33 (0.21)	NA	NA
Czech post-MONICA	NA	NA	NA	NA	2346	0.00 (1.01)	NA	NA	2524	27.20 (4.71)	2525	89.67 (13.03)	NA	NA	NA	NA
DCH	NA	NA	NA	NA	NA	NA	NA	NA	2736	26.53 (4.00)	2736	91.67 (12.7)	NA	NA	NA	NA
EAS	854	0.96 (0.25)	790	111.74(43.7)	640	0.66 (1.09)	619	0.80(0.74)	873	25.53 (3.84)	NA	NA	825	-0.33 (0.32)	NA	NA
ELSA	1823	1.01 (0.24)	NA	NA	5416	0.72 (1.11)	NA	NA	5144	27.46 (4.37)	4415	92.68 (12.71)	NA	NA	NA	NA
EPIC InterAct	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EPIC Nether-lands	NA	NA	NA	NA	4338	0.83 (1.69)	NA	NA	5184	26.77 (4.45)	5180	88.47 (12.49)	NA	NA	NA	NA
EPIC Norfolk	18654	1.05 (0.28)	NA	NA	15709	0.49 (1.06)	NA	NA	20169	26.30 (3.81)	20182	88.42 (12.32)	NA	NA	NA	NA

Continued on next page

Table C.8 – *Continued from previous page*

Study	ln Fibrinogen (g/l)		von Willebrand factor (IU/dl)		ln CRP (mg/l)		ln IL6 (pg/ml)		BMI (kg/m2)		Waist circumference (cm)		ln CIMT (mm)		ln BNP (ng/l)	
	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
EPIC-Potsdam	NA	NA	NA	NA	2253	-0.25 (1.39)	NA	NA	2249	26.19 (4.29)	2252	86.41 (12.96)	NA	NA	NA	NA
EPIC Turin	NA	NA	NA	NA	NA	NA	NA	NA	4467	25.85 (3.59)	4296	89.02 (11.55)	NA	NA	NA	NA
FHS	308	1.26 (0.21)	689	131.77(47	312	0.41 (1.26)	693	1.11(0.72)	312	27.72 (5.73)	728	90.04 (14.55)	671	-0.53 (0.26)	NA	NA
HAPIEE Czech	NA	NA	NA	NA	6540	0.30 (1.03)	NA	NA	6673	28.20 (4.56)	6671	93.32 (12.73)	NA	NA	NA	NA
HAPIEE Lithuania	NA	NA	NA	NA	926	0.42 (1.11)	NA	NA	6930	29.36 (5.29)	6903	92.61 (13.49)	NA	NA	NA	NA
HAPIEE Poland	NA	NA	NA	NA	906	0.47 (1.06)	NA	NA	8767	28.15 (4.58)	8772	92.65 (12.38)	NA	NA	NA	NA
HAPIEE Russia	NA	NA	NA	NA	1045	0.49 (1.2)	NA	NA	7082	28.61 (5.45)	7080	92.89 (12.84)	NA	NA	NA	NA
HIMS	NA	NA	NA	NA	3834	0.70 (1.04)	NA	NA	4188	26.77 (3.41)	4191	98.65 (9.81)	NA	NA	NA	NA
HPFS	756	1.37 (0.20)	NA	NA	1262	0.16 (1.11)	755	0.66(0.94)	1264	25.78 (3.31)	1170	98.29 (10.01)	NA	NA	NA	NA
IMPROVE Groeningen	NA	NA	NA	NA	420	0.88 (1.12)	NA	NA	420	29.40 (4.76)	415	101.71 (12.28)	421	-0.13 (0.24)	NA	NA
IMPROVE Kuopio 1	NA	NA	NA	NA	480	0.14 (1.29)	NA	NA	481	27.72 (4.02)	481	93.19 (11.06)	480	-0.07 (0.21)	NA	NA
IMPROVE Kuopio 2	NA	NA	NA	NA	440	0.32 (1.25)	NA	NA	440	29.12 (4.62)	439	99.17 (13.43)	439	-0.07 (0.20)	NA	NA
IMPROVE Milan	NA	NA	NA	NA	514	0.35 (1.17)	NA	NA	514	25.24 (3.28)	512	88.07 (10.49)	514	-0.15 (0.20)	NA	NA
IMPROVE Paris	NA	NA	NA	NA	436	0.27 (1.26)	NA	NA	436	26.34 (3.83)	435	93.08 (12.79)	436	-0.22 (0.18)	NA	NA
IMPROVE Perugia	NA	NA	NA	NA	464	0.66 (1.07)	NA	NA	464	26.27 (3.51)	464	88.34 (10.30)	464	-0.24 (0.18)	NA	NA
IMPROVE Stockholm	NA	NA	NA	NA	480	0.43 (1.27)	NA	NA	480	26.82 (4.13)	480	95.27 (11.77)	480	-0.09 (0.19)	NA	NA
Inter99	718	0.55 (0.72)	NA	NA	5629	-0.12 (1.29)	621	1.08 (1.02)	6328	26.31 (4.62)	6318	86.55 (13.34)	NA	NA	NA	NA
ISGS-SWISS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Izhevsk	NA	NA	NA	NA	NA	NA	NA	NA	649	26.71 (4.97)	653	94.79 (11.79)	NA	NA	NA	NA
MDC	NA	NA	NA	NA	NA	BA	NA	NA	1936	24.39 (3.31)	1936	81.24 (14.46)	NA	NA	NA	NA

Continued on next page

Table C.8 – *Continued from previous page*

Study	ln Fibrinogen (g/l)		von Willebrand factor (IU/dl)		ln CRP (mg/l)		ln IL6 (pg/ml)		BMI (kg/m2)		Waist circumference (cm)		ln CIMT (mm)		ln BNP (ng/l)	
	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)
MESA	2283	1.19 (0.20)	415	134.55 (54.34)	2284	0.58 (1.15)	2251	0.15 (0.66)	2293	27.75 (5.06)	2293	97.97 (14.53)	2269	-0.34 (0.26)	NA	NA
MRC 1958BC	2512	1.07 (0.20)	2513	124.32 (41.74)	2513	0.02 (1.20)	NA	NA	2585	27.26 (4.82)	2580	92.00 (13.58)	NA	NA	NA	NA
MRC NSHD	NA	NA	NA	NA	NA	NA	NA	NA	2671	27.34 (4.65)	2682	91.62 (13.06)	NA	NA	NA	NA
NHANES III	NA	NA	NA	NA	NA	NA	NA	NA	2388	26.60 (5.60)	2300	92.90 (14.70)	NA	NA	NA	NA
NHS	707	1.23 (0.27)	NA	NA	1322	0.72 (1.15)	675	0.63(0.68)	1321	25.66 (4.75)	942	80.06 (11.10)	NA	NA	NA	NA
NORDIL	NA	NA	NA	NA	NA	NA	NA	NA	1883	28.27 (4.57)	NA	NA	NA	NA	NA	NA
NPHSII	2646	1.00 (0.19)	170	110.46 (34.61)	2205	0.92 (1.00)	NA	NA	2656	26.45 (3.5)	NA	NA	NA	NA	NA	NA
Portuguese Stroke Study	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PREVEND	NA	NA	NA	NA	7327	0.25 (1.13)	NA	NA	7653	26.08 (4.21)	7654	0.88 (0.10)	712	-0.28 (0.23)	NA	NA
PRO-CARDIS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PROSPER	5357	1.26 (0.21)	5327	140.79 (45.9)	5400	1.13 (1.12)	5374	0.98 (0.66)	5504	26.84 (4.19)	NA	NA	NA	NA	NA	NA
Rotterdam	2354	0.99 (0.24)	3380	137.20 (63.63)	5458	0.62 (1.04)	NA	NA	5635	26.29 (3.68)	5341	90.62 (11.12)	4745	0.00 (0.19)	3355	4.74 (1.06)
SMART	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7917	-0.17 (0.28)	2700	2.47 (0.98)
TPT	3109	1.09 (0.19)	NA	NA	NA	NA	NA	NA	3162	27.47 (3.56)	NA	NA	NA	NA	NA	NA
UCP	NA	NA	NA	NA	NA	NA	NA	NA	1480	27.14 (4.15)	NA	NA	NA	NA	NA	NA
ULSAM	319	1.27 (0.27)	NA	NA	435	0.64 (1.01)	405	1.39 (0.84)	452	26.02 (3.24)	445	94.59 (9.21)	NA	NA	444	4.83 (1.15)
Whitehall II	1456	0.98 (0.20)	4306	104.80 (38.84)	4488	-0.16 (1.16)	4466	0.37 (0.59)	5022	24.35 (3.30)	4691	84.78 (11.35)	3249	-0.26 (0.19)	NA	NA
WHI	2453	1.08 (0.25)	NA	NA	4681	0.69 (1.36)	3546	0.81 (0.74)	7814	28.35 (6.26)	NA	NA	NA	NA	NA	NA

Table C.9: Meta-analysis pooled linear and quadratic coefficients of the association between alcohol and traits on observational analysis, adjusted for age and gender

Trait	Number	Linear beta (95%CI)	P-value	Quadratic beta (95%CI)	P-value
BMI	130909	-0.14(-0.16,-0.13)	1.5E-92	0.03(0.03,0.03)	8.6E-54
SBP	129573	-0.10(-0.12,-0.09)	1.1E-51	0.04(0.03,0.04)	5.4E-84
DBP	129557	-0.06(-0.08,-0.05)	6.5E-20	0.03(0.03,0.03)	3E-52
ln TG	112496	-0.12(-0.14,-0.11)	1.7E-58	0.03(0.02,0.03)	3.3E-43
HDL-C	109998	0.11(0.09,0.12)	3E-48	0.02(0.01,0.02)	8.9E-21
Non-HDL	109910	-0.05(-0.06,-0.03)	1E-09	0.01(0.00,0.01)	0.000033
Waist circumference	108381	-0.14(-0.15,-0.12)	2.7E-82	0.04(0.03,0.04)	5.6E-79
ln CRP	90131	-0.15(-0.16,-0.13)	4E-66	0.04(0.03,0.04)	3.9E-56
Physical activity	88630	0.07(0.05,0.09)	6.6E-16	-0.01(-0.02,-0.01)	9.5E-09
Pack years	69727	-0.16(-0.18,-0.15)	1.4E-69	0.07(0.06,0.07)	7E-150
Education	66022	0.25(0.23,0.27)	2E-140	-0.04(-0.05,-0.04)	3.7E-63
Glucose	65571	-0.07(-0.09,-0.05)	1E-12	0.02(0.01,0.02)	7.1E-11
ln Fibrinogen	64288	-0.12(-0.14,-0.10)	1E-29	0.01(0.00,0.01)	0.0053
Cigarettes/day	48323	-0.19(-0.21,-0.17)	4.8E-72	0.07(0.06,0.07)	2E-127
ln GGT	31588	-0.15(-0.18,-0.13)	9.5E-30	0.09(0.08,0.09)	3E-118
ln Lp(a)	29319	0.01(-0.02,0.04)	0.57	-0.01(-0.01,0.00)	0.28
ApoA-1	26153	0.04(0.00,0.07)	0.024	0.04(0.03,0.05)	1.7E-15
ApoB	26146	-0.08(-0.11,-0.04)	0.000015	0.02(0.01,0.03)	1.2E-06
lnIL6	23535	-0.20(-0.23,-0.17)	1.4E-31	0.05(0.04,0.06)	2.3E-24
Von Willebrand factor	17983	-0.11(-0.15,-0.07)	2.4E-08	0.02(0.01,0.03)	0.00065
Factor VII	17305	-0.05(-0.09,-0.01)	0.016	0.01(0.00,0.03)	0.012
ln CIMT	14797	-0.08(-0.12,-0.04)	0.000045	0.02(0.01,0.03)	0.000028
ln Cotinine	6960	-0.27(-0.33,-0.21)	8E-18	0.09(0.07,0.11)	8.4E-23
ln NT-proBNP	4553	-0.06(-0.14,0.01)	0.092	0.02(-0.00,0.04)	0.056

Footnote: traits were standardized prior to analysis thus beta coefficients represent the difference in standard deviation for each trait

Table C.10: Linkage disequilibrium between rs1229984 and SNPs on chromosome 4 that are associated with cardiometabolic traits from previous GWAs and gene-centric array analyses

SNP	Platform	Primary phenotype	Base pair position	Distance from rs1229984	R^2 with rs1229984
rs1458038	GWAS	Systolic BP	81164723	-19074596	N/A
rs16998073	GWAS	Diastolic BP	81184341	-19054978	N/A
rs871606	GWAS	BP	54799245	-45440074	N/A
rs442177	GWAS	Triglycerides	88030261	-12209058	N/A
rs1878406	GWAS	Carotid intima media thickness	148000000	48154345	N/A
rs2200733	GWAS	Stroke (ischemic)	112000000	11470850	0.00119
rs4688985	Gene Centric	Type 2 diabetes	6285715	-93953604	0.00188
rs4689388	GWAS	Type 2 diabetes and other traits	6270056	-93969263	0.0000387
rs7659604	GWAS	Type 2 diabetes	123000000	22426195	0.0000246

Footnote: The analysis was conducted in Whitehall II, restricted to Europeans using PLINK. N/A: not available as SNP on Metabochip and not IBC 50K CardioChip. BP: blood pressure. For those SNPs with R^2 annotated as NA, the distance from the rs1229984 makes it unlikely that LD value between the SNP with rs1229984 would be high. GWAs: genome wide association study

C.3 Supplemental Figures

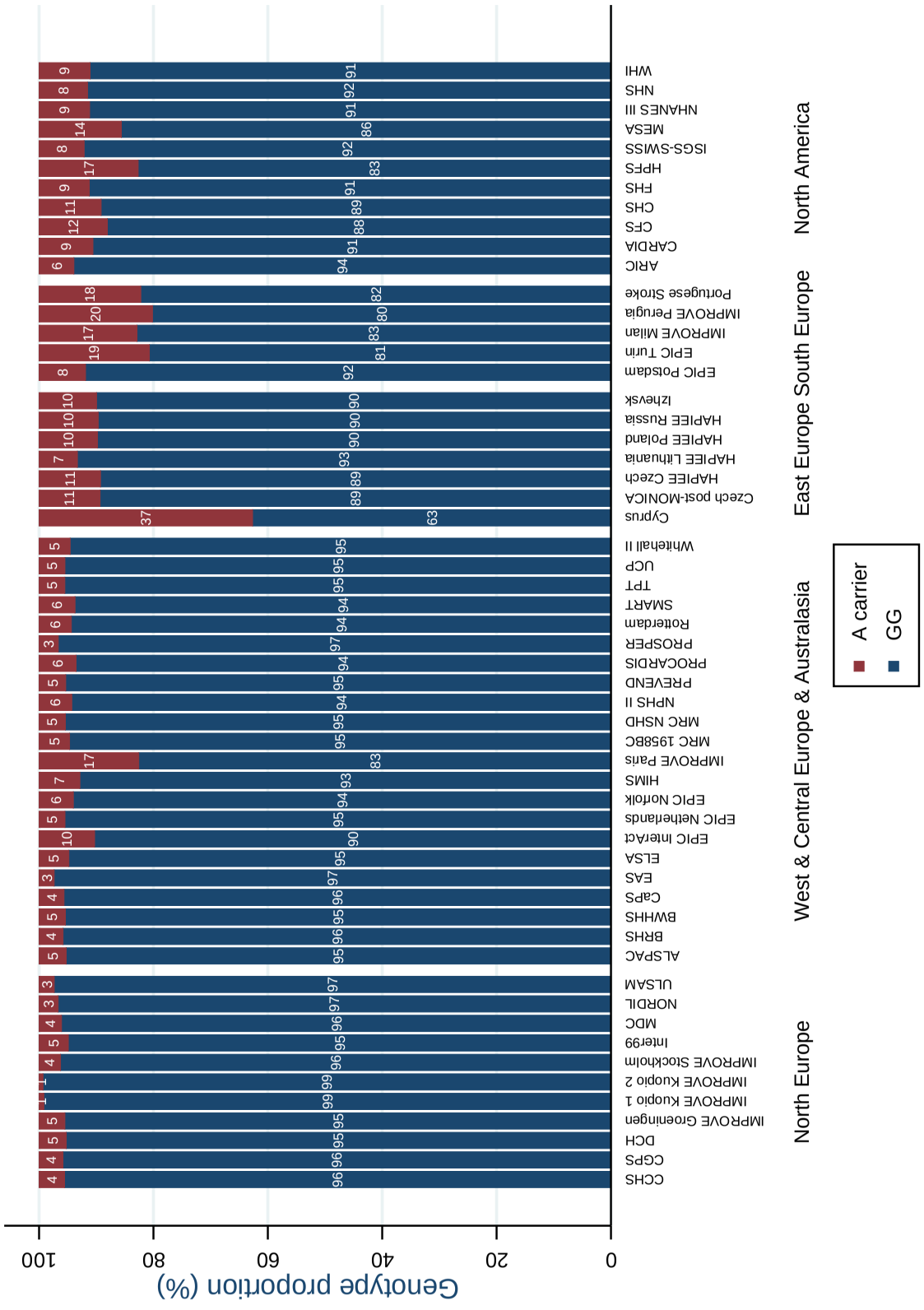


Figure C.1: *ADH1B* rs1229984 A-allele frequency in the 56 collaborating studies, arranged by geographical region

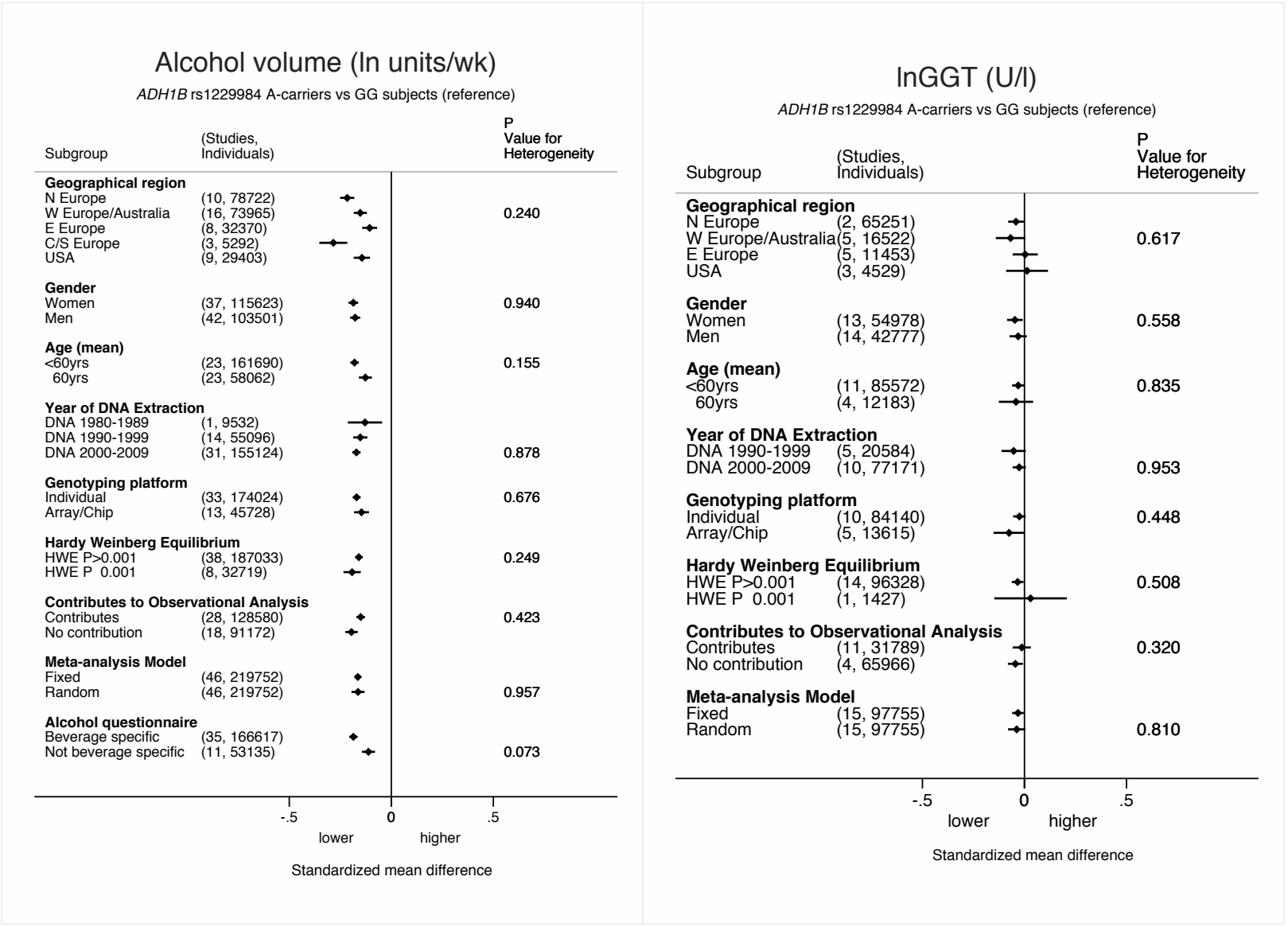


Figure C.2: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and alcohol traits.

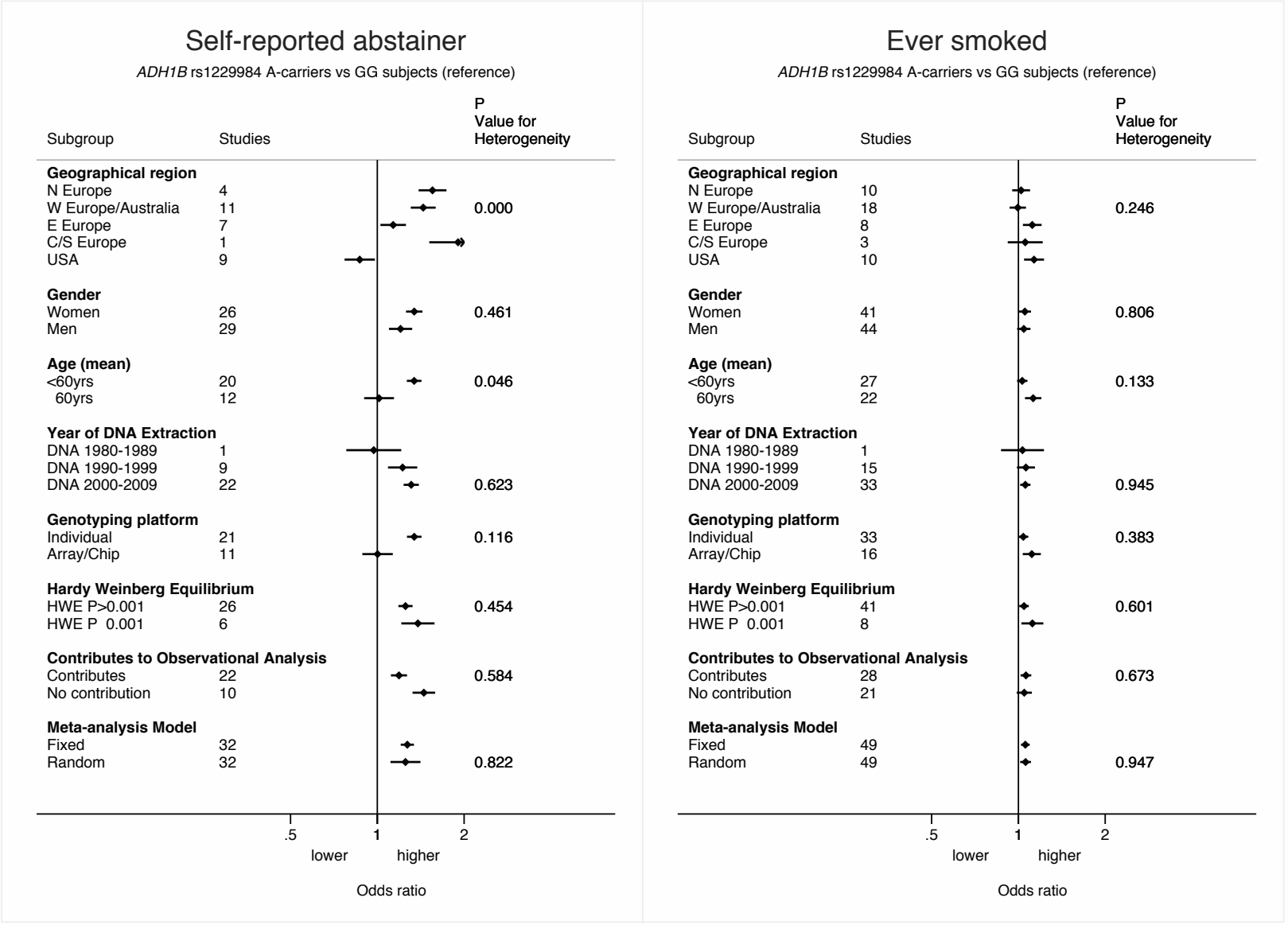


Figure C.3: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and alcohol traits.

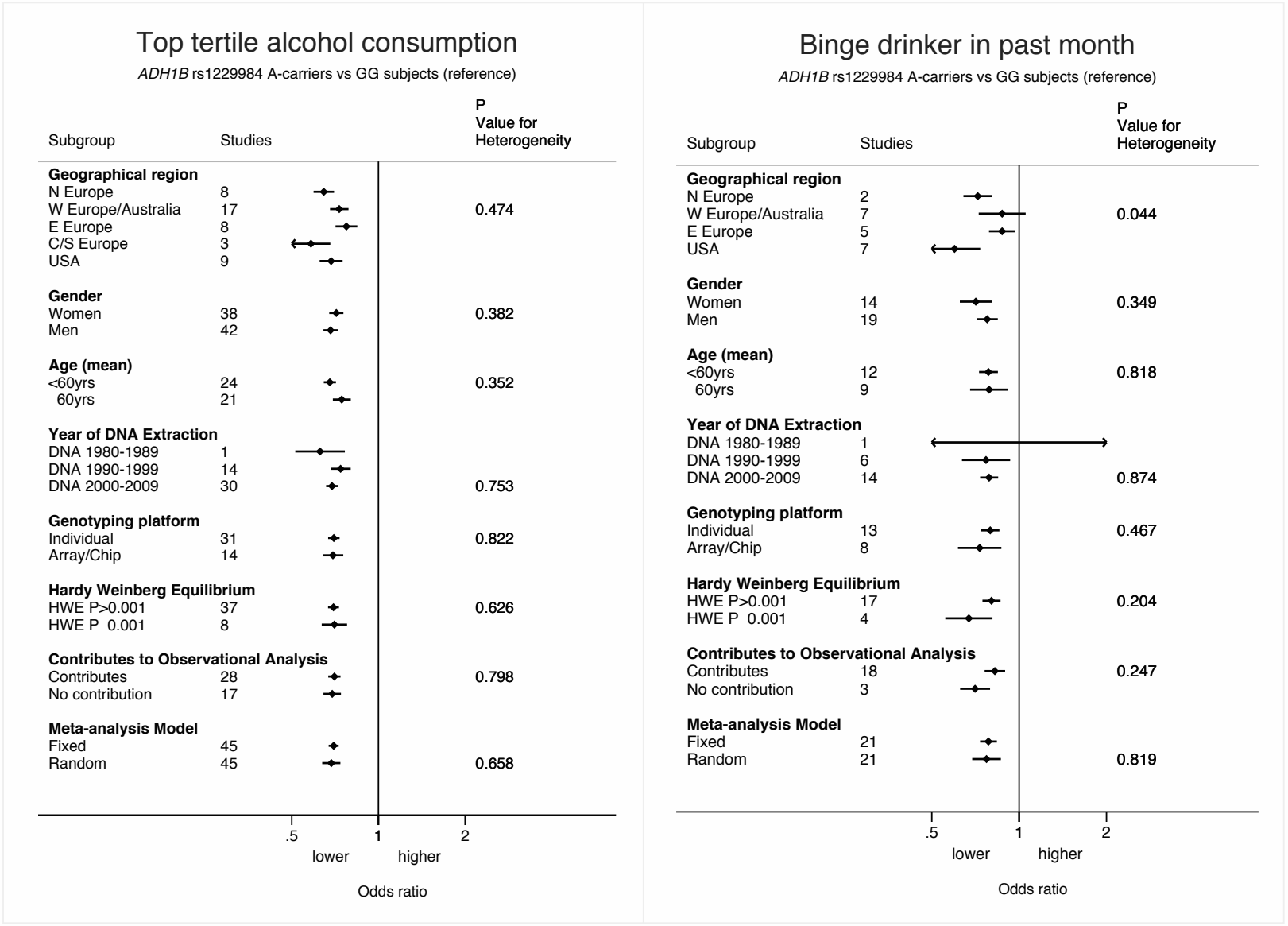


Figure C.4: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and alcohol traits.

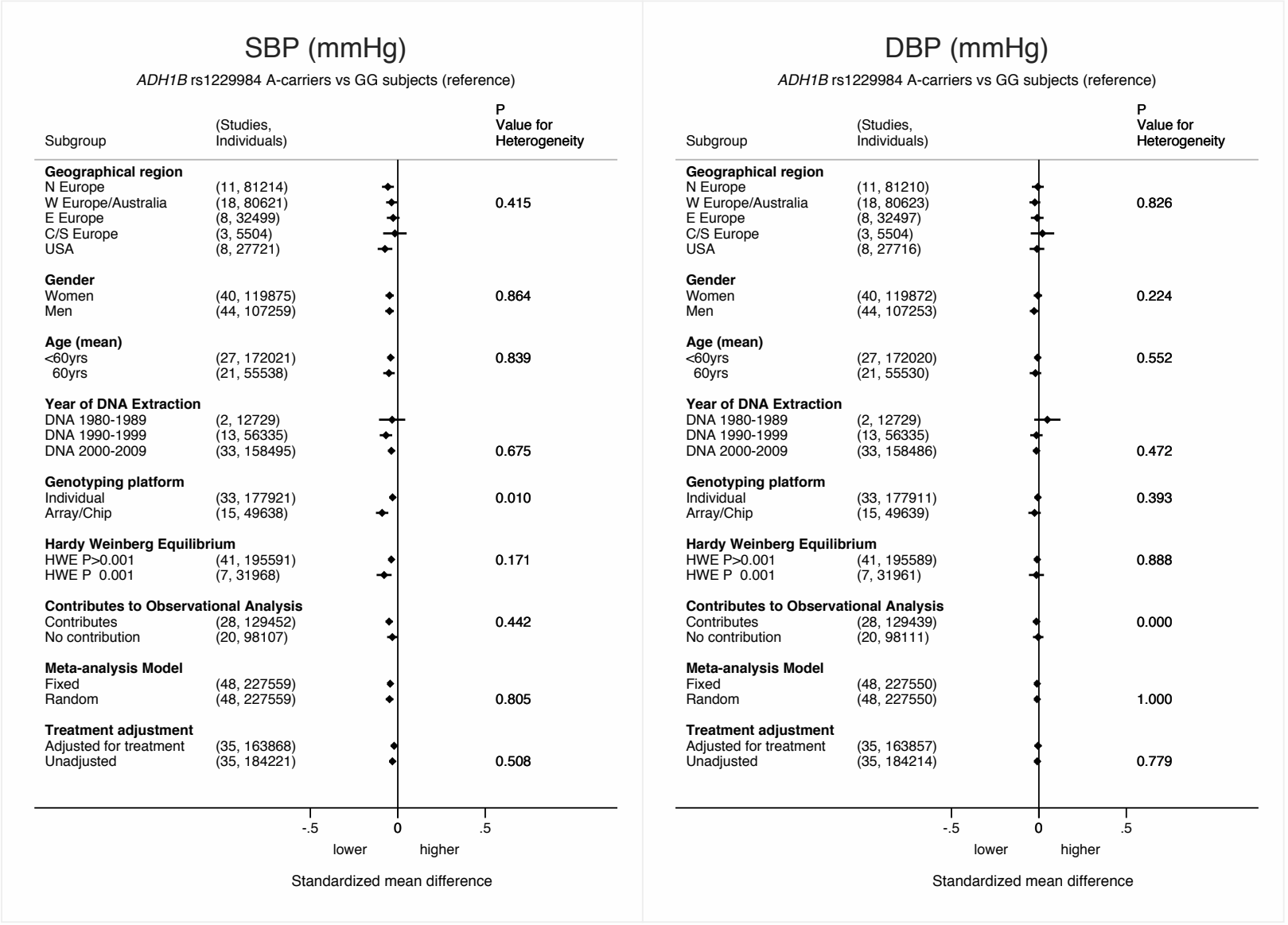


Figure C.5: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

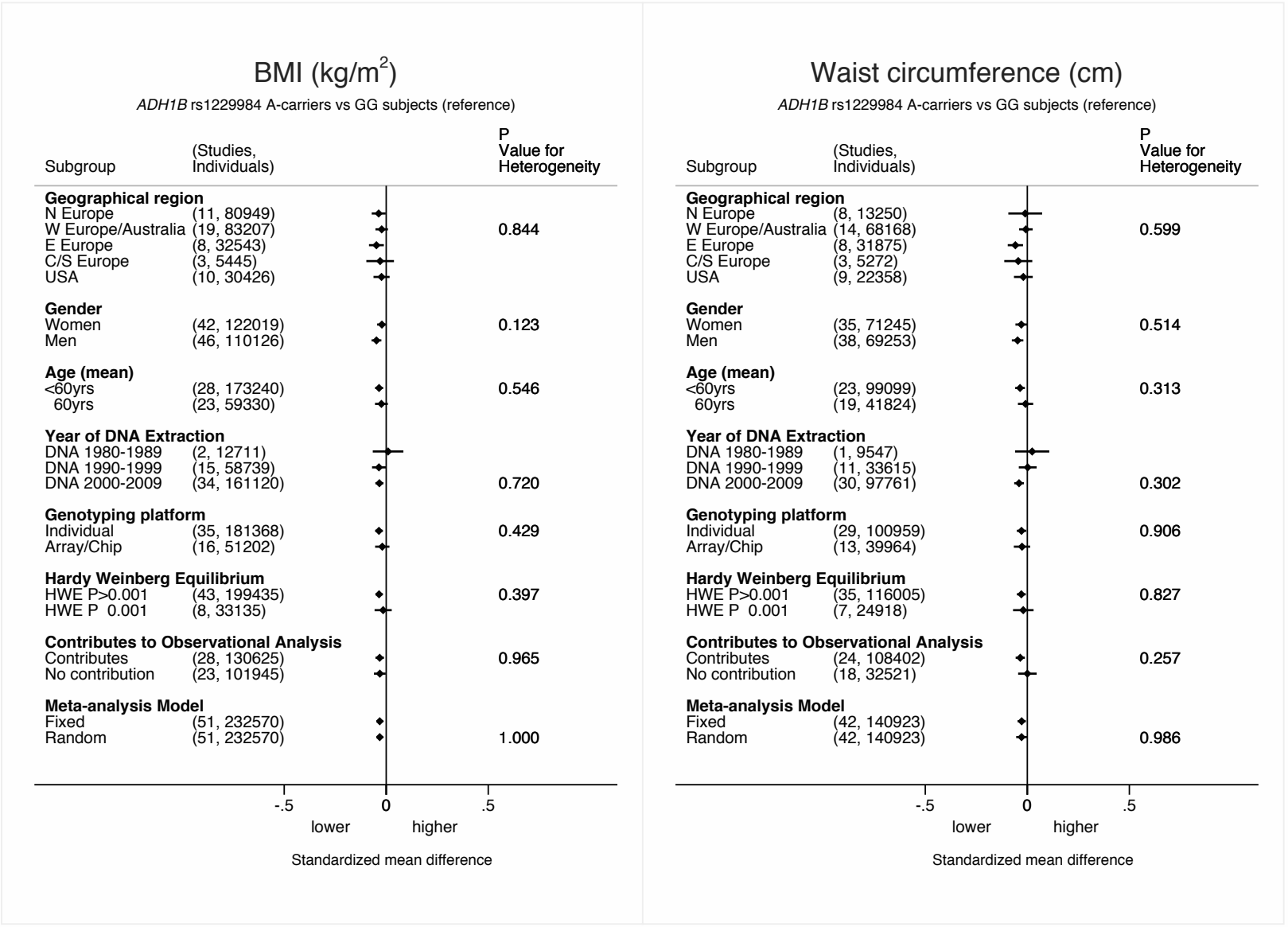


Figure C.6: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

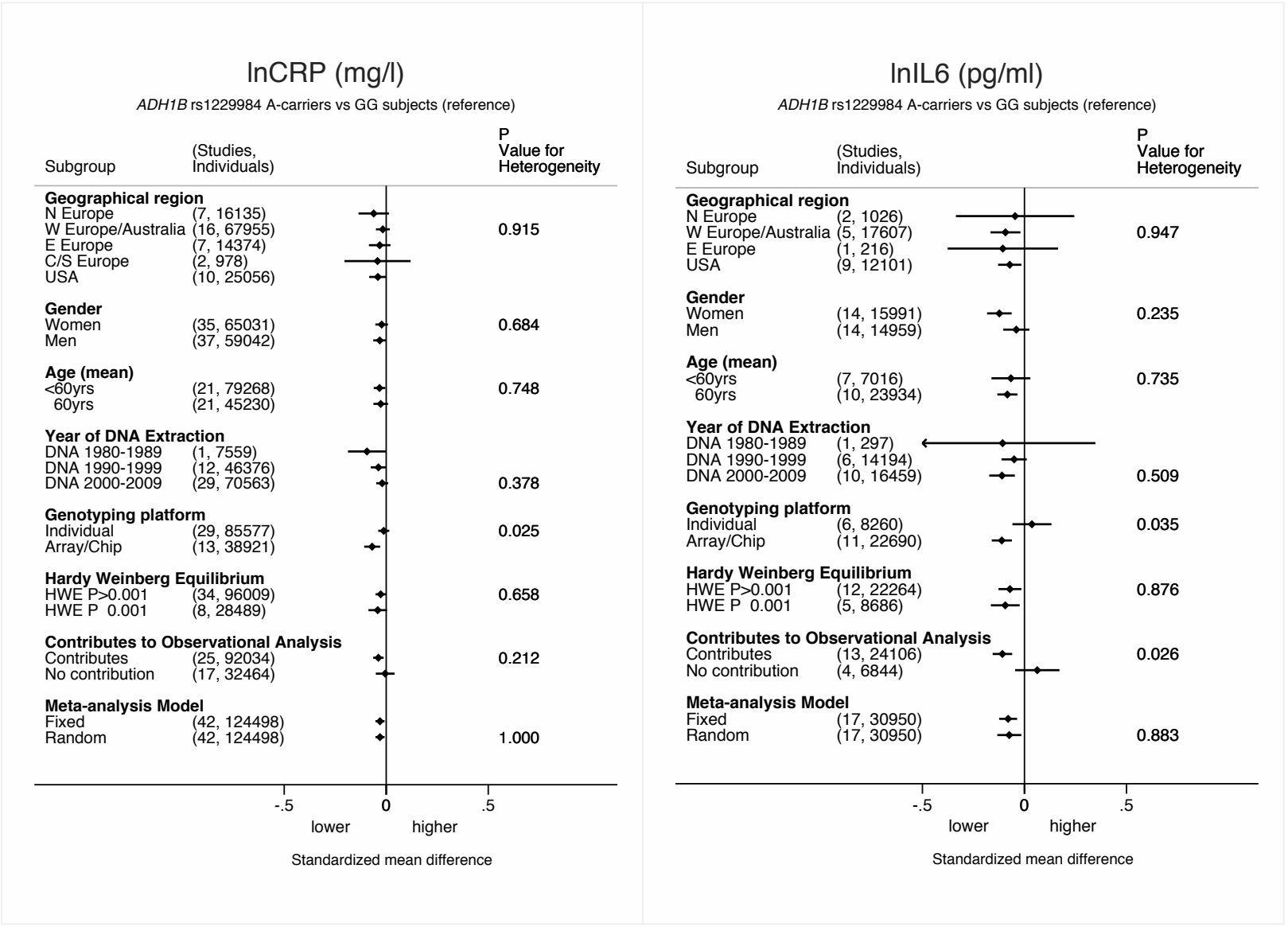


Figure C.7: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

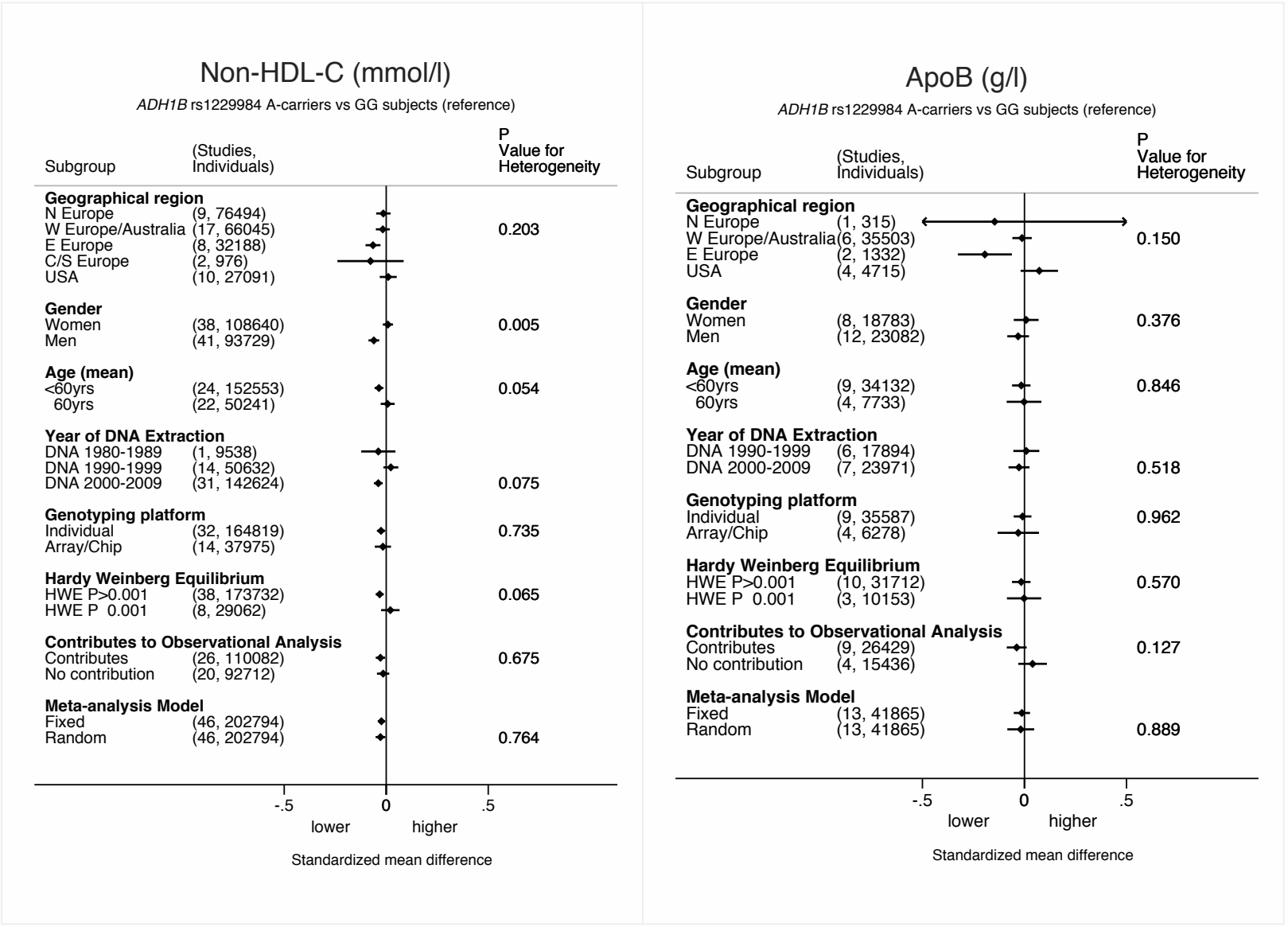


Figure C.8: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

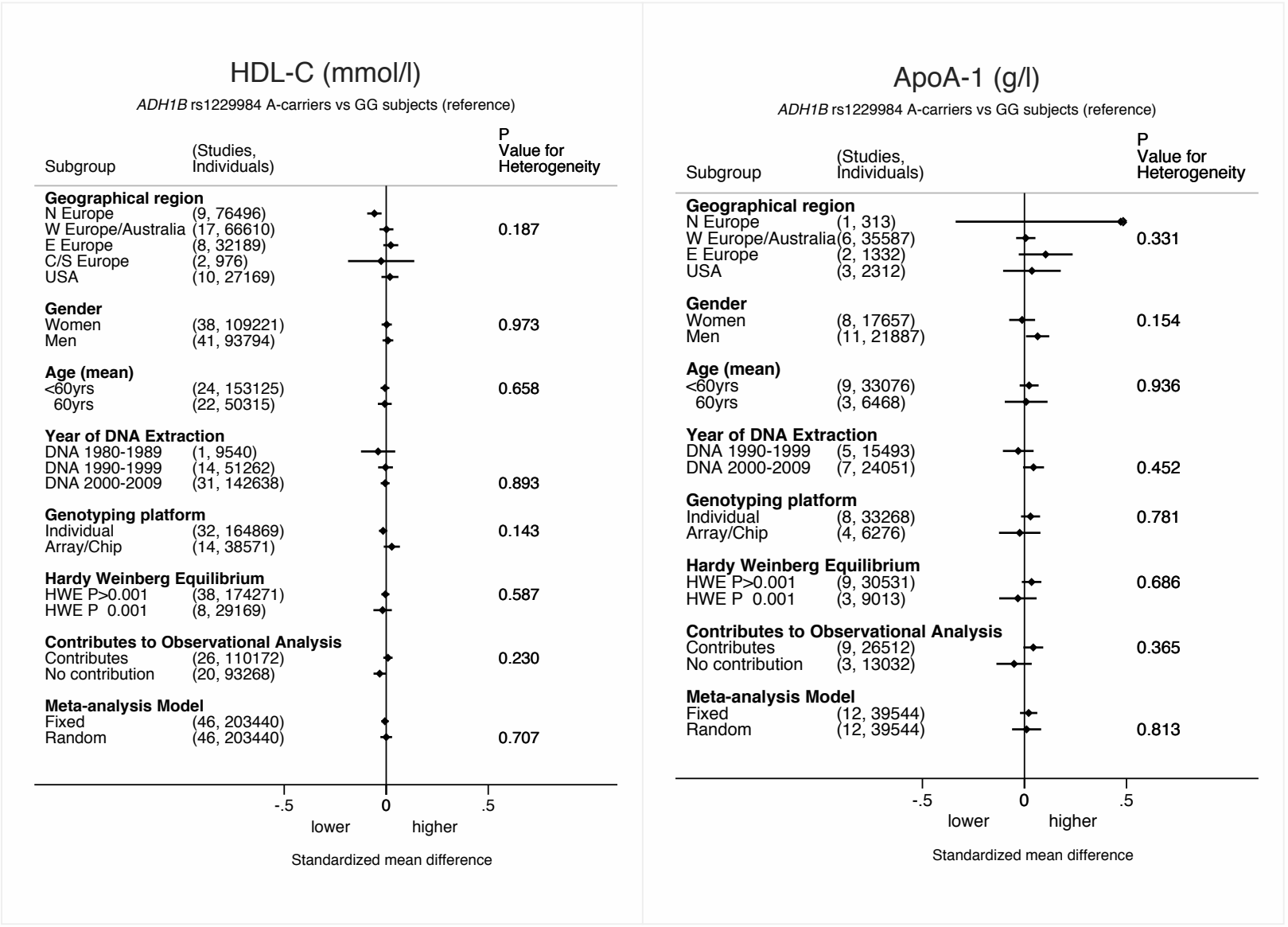


Figure C.9: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

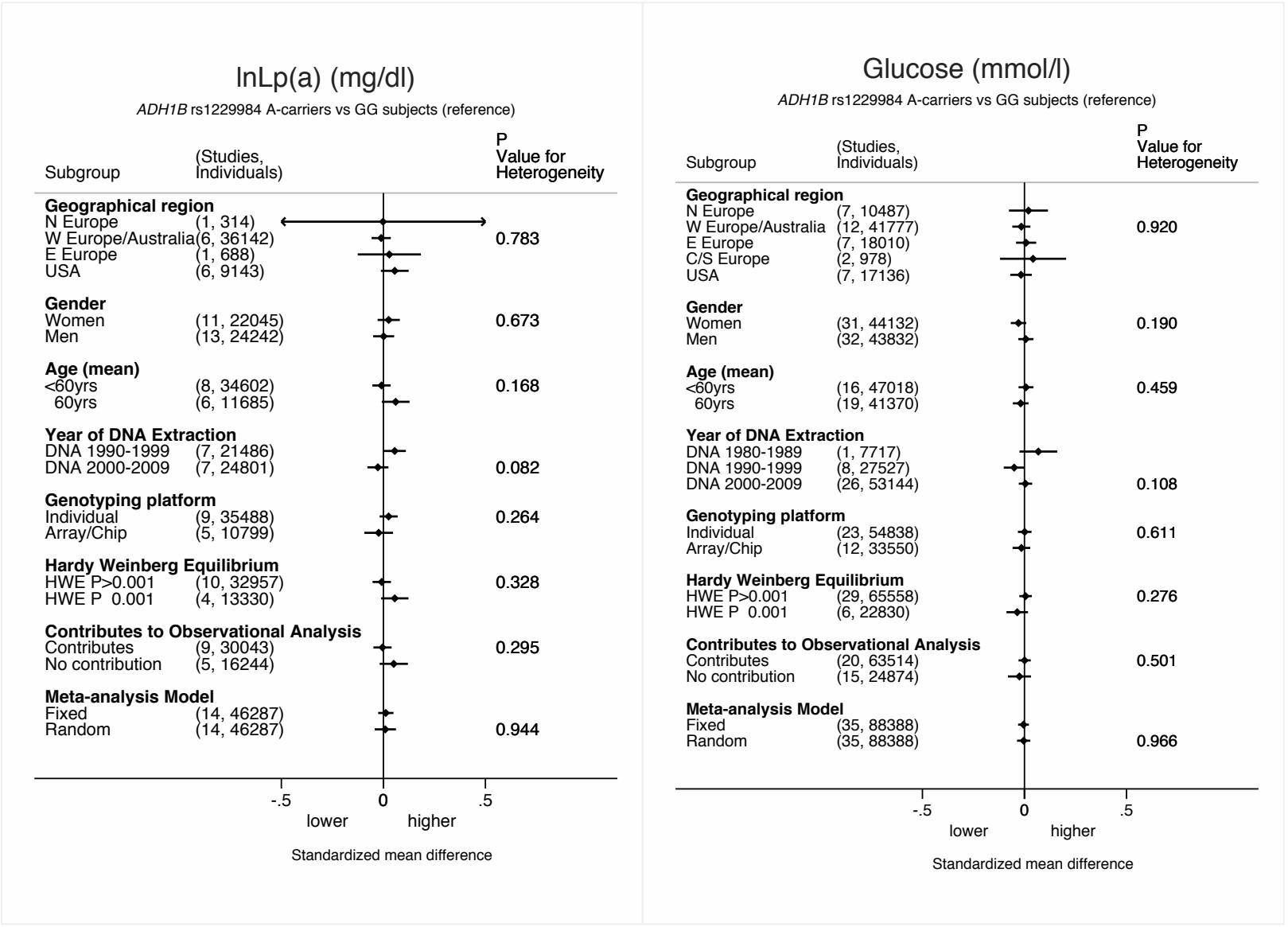


Figure C.10: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

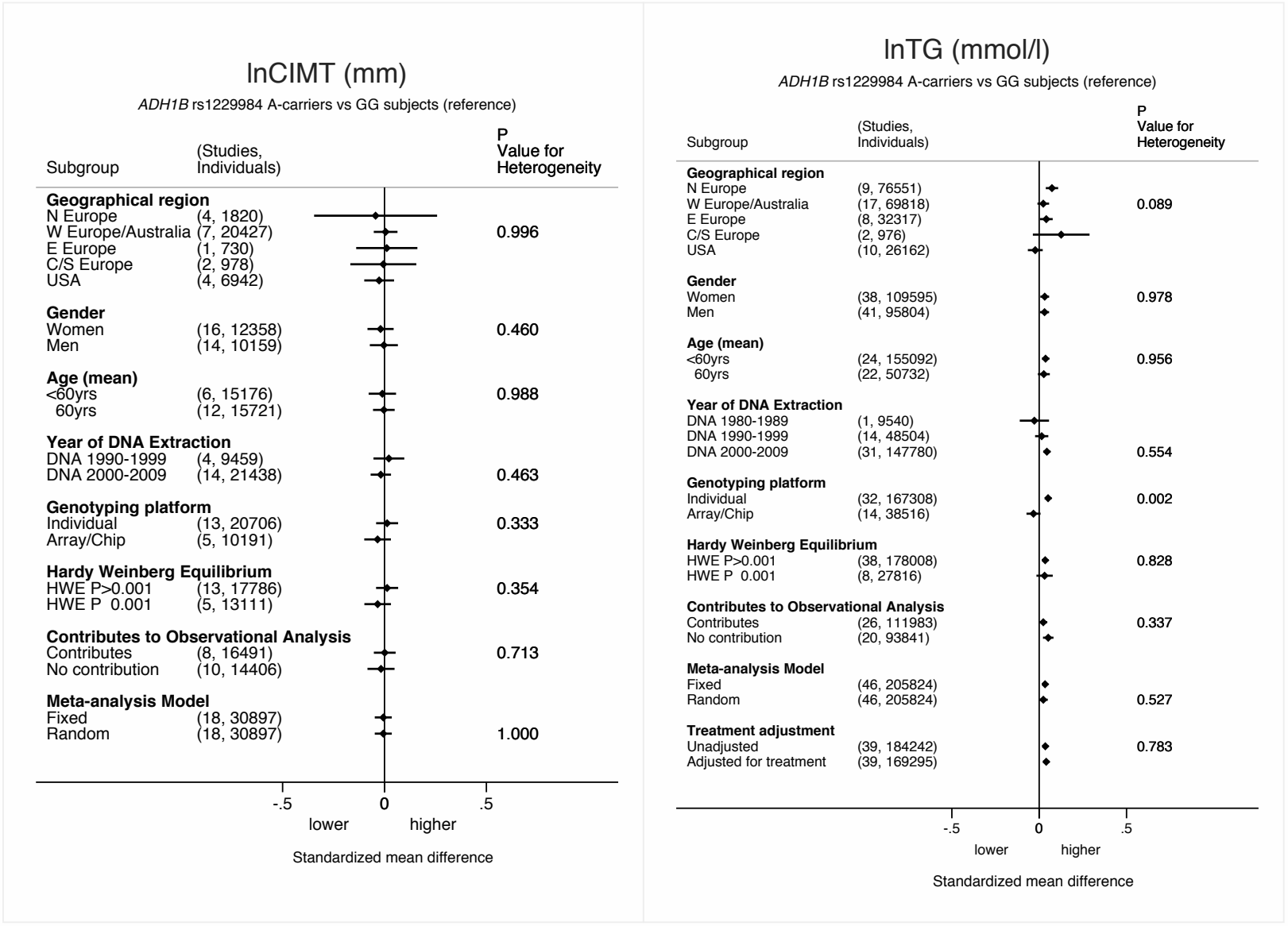


Figure C.11: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

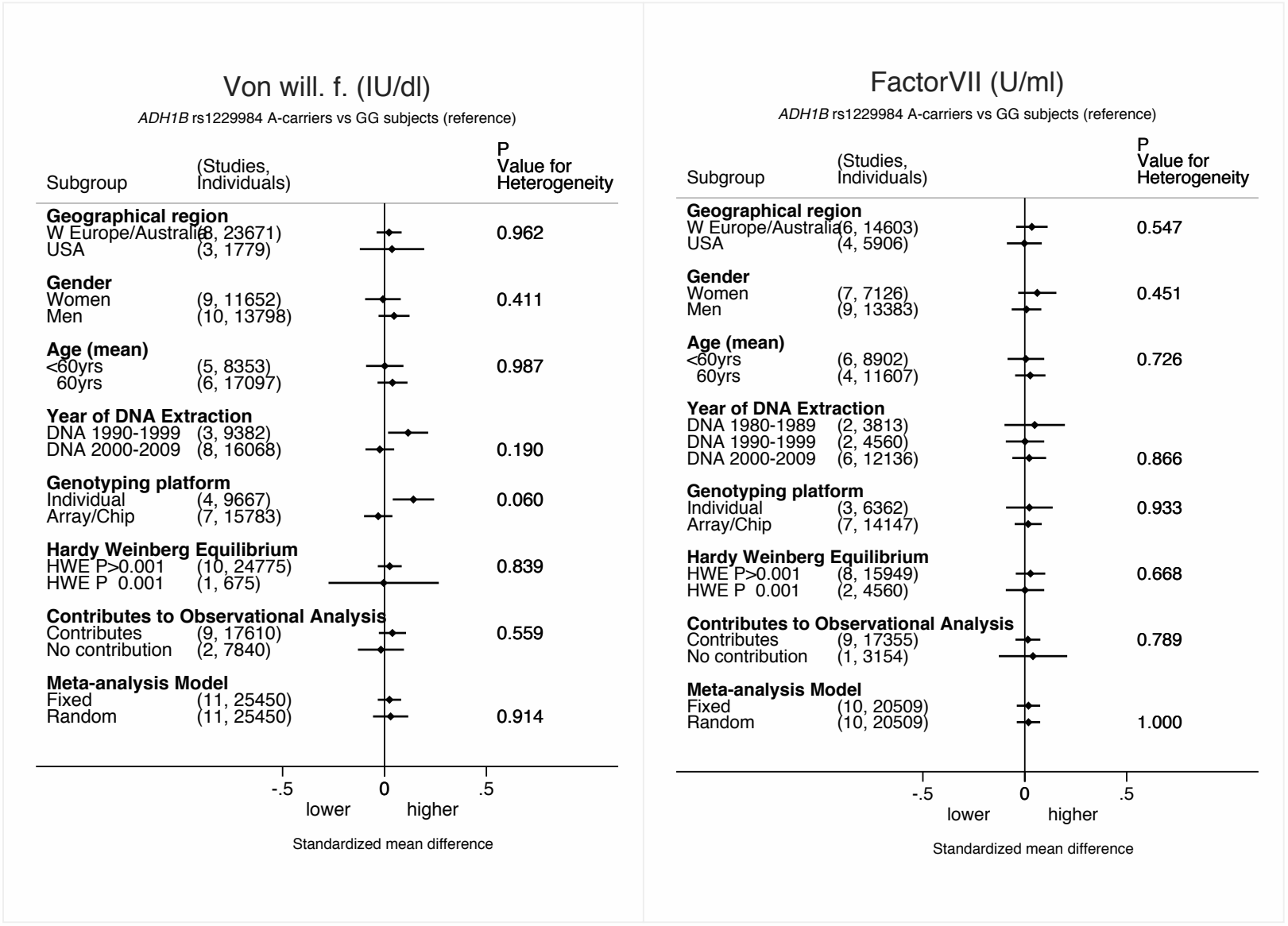


Figure C.12: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.

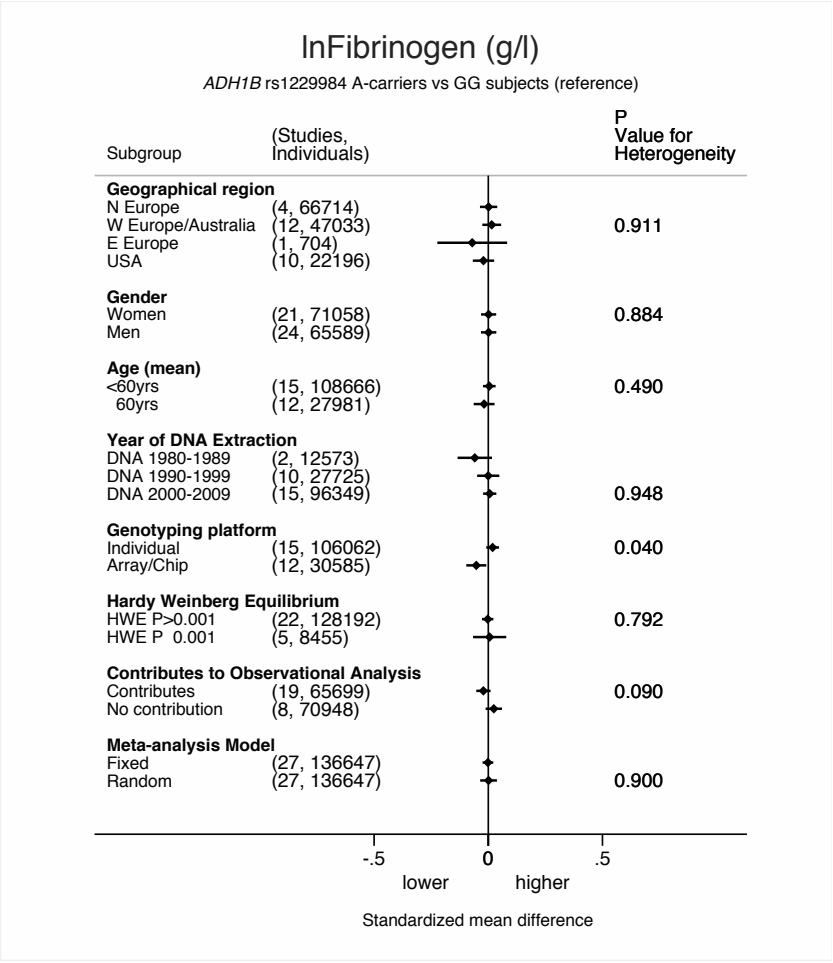
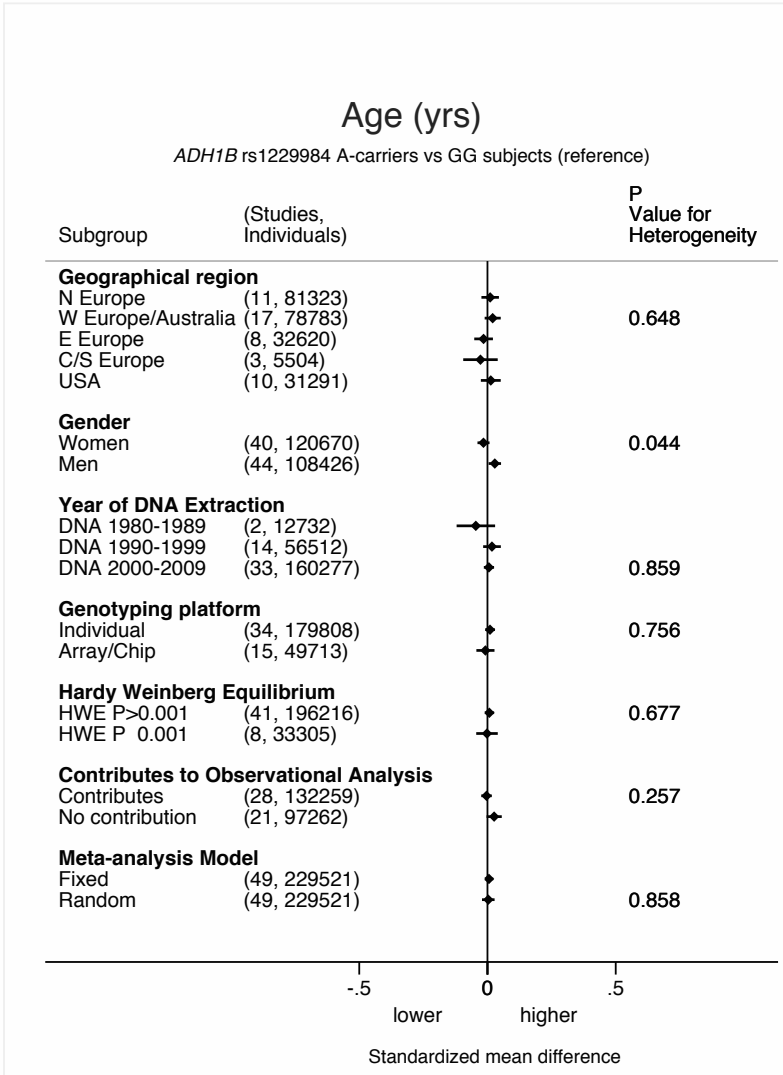


Figure C.13: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiovascular traits.



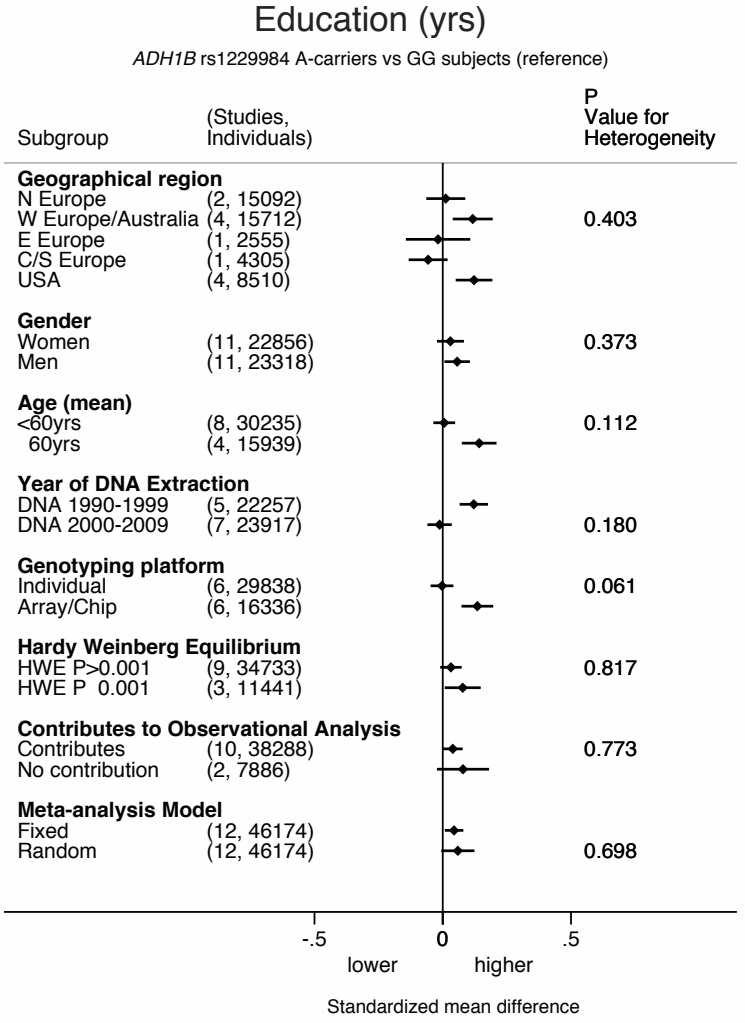


Figure C.14: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits.

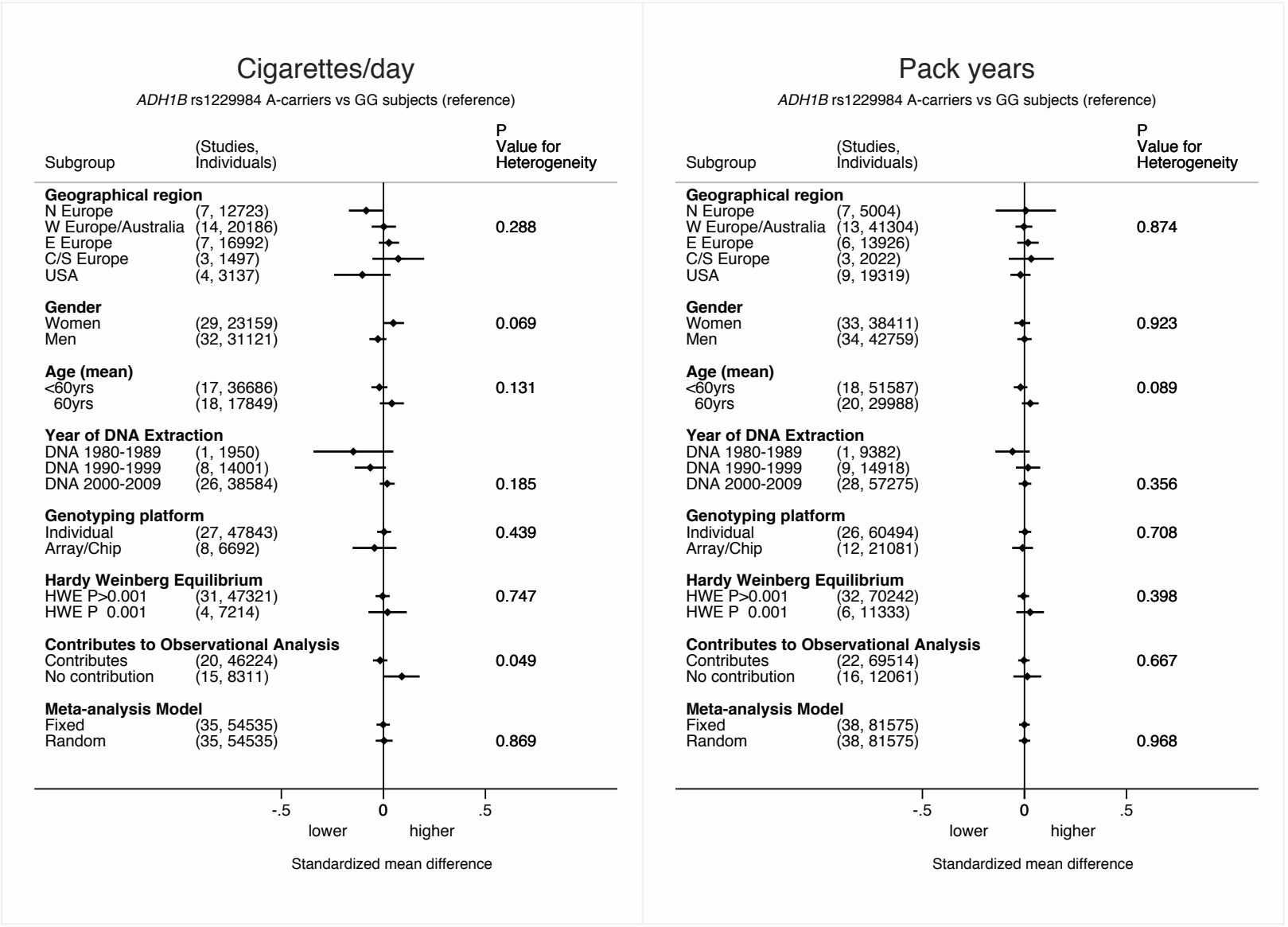
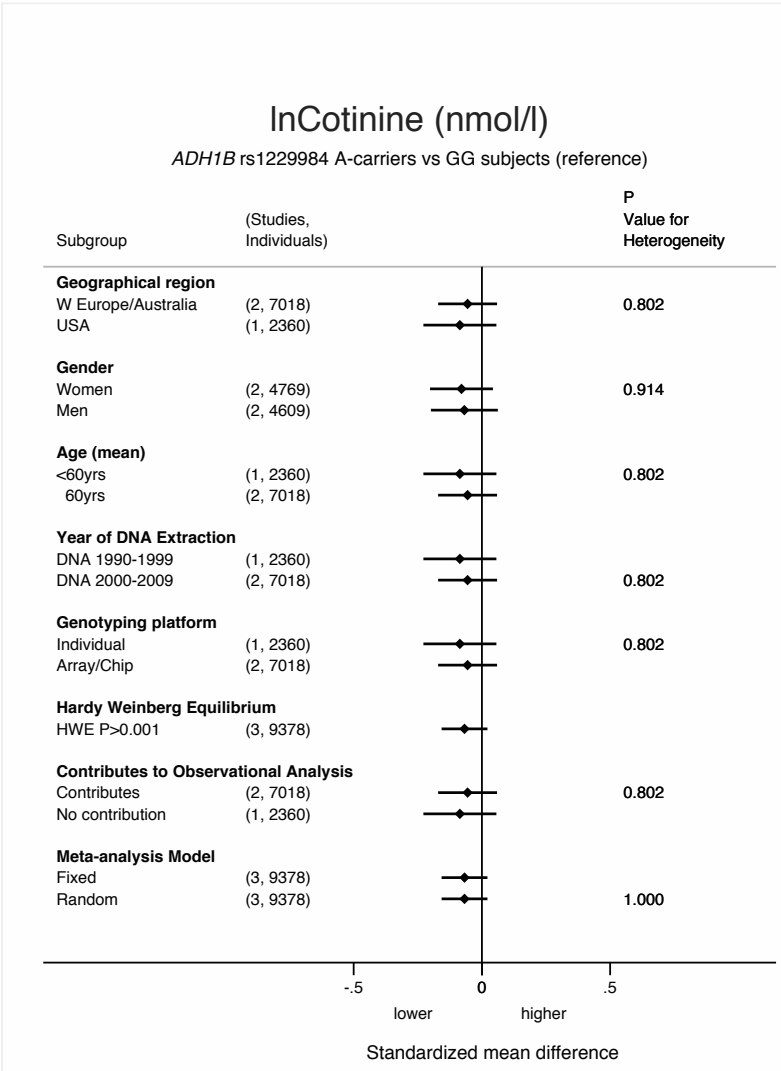


Figure C.15: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits.



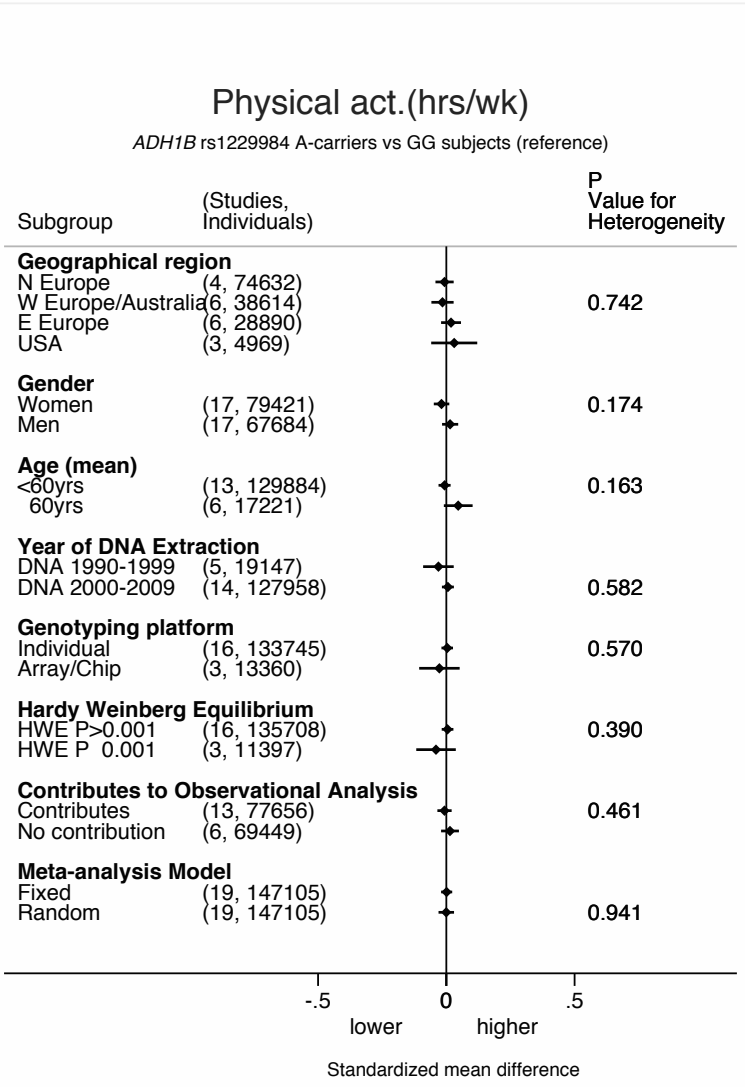


Figure C.16: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and lifestyle traits.

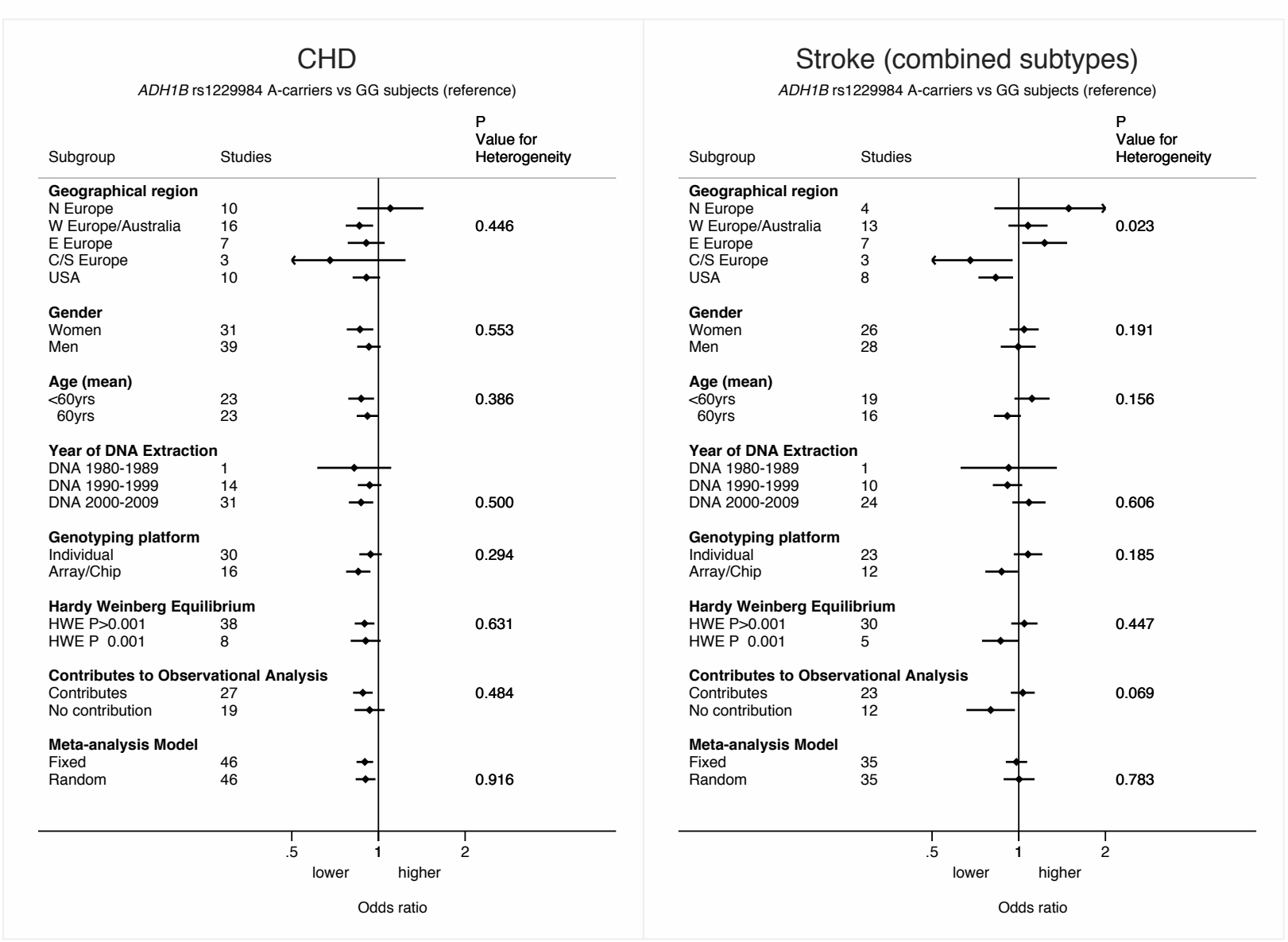


Figure C.17: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiometabolic disorders.

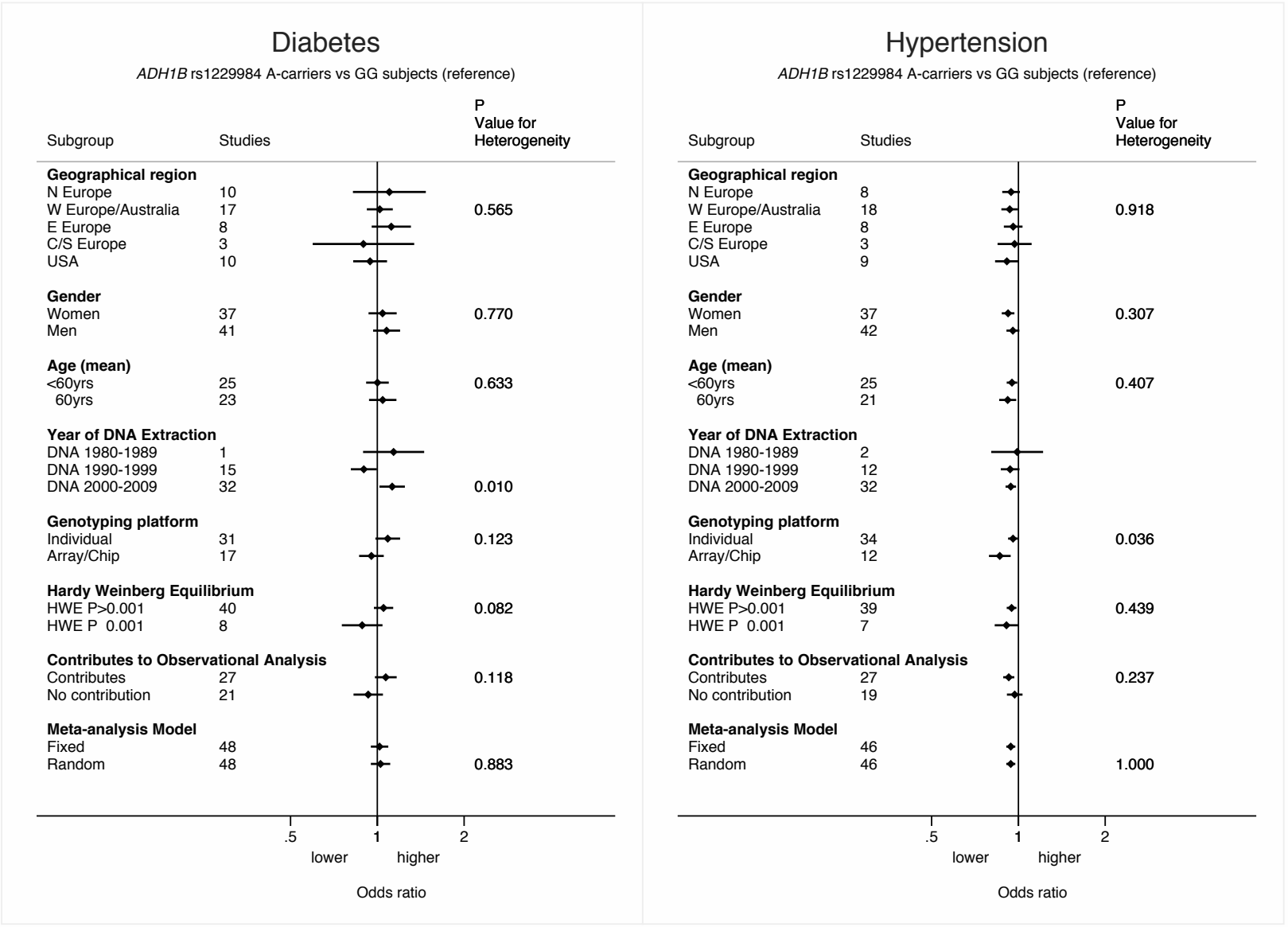


Figure C.18: Subgroup analysis of the association between *ADH1B* rs1229984 (A-allele carriers vs. GG-subjects) and cardiometabolic disorders.

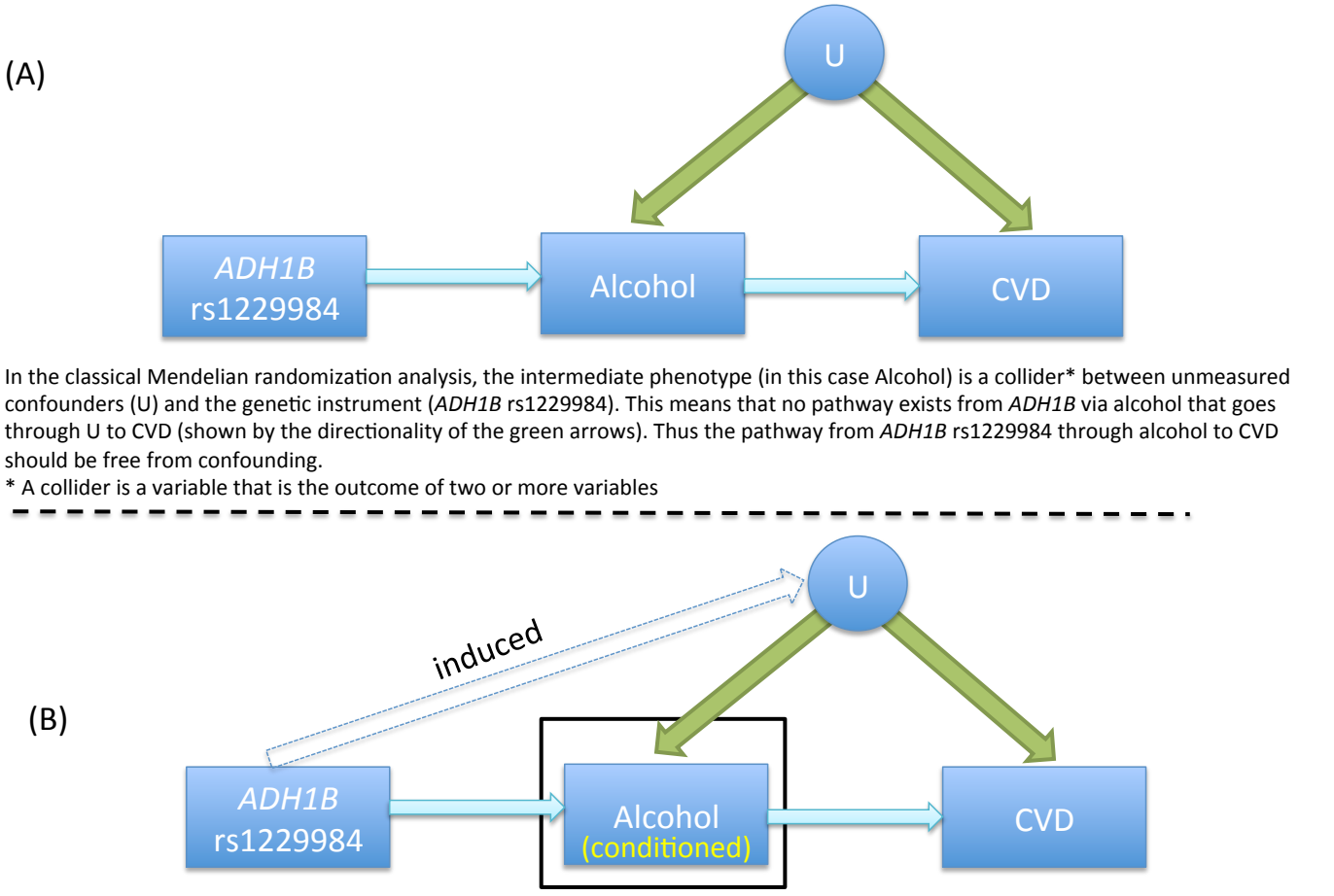


Figure C.19: Directed acyclic graphs to illustrate potential collider bias in Mendelian randomization analysis of alcohol and CVD using the *ADH1B* rs1229984 variant.

Footnote: Collider bias is the principle that adjusting or stratifying for a trait that is a collider may introduce bias by opening a new pathway through the confounder.

C.4 Analysis plan

ADH1B (rs1229984) Alcohol & Cardiovascular Disease

Analysis Plan

Overview

The collaboration will utilise the ADH1B-rs1229984 variant as an instrument to investigate the effect of alcohol consumption on cardiovascular traits and disease. To accomplish this, we will conduct the following steps:

i. First, to confirm the association between ADH1B variant (rs1229984) and the multiple alcohol phenotypes.

ii. The association between ADH1B-rs1229984 variant and cardiovascular biomarkers and prevalent disease will be meta-analysed across studies.

iii. To perform instrumental variable (IV) analysis to estimate the unbiased effect of alcohol consumption on cardiovascular biomarker and prevalent disease, by combining the pooled gene-alcohol and gene-biomarker estimates.

Background

The detrimental effects of alcohol consumption are wide and varied, with consumption linked to over 60 conditions and diseases.[11] Despite this, there is great interest about the potential cardio-protective effect of moderate drinking, which is thought to operate, at least in part, via a favourable impact on lipid profiles most notably raised HDL-C.[1] Other proposed mechanisms include lower levels of LDL-C, lower plasma apolipoprotein A concentration, prevention of clot formation and reduction in platelet aggregation.[1-5]

The potential for alcohol metabolising genes to act as instruments, or proxy measures of alcohol consumption, has been previously described.[15] A significant association has been found between variants of alcohol metabolising genes and several alcohol-related conditions including hypertension, oesophageal cancer and head & neck cancer.[16-18] To date, Mendelian randomization studies have most successfully used ALDH2*2 as the instrument for lifetime alcohol exposure. ALDH2*2 is common in Asian populations but absent in populations of European descent.[19] ALDH2*2 is an inactive variant of the alcohol-metabolizing enzyme aldehyde dehydrogenase (ALDH) which is responsible for oxidising acetaldehyde to acetate and water. Acetaldehyde is a toxic and volatile compound that can cause considerable damage if not quickly converted and eliminated from the body. But because acetaldehyde also causes uncomfortable physical symptoms such as facial flushing, palpitations and hangovers, individuals predisposed to high concentrations are protected from excessive drinking.

The other major enzyme of the alcohol metabolizing pathway is alcohol dehydrogenase (ADH) which oxidizes alcohol to acetaldehyde. Seven genes coding for ADH are found in a tight cluster on chromosome 4 and some are polymorphic in white European populations.[20] More active variants of ADH cause higher concentrations of acetaldehyde in the body following alcohol consumption and are therefore protective against drinking. Functional variants in both ADH1B and ADH1C have been associated with alcohol consumption or alcohol dependence.[21-25] The ADH1B variant (rs1229984) has emerged as the strongest ADH1B SNP to associate with alcohol phenotypes and is therefore the most suitable instrument for Mendelian randomization studies in Europeans.[22] The protective A allele has an allele frequency of approximately 2-5% in Europeans.[26-27]

Objectives

1. To confirm the association between rs1229984 variant and multiple alcohol phenotypes

2. To investigate if the ADH1B (rs1229984) variant is associated with potential confounders (e.g. smoking, education, socio-economic status and physical activity).

3. To investigate the association between rs1229984 variant and cardiovascular biomarkers and disease

Data

The final analysis will be based on meta-analysis of individual-participant data and summary genetic data provided by individual studies (**See Table**). Designated members from each study will be responsible for arranging analysis of their data according to this plan, and for reporting agreed summaries to the meta-analysis co-ordinating group.

Investigators are asked to provide basic information about their study including sampling details and genotyping procedure. A spreadsheet detailing all the information required is provided with this analysis plan (**appendix A**). Please note that automatic genotype coding using standard coding algorithms is preferred to manual coding.

Table. List of studies currently involved in the collaboration

Cohort Name	Number of participants
ALSPAC	6108
ARIC	9557
BRHS	3843
BWHHS	3412
CARDIA	1433
CCHS	9081
CFS	134
CHS	3936
CYPRUS	730
CaPS	1102
DCH	2736
EAS	873
ELSA	5450
EPIC Potsdam	2253
EPIC Netherlands	5186
EPIC Norfolk	20195
FHS	1082
HIMS	4191
HAPIEE Czech	6678
HAPIEE Lithuania	6936
HAPIEE Poland	8779
HAPIEE Russia	7083
Health Professional's Study	1264
IMPROVE Groeningen	421
IMPROVE Kuopio 1	481
IMPROVE Kuopio 2	440
IMPROVE Milan	514
IMPROVE Paris	436
IMPROVE Perugia	464
IMPROVE Stockholm	480
ISGS	1124
Inter99	6332
Izhevsk	653
MESA	2293
MONICA	2558
MRC 1946	2696
NHANESII	2388
NPHSII	2659
Nurse's Health	1322
PREVEND	7729
PROSPER	5504
Portugese Stroke Study	1002

Rotterdam	5827
SMART	7917
TPT	3175
Turin	4526
UCP	1615
ULSAM	453
WH2	5029
WHI	7882
TOTAL	**187,962**

Statistical analysis plan

NB: For specific details of the analysis please see the **Stata do-file** document that is attached to this invitation.

*Data preparation (**please note that almost all of these steps are undertaken in the Stata do-file** with the exception of selection of 'wave' in studies with multiple waves [repeated measures])*

- Repeated measures: Whenever phenotypes are available at more than one time-point, analyses should be undertaken on the wave that maximises inclusion of subjects for key variables (usually the baseline).

- Coding of genotypes: According to the dominant model (carriers of the rare allele to be compared to non-carriers). Rare homozygotes should be grouped together with heterozygotes.

- All alcohol units refer to British units equivalent to 7.9g of ethanol. Units of alcohol can be calculated by multiplying the volume of alcohol in litres by the alcohol beverage percentage.

- Treatment of missing data: All analyses should be limited to subjects who have complete data for sex, age, gene-variant and *any one* of the cardiovascular biomarkers of interest (e.g. blood pressure, lipid profile, apolipoproteins, fibrinogen). It is understood that analyses of other phenotypes including alcohol consumption might be limited to a smaller group of people because of further missing data in the specific phenotype analysed.

- Ethnicity: analysis is limited to individuals of *white European* ancestry

- Family members: where individuals are related, please limit analysis to the oldest person in the relation and discard the younger relative(s)

Analyses:
Analyses are performed automatically by the Stata do-file. For a comprehensive list of traits that are used, please refer to "ADH1B project variables.xls" excel sheet, in which the units for each traits are documented. Conversion factors for traits are provided in the Stata do-file.

Wherever possible analyses should be undertaken using the standardised do file provided.

Meta analysis

Meta-analysis (forest plots etc.) of individual study regression coefficients is conducted by the analysis coordinating group at LSTHM/UCL after submission and preparation of the data.

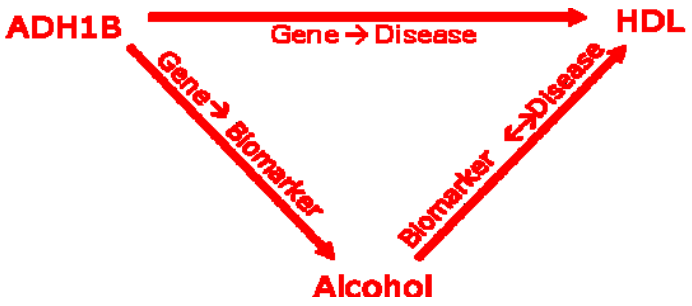
Observational analysis

A pooled dataset is being created to establish the comparable observational effects of weekly units of alcohol consumption on CVD biomarkers.

Instrumental variable analysis

The instrumental variable analysis will take 2 forms:

(1) Assuming that the curvilinear relationship is entirely due to confounding, the standard two-state least squared IV regress will be performed using summary data from each collaborating study
(2) A non-linear Mendelian randomization model has been developed by Professor Frank Dudbidge at LSHTM. This requires an additional analysis, and the analysis files are in development and will be distributed shortly.



The *ADH1B*-CVD collaboration

LSTHM/UCL co-ordinating centre: Michael Holmes, Caroline Dale, Richard Silverwood, Aroon Hingorani, Dave Leon, Frank Dudbridge, Juan P Casas
Email inquiries: m.holmes@ucl.ac.uk or Caroline.Dale@lshtm.ac.uk

References

1. Kloner RA, Rezkalla SH. To drink or not to drink? That is the question. Circulation. 2007 Sep 11;116(11):1306-17.
2. Savolainen MJ, Kesaniemi YA. Effects of alcohol on lipoproteins in relation to coronary heart disease. Curr Opin Lipidol. 1995 Aug;6(4):243-50.
3. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation. 1999 Sep 7;100(10):1050-5.
4. Agarwal DP. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. Alcohol Alcohol. 2002 Sep-Oct;37(5):409-15.
5. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. Bmj. 1999 Dec 11;319(7224):1523-8.
6. Rimm EB, Moats C. Alcohol and coronary heart disease: Drinking patterns and mediators of effect. Annals of Epidemiology 2007;17(5):S3 - S7.
7. Britton A, McKee M. The relation between alcohol and cardiovascular disease in Eastern Europe: explaining the paradox. J Epidemiol Community Health. 2000 May;54(5):328-32.
8. McKee M, Britton A. The positive relationship between alcohol and heart disease in eastern Europe: potential physiological mechanisms. J R Soc Med. 1998 Aug;91(8):402-7.
9. Peasey A, Bobak M, Malyutina S, Pajak A, Kubinova R, Pikhart H, et al. Do lipids contribute to the lack of cardio-protective effect of binge drinking: alcohol consumption and lipids in three eastern European countries. Alcohol Alcohol. 2005 Sep-Oct;40(5):431-5.
10. Fuchs FD, Chambless LE. Is the cardioprotective effect of alcohol real? Alcohol. 2007 Sep;41(6):399-402.
11. Rehm J, Room R, Graham K, Monteiro M, Gmel G, Sempos CT. The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease: an overview. Addiction. 2003 Sep;98(9):1209-28.
12. Sesso HD, Cook NR, Buring JE, Manson JE, Gaziano JM. Alcohol consumption and the risk of hypertension in women and men. Hypertension. 2008 Apr;51(4):1080-7.
13. Asia Pacific Cohort Studies Collaboration. Joint effects of systolic blood pressure and serum cholesterol on cardiovascular disease in the Asia Pacific region. Circulation. 2005 Nov 29;112(22):3384-90.
14. Lewington S, Clarke R. Combined effects of systolic blood pressure and total cholesterol on cardiovascular disease risk. Circulation. 2005 Nov 29;112(22):3373-4.
15. Davey Smith G, Ebrahim S. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? Bmj. 2005 May 7;330(7499):1076-9.
16. Lewis SJ, Smith GD. Alcohol, ALDH2, and esophageal cancer: a meta-analysis which illustrates the potentials and limitations of a Mendelian randomization approach. Cancer Epidemiol Biomarkers Prev. 2005 Aug;14(8):1967-71.
17. Boccia S, Hashibe M, Galli P, De Feo E, Asakage T, Hashimoto T, et al. Aldehyde dehydrogenase 2 and head and neck cancer: a meta-analysis implementing a Mendelian randomization approach. Cancer Epidemiol Biomarkers Prev. 2009 Jan;18(1):248-54.
18. Chen L, Davey Smith G, Harbord RM, Lewis SJ. Alcohol intake and blood pressure: a systematic review implementing a Mendelian randomization approach. PLoS Med. 2008 Mar 4;5(3):e52.
19. Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health. 2007;30(1):5-13.
20. Agarwal DP. Genetic polymorphisms of alcohol metabolizing enzymes. Pathol Biol (Paris). 2001 Nov;49(9):703-9.
21. Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A, Gronbaek M. [Genetic variations in alcohol dehydrogenase, drinking habits and alcoholism]. Ugeskr Laeger. 2008 Aug 25;170(35):2672-5.
22. Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PA, Richter MM, et al. Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet. 2009 Feb 1;18(3):580-93.
23. Kuo PH, Kalsi G, Prescott CA, Hodgkinson CA, Goldman D, van den Oord EJ, et al. Association of ADH and ALDH genes with alcohol dependence in the Irish Affected Sib Pair Study of alcohol dependence (IASPSAD) sample. Alcohol Clin Exp Res. 2008 May;32(5):785-95.
24. Carr LG, Foroud T, Stewart T, Castelluccio P, Edenberg HJ, Li TK. Influence of ADH1B polymorphism on alcohol use and its subjective effects in a Jewish population. Am J Med Genet. 2002 Oct 1;112(2):138-43.
25. Zuccolo L, Fitz-Simon N, Gray R, Ring SM, Sayal K, Davey Smith G, et al. A non-synonymous variant in ADH1B is strongly associated with prenatal alcohol use in a European sample of pregnant women. Hum Mol Genet. 2009 Aug 17.
26. Borras E, Coutelle C, Rosell A, Fernandez-Muixi F, Broch M, Crosas B, et al. Genetic polymorphism of alcohol dehydrogenase in europeans: the ADH2*2 allele decreases the risk for alcoholism and is associated with ADH3*1. Hepatology. 2000 Apr;31(4):984-9.
27. Li H, Mukherjee N, Soundararajan U, Tarnok Z, Barta C, Khaliq S, et al. Geographically separate increases in the frequency of the derived ADH1B*47His allele in eastern and western Asia. Am J Hum Genet. 2007 Oct;81(4):842-6.
28. Hines LM, Stampfer MJ, Ma J, Gaziano JM, Ridker PM, Hankinson SE, et al. Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N Engl J Med. 2001 Feb 22;344(8):549-55.

29. Heidrich J, Wellmann J, Doring A, Illig T, Keil U. Alcohol consumption, alcohol dehydrogenase and risk of coronary heart disease in the MONICA/KORA-Augsburg cohort 1994/1995-2002. Eur J Cardiovasc Prev Rehabil. 2007 Dec;14(6):769-74.
30. Ebrahim S, Lawlor DA, Shlomo YB, Timpson N, Harbord R, Christensen M, et al. Alcohol dehydrogenase type 1C (ADH1C) variants, alcohol consumption traits, HDL-cholesterol and risk of coronary heart disease in women and men: British Women's Heart and Health Study and Caerphilly cohorts. Atherosclerosis. 2008 Feb;196(2):871-8.
31. Djousse L, Levy D, Herbert AG, Wilson PW, D'Agostino RB, Cupples LA, et al. Influence of alcohol dehydrogenase 1C polymorphism on the alcohol-cardiovascular disease association (from the Framingham Offspring Study). Am J Cardiol. 2005 Jul 15;96(2):227-32.
32. Younis J, Cooper JA, Miller GJ, Humphries SE, Talmud PJ. Genetic variation in alcohol dehydrogenase 1C and the beneficial effect of alcohol intake on coronary heart disease risk in the Second Northwick Park Heart Study. Atherosclerosis. 2005 Jun;180(2):225-32.
33. Husemoen LL, Fenger M, Friedrich N, Tolstrup JS, Beenfeldt Fredriksen S, Linneberg A. The association of ADH and ALDH gene variants with alcohol drinking habits and cardiovascular disease risk factors. Alcohol Clin Exp Res. 2008 Nov;32(11):1984-91.
34. Whitfield JB, O'Brien ME, Nightingale BN, Zhu G, Heath AC, Martin NG. ADH genotype does not modify the effects of alcohol on high-density lipoprotein. Alcohol Clin Exp Res. 2003 Mar;27(3):509-14.
35. Druesne-Pecollo N, Tehard B, Mallet Y, Gerber M, Norat T, Hercberg S, et al. Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. Lancet Oncol. 2009 Feb;10(2):173-80.
36. Tolstrup JS, Gronbaek M, Nordestgaard BG. Alcohol Intake, Myocardial Infarction, Biochemical Risk Factors, and Alcohol Dehydrogenase Genotypes. Circulation Cardiovascular Genetics. 2009;2:507-14.
37. Hellerstedt WL, Jeffery RW, Murray DM. The association between alcohol intake and adiposity in the general population. Am J Epidemiol. 1990 Oct;132(4):594-611.
38. Arif AA, Rohrer JE. Patterns of alcohol drinking and its association with obesity: data from the Third National Health and Nutrition Examination Survey, 1988-1994. BMC Public Health. 2005;5:126.
39. Lukasiewicz E, Mennen LI, Bertrais S, Arnault N, Preziosi P, Galan P, et al. Alcohol intake in relation to body mass index and waist-to-hip ratio: the importance of type of alcoholic beverage. Public Health Nutr. 2005 May;8(3):315-20.
40. Bobak M, Skodova Z, Marmot M. Beer and obesity: a cross-sectional study. Eur J Clin Nutr. 2003 Oct;57(10):1250-3.
41. Lindgren CM, Heid IM, Randall JC, Lamina C, Steinthorsdottir V, Qi L, et al. Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet. 2009 Jun;5(6):e1000508.
42. Heeb JL, Gmel G. Measuring alcohol consumption: a comparison of graduated frequency, quantity frequency, and weekly recall diary methods in a general population survey. Addict Behav. 2005 Mar;30(3):403-13.
43. Bloomfield K, Gmel G, Wilsnack S. Introduction to special issue 'gender, culture and alcohol problems: a multi-national study'. Alcohol Alcohol Suppl. 2006 Oct-Nov;41(1):i3-i7.
44. Paillard F. High-density lipoprotein cholesterol and triglycerides in the statin era: a pending issue? Arch Cardiovasc Dis. 2009 Mar;102(3):165-7.
45. Laforest L, Souchet T, Moulin P, Ritleng C, Desamericq G, Le Jeunne P, et al. Prevalence of low high-density lipoprotein cholesterol and hypertriglyceridaemia in patients treated with hypolipidaemic drugs. Arch Cardiovasc Dis. 2009 Jan;102(1):43-50.

Appendix D

Published papers

References

[1] D. Lloyd-Jones, R. Adams, M. Carnethon, G. De Simone, T. B. Ferguson, K. Flegal, E. Ford, K. Furie, A. Go, K. Greenlund, N. Haase, S. Hailpern, M. Ho, V. Howard, B. Kissela, S. Kittner, D. Lackland, L. Lisabeth, A. Marelli, M. McDermott, J. Meigs, D. Mozaffarian, G. Nichol, C. O'Donnell, V. Roger, W. Rosamond, R. Sacco, P. Sorlie, R. Stafford, J. Steinberger, T. Thom, S. Wasserthiel-Smoller, N. Wong, J. Wylie-Rosett, and Y. Hong. Heart disease and stroke statistics–2009 update: a report from the american heart association statistics committee and stroke statistics subcommittee. *Circulation*, 119(3):480–6, 2009. American Heart Association Statistics Committee and Stroke Statistics Subcommittee Journal Article United States.

[2] D. M. Lloyd-Jones, M. G. Larson, A. Beiser, and D. Levy. Lifetime risk of developing coronary heart disease. *Lancet*, 353(9147):89–92, 1999. Journal Article England.

[3] Paul A. Heidenreich, Justin G. Trogdon, Olga A. Khavjou, Javed Butler, Kathleen Dracup, Michael D. Ezekowitz, Eric Andrew Finkelstein, Yuling Hong, S Claiborne Johnston, Amit Khera, Donald M. Lloyd-Jones, Sue A. Nelson, Graham Nichol, Diane Orenstein, Peter W F. Wilson, Y Joseph Woo, American Heart Association Advocacy Coordinating Committee , Stroke Council , Council on Cardiovascular Radiology , Intervention, Council on Clinical Cardiology , Council on Epidemiology , Prevention, Council on Arteriosclerosis , Thrombosis , Vascular Biology, Council on Cardiopulmonary , Critical Care , Perioperative , Resuscitation, Council on Cardiovascular Nursing , Council on the Kidney in Cardiovascular Disease , Council on Cardiovascular Surgery , Anesthesia, Interdisciplinary Council on Quality of Care, and Outcomes Research. Forecasting the future of cardiovascular disease in the united states: a policy statement from the american heart association. *Circulation*, 123(8):933–944, Mar 2011.

[4] M. E. Marenberg, N. Risch, L. F. Berkman, B. Floderus, and U. de Faire. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med*, 330(15):1041–6, 1994. 2-T32-MH-14235/MH/NIMH NIH HHS/United States HG-00348/HG/NHGRI NIH HHS/United States Journal Article Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. United states.

[5] A. Wienke, N. V. Holm, A. Skytthe, and A. I. Yashin. The heritability of mortality due to heart diseases: a correlated frailty model applied to danish twins. *Twin Res*, 4(4):266–74, 2001. 7PO1AG08761-09/AG/NIA NIH HHS/United States Comparative Study Journal Article Research Support, U.S. Gov't, P.H.S. Twin Study England the official journal of the International Society for Twin Studies.

[6] S. Zdravkovic, A. Wienke, N. L. Pedersen, M. E. Marenberg, A. I. Yashin, and U. De Faire. Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 swedish twins. *J Intern Med*, 252(3):247–54, 2002. 7P01 AG08761/AG/NIA NIH HHS/United States Journal

Article Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Twin Study England.

[7] H. C. Bucher and D. R. Ragland. Socioeconomic indicators and mortality from coronary heart disease and cancer: a 22-year follow-up of middle-aged men. *Am J Public Health*, 85(9):1231–6, 1995. Journal Article Multicenter Study Research Support, Non-U.S. Gov't United states.

[8] H. Hemingway, M. Shipley, P. Macfarlane, and M. Marmot. Impact of socioeconomic status on coronary mortality in people with symptoms, electrocardiographic abnormalities, both or neither: the original whitehall study 25 year follow up. *J Epidemiol Community Health*, 54(7):510–6, 2000. Journal Article Research Support, Non-U.S. Gov't England.

[9] A. E. Kunst, F. Groenhof, O. Andersen, J. K. Borgan, G. Costa, G. Desplanques, H. Filakti, R. Giraldes Mdo, F. Faggiano, S. Harding, C. Junker, P. Martikainen, C. Minder, B. Nolan, F. Pagnanelli, E. Regidor, D. Vagero, T. Valkonen, and J. P. Mackenbach. Occupational class and ischemic heart disease mortality in the united states and 11 european countries. *Am J Public Health*, 89(1):47–53, 1999. Comparative Study Journal Article Research Support, Non-U.S. Gov't United states.

[10] J. P. Strong, G. T. Malcom, C. A. McMahan, R. E. Tracy, 3rd Newman, W. P., E. E. Herderick, and J. F. Cornhill. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the pathobiological determinants of atherosclerosis in youth study. *Jama*, 281(8):727–35, 1999. HL-33728/HL/NHLBI NIH HHS/United States HL-33733/HL/NHLBI NIH HHS/United States HL-33765/HL/NHLBI NIH HHS/United States etc. Journal Article Multicenter Study Research Support, U.S. Gov't, P.H.S. United states the journal of the American Medical Association.

[11] P. Puska, K. Koskela, H. Pakarinen, P. Puumalainen, V. Soininen, and J. Tuomilehto. The north karelia project: a programme for community control of cardiovascular diseases. *Scand J Soc Med*, 4(2):57–60, 1976.

[12] Comprehensive cardiovascular community control programmes in europe. *EURO Rep Stud*, (106):1–91, 1988.

[13] P. Pietinen, E. Vartiainen, R. Seppanen, A. Aro, and P. Puska. Changes in diet in finland from 1972 to 1992: impact on coronary heart disease risk. *Prev Med*, 25(3):243–50, 1996. Journal Article Research Support, Non-U.S. Gov't United states.

[14] S. Ebrahim and G. D. Smith. Exporting failure? coronary heart disease and stroke in developing countries. *Int J Epidemiol*, 30(2):201–205, Apr 2001.

[15] U. Uusitalo, E. J. Feskens, J. Tuomilehto, G. Dowse, U. Haw, D. Fareed, F. Hemraj, H. Gareeboo, K. G. Alberti, and P. Zimmet. Fall in total cholesterol concentration over five years in association with changes in fatty acid composition of cooking oil in mauritius: cross sectional survey. *BMJ*, 313(7064):1044–1046, Oct 1996.

[16] Stephen S. Lim, Theo Vos, Abraham D. Flaxman, Goodarz Danaei, Kenji Shibuya, Heather Adair-Rohani, Markus Amann, H Ross Anderson, Kathryn G. Andrews, Martin Aryee, Charles Atkinson, Loraine J. Bacchus, Adil N. Bahalim, Kalpana Balakrishnan, John Balmes, Suzanne Barker-Collo, Amanda Baxter, Michelle L. Bell, Jed D. Blore, Fiona Blyth, Carissa Bonner, Guilherme Borges, Rupert Bourne, Michel Boussinesq, Michael Brauer, Peter Brooks, Nigel G. Bruce, Bert Brunekreef, Claire Bryan-Hancock, Chiara Bucello, Rachelle Buchbinder, Fiona Bull, Richard T.

Burnett, Tim E. Byers, Bianca Calabria, Jonathan Carapetis, Emily Carnahan, Zoe Chafe, Fiona Charlson, Honglei Chen, Jian Shen Chen, Andrew Tai-Ann Cheng, Jennifer Christine Child, Aaron Cohen, K Ellicott Colson, Benjamin C. Cowie, Sarah Darby, Susan Darling, Adrian Davis, Louisa Degenhardt, Frank Dentener, Don C. Des Jarlais, Karen Devries, Mukesh Dherani, Eric L. Ding, E Ray Dorsey, Tim Driscoll, Karen Edmond, Suad Eltahir Ali, Rebecca E. Engell, Patricia J. Erwin, Saman Fahimi, Gail Falder, Farshad Farzadfar, Alize Ferrari, Mariel M. Finucane, Seth Flaxman, Francis Gerry R. Fowkes, Greg Freedman, Michael K. Freeman, Emmanuela Gakidou, Santu Ghosh, Edward Giovannucci, Gerhard Gmel, Kathryn Graham, Rebecca Grainger, Bridget Grant, David Gunnell, Hialy R. Gutierrez, Wayne Hall, Hans W. Hoek, Anthony Hogan, H Dean Hosgood, 3rd, Damian Hoy, Howard Hu, Bryan J. Hubbell, Sally J. Hutchings, Sydney E. Ibeanusi, Gemma L. Jacklyn, Rashmi Jasrasaria, Jost B. Jonas, Haidong Kan, John A. Kanis, Nicholas Kassebaum, Norito Kawakami, Young-Ho Khang, Shahab Khatibzadeh, Jon-Paul Khoo, Cindy Kok, Francine Laden, Ratilal Lalloo, Qing Lan, Tim Lathlean, Janet L. Leasher, James Leigh, Yang Li, John Kent Lin, Steven E. Lipshultz, Stephanie London, Rafael Lozano, Yuan Lu, Joelle Mak, Reza Malekzadeh, Leslie Mallinger, Wagner Marcenes, Lyn March, Robin Marks, Randall Martin, Paul McGale, John McGrath, Sumi Mehta, George A. Mensah, Tony R. Merriman, Renata Micha, Catherine Michaud, Vinod Mishra, Khayriyyah Mohd Hanafiah, Ali A. Mokdad, Lidia Morawska, Dariush Mozaffarian, Tasha Murphy, Mohsen Naghavi, Bruce Neal, Paul K. Nelson, Joan Miquel Nolla, Rosana Norman, Casey Olives, Saad B. Omer, Jessica Orchard, Richard Osborne, Bart Ostro, Andrew Page, Kiran D. Pandey, Charles D H. Parry, Erin Passmore, Jayadeep Patra, Neil Pearce, Pamela M. Pelizzari, Max Petzold, Michael R. Phillips, Dan Pope, C Arden Pope, 3rd, John Powles, Mayuree Rao, Homie Razavi, Eva A. Rehfuess, Jrgen T. Rehm, Beate Ritz, Frederick P. Rivara, Thomas Roberts, Carolyn Robinson, Jose A. Rodriguez-Portales, Isabelle Romieu, Robin Room, Lisa C. Rosenfeld, Ananya Roy, Lesley Rushton, Joshua A. Salomon, Uchechukwu Sampson, Lidia Sanchez-Riera, Ella Sanman, Amir Sapkota, Soraya Seedat, Peilin Shi, Kevin Shield, Rupak Shivakoti, Gitanjali M. Singh, David A. Sleet, Emma Smith, Kirk R. Smith, Nicolas J C. Stapelberg, Kyle Steenland, Heidi Stckl, Lars Jacob Stovner, Kurt Straif, Lahn Straney, George D. Thurston, Jimmy H. Tran, Rita Van Dingenen, Aaron van Donkelaar, J Lennert Veerman, Lakshmi Vijayakumar, Robert Weintraub, Myrna M. Weissman, Richard A. White, Harvey Whiteford, Steven T. Wiersma, James D. Wilkinson, Hywel C. Williams, Warwick Williams, Nicholas Wilson, Anthony D. Woolf, Paul Yip, Jan M. Zielinski, Alan D. Lopez, Christopher J L. Murray, Majid Ezzati, Mohammad A. AlMazroa, and Ziad A. Memish. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. *Lancet*, 380(9859):2224–2260, Dec 2012.

[17] R. DOLL and A. B. HILL. The mortality of doctors in relation to their smoking habits; a preliminary report. *Br Med J*, 1(4877):1451–1455, Jun 1954.

[18] Carey Conley Thomson, Michael Siegel, Jonathan Winickoff, Lois Biener, and Nancy A. Rigotti. Household smoking bans and adolescents' perceived prevalence of smoking and social acceptability of smoking. *Prev Med*, 41(2):349–356, Aug 2005.

[19] Nicholas A. Christakis and James H. Fowler. The collective dynamics of smoking in a large social network. *N Engl J Med*, 358(21):2249–2258, May 2008.

[20] Smoking ban gets seal of public approval. *The Scottish Goverment. Available at: http://www.scotland.gov.uk/News/Releases/2006/06/26080617*, 2006.

[21] Health act 2006. available at: http://www.legislation.gov.uk/ukpga/2006/28/contents. *The National Archives*, 2006.

[22] Michelle Sims, Roy Maxwell, Linda Bauld, and Anna Gilmore. Short term impact of smoke-free legislation in england: retrospective analysis of hospital admissions for myocardial infarction. *BMJ*, 340:c2161, 2010.

[23] Giulia Cesaroni, Francesco Forastiere, Nera Agabiti, Pasquale Valente, Piergiorgio Zuccaro, and Carlo A. Perucci. Effect of the italian smoking ban on population rates of acute coronary events. *Circulation*, 117(9):1183–1188, Mar 2008.

[24] Sadik A. Khuder, Sheryl Milz, Timothy Jordan, James Price, Kathi Silvestri, and Pam Butler. The impact of a smoking ban on hospital admissions for coronary heart disease. *Prev Med*, 45(1):3–8, Jul 2007.

[25] Smoke-free policies improve health. available at $www.cdc.gov/tobacco/data_statistics/fact_sheets/secondhand_smoke/$ *Centers for Disease Control and Prevention*, 2011.

[26] Public health guidance, ph25 - issued: June 2010. prevention of cardiovascular disease at the population level. *NICE*, 2010.

[27] Mahshid Dehghan, Noori Akhtar-Danesh, and Anwar T. Merchant. Childhood obesity, prevalence and prevention. *Nutr J*, 4:24, 2005.

[28] A. Must and R. S. Strauss. Risks and consequences of childhood and adolescent obesity. *Int J Obes Relat Metab Disord*, 23 Suppl 2:S2–11, Mar 1999.

[29] Minimum alcohol price planned for england and wales. availeble at: http://www.bbc.co.uk/news/uk-17482035. *BBC News*.

[30] Donna F. Stroup, Valerie Johnson, Robert S. Hahn, and Dwayne C. Proctor. Reversing the trend of childhood obesity. *Prev Chronic Dis*, 6(3):A83, Jul 2009.

[31] M. R. Law, J. K. Morris, and N. J. Wald. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ*, 338:b1665, 2009.

[32] R. Collins, R. Peto, S. MacMahon, P. Hebert, N. H. Fiebach, K. A. Eberlein, J. Godwin, N. Qizilbash, J. O. Taylor, and C. H. Hennekens. Blood pressure, stroke, and coronary heart disease. part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet*, 335(8693):827–38, 1990. Clinical Trial Journal Article Randomized Controlled Trial Research Support, Non-U.S. Gov't Review England.

[33] C. Baigent, L. Blackwell, J. Emberson, L. E. Holland, C. Reith, N. Bhala, R. Peto, E. H. Barnes, A. Keech, J. Simes, and R. Collins. Efficacy and safety of more intensive lowering of ldl cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet*, 376(9753):1670–81, 2010. Cholesterol Treatment Trialists' (CTT) Collaboration Baigent, C Blackwell, L Emberson, J Holland, L E Reith, C Bhala, N Peto, R Barnes, E H Keech, A Simes, J Collins, R British Heart Foundation/United Kingdom Medical Research Council/United Kingdom Meta-Analysis Research Support, Non-U.S. Gov't England Lancet Lancet. 2010 Nov 13;376(9753):1670-81. Epub 2010 Nov 8.

[34] C. Baigent, A. Keech, P. M. Kearney, L. Blackwell, G. Buck, C. Pollicino, A. Kirby, T. Sourjina, R. Peto, R. Collins, and R. Simes. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*, 366(9493):1267–78, 2005. Cholesterol Treatment Trialists' (CTT) Collaborators WT074327/Wellcome Trust/United Kingdom Wellcome Trust/United Kingdom Journal Article Meta-Analysis Research Support, Non-U.S. Gov't England.

[35] G. Rose. Sick individuals and sick populations. *Int J Epidemiol*, 14(1):32–8, 1985. Journal Article England.

[36] R. Jackson, C. M. Lawes, D. A. Bennett, R. J. Milne, and A. Rodgers. Treatment with drugs to lower blood pressure and blood cholesterol based on an individual's absolute cardiovascular risk. *Lancet*, 365(9457):434–41, 2005. Journal Article Research Support, Non-U.S. Gov't England.

[37] Piotr Bandosz, Martin O'Flaherty, Wojciech Drygas, Marcin Rutkowski, Jacek Koziarek, Bogdan Wyrzykowski, Kathleen Bennett, Tomasz Zdrojewski, and Simon Capewell. Decline in mortality from coronary heart disease in poland after socioeconomic transformation: modelling study. *BMJ*, 344:d8136, 2012.

[38] S. Yusuf, S. Hawken, S. Ounpuu, T. Dans, A. Avezum, F. Lanas, M. McQueen, A. Budaj, P. Pais, J. Varigos, and L. Lisheng. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the interheart study): case-control study. *Lancet*, 364(9438):937–52, 2004. Yusuf, Salim Hawken, Steven Ounpuu, Stephanie Dans, Tony Avezum, Alvaro Lanas, Fernando McQueen, Matthew Budaj, Andrzej Pais, Prem Varigos, John Lisheng, Liu INTERHEART Study Investigators Comparative Study Multicenter Study Research Support, Non-U.S. Gov't England Lancet Lancet. 2004 Sep 11-17;364(9438):937-52.

[39] K. Giskes, A. E. Kunst, J. Benach, C. Borrell, G. Costa, E. Dahl, J A A. Dalstra, B. Federico, U. Helmert, K. Judge, E. Lahelma, K. Moussa, P. O. Ostergren, S. Platt, R. Prattala, N. K. Rasmussen, and J. P. Mackenbach. Trends in smoking behaviour between 1985 and 2000 in nine european countries by education. *J Epidemiol Community Health*, 59(5):395–401, May 2005.

[40] A. R. Omran. The epidemiologic transition. a theory of the epidemiology of population change. *Milbank Mem Fund Q*, 49(4):509–538, Oct 1971.

[41] Bernard J. Gersh, Karen Sliwa, Bongani M. Mayosi, and Salim Yusuf. Novel therapeutic concepts: the epidemic of cardiovascular disease in the developing world: global implications. *Eur Heart J*, 31(6):642–648, Mar 2010.

[42] Neil J. Stone. Reducing residual risk in secondary prevention of cardiovascular disease. *Circulation*, 125(16):1958–1960, Apr 2012.

[43] P. N. Hopkins and R. R. Williams. A survey of 246 suggested coronary risk factors. *Atherosclerosis*, 40(1):1–52, 1981.

[44] F. M. Sacks, M. A. Pfeffer, L. A. Moye, J. L. Rouleau, J. D. Rutherford, T. G. Cole, L. Brown, J. W. Warnica, J. M. Arnold, C. C. Wun, B. R. Davis, and E. Braunwald. 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*, 335(14):1001–9, 1996. Clinical Trial Journal Article Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United states.

[45] J. Shepherd, S. M. Cobbe, I. Ford, C. G. Isles, A. R. Lorimer, P. W. MacFarlane, J. H. McKillop, and C. J. Packard. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. west of scotland coronary prevention study group. *N Engl J Med*, 333(20):1301–7, 1995. Clinical Trial Journal Article Randomized Controlled Trial Research Support, Non-U.S. Gov't United states.

[46] Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the scandinavian simvastatin survival study (4s). *Lancet*, 344(8934):1383–9, 1994. Clinical Trial Journal Article Randomized Controlled Trial Research Support, Non-U.S. Gov't England.

[47] Timothy J. Aitman, Charles Boone, Gary A. Churchill, Michael O. Hengartner, Trudy F C. Mackay, and Derek L. Stemple. The future of model organisms in human disease research. *Nat Rev Genet*, 12(8):575–582, Aug 2011.

[48] Stewart C. Whitman. A practical approach to using mice in atherosclerosis research. *Clin Biochem Rev*, 25(1):81–93, Feb 2004.

[49] Alan Daugherty. Mouse models of atherosclerosis. *Am J Med Sci*, 323(1):3–10, Jan 2002.

[50] Daniel G. Hackam and Donald A. Redelmeier. Translation of research evidence from animals to humans. *JAMA*, 296(14):1731–1732, Oct 2006.

[51] H Bart van der Worp, David W. Howells, Emily S. Sena, Michelle J. Porritt, Sarah Rewell, Victoria O'Collins, and Malcolm R. Macleod. Can animal models of disease reliably inform human studies? *PLoS Med*, 7(3):e1000245, Mar 2010.

[52] Emily S. Sena, H Bart van der Worp, Philip M W. Bath, David W. Howells, and Malcolm R. Macleod. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*, 8(3):e1000344, Mar 2010.

[53] M. J. Stampfer and G. A. Colditz. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Prev Med*, 20(1):47–63, Jan 1991.

[54] Jacques E. Rossouw, Garnet L. Anderson, Ross L. Prentice, Andrea Z. LaCroix, Charles Kooperberg, Marcia L. Stefanick, Rebecca D. Jackson, Shirley A A. Beresford, Barbara V. Howard, Karen C. Johnson, Jane Morley Kotchen, Judith Ockene, and Writing Group for the Women's Health Initiative Investigators . Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. *JAMA*, 288(3):321–333, Jul 2002.

[55] E. B. Rimm, M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz, and W. C. Willett. Vitamin e consumption and the risk of coronary heart disease in men. *N Engl J Med*, 328(20):1450–1456, May 1993.

[56] M. J. Stampfer, C. H. Hennekens, J. E. Manson, G. A. Colditz, B. Rosner, and W. C. Willett. Vitamin e consumption and the risk of coronary disease in women. *N Engl J Med*, 328(20):1444–1449, May 1993.

[57] A. B. HILL. The environment and disease: Association or causation? *Proc R Soc Med*, 58:295–300, May 1965.

[58] Rachel S. Eidelman, Danielle Hollar, Patricia R. Hebert, Gervasio A. Lamas, and Charles H. Hennekens. Randomized trials of vitamin e in the treatment and prevention of cardiovascular disease. *Arch Intern Med*, 164(14):1552–1556, Jul 2004.

[59] Edgar R Miller, 3rd, Roberto Pastor-Barriuso, Darshan Dalal, Rudolph A. Riemersma, Lawrence J. Appel, and Eliseo Guallar. Meta-analysis: high-dosage vitamin e supplementation may increase all-cause mortality. *Ann Intern Med*, 142(1):37–46, Jan 2005.

[60] J. P. Ioannidis. Why most published research findings are false. *PLoS Med*, 2(8):e124, 2005. Journal Article United States.

[61] Neal S. Young, John P A. Ioannidis, and Omar Al-Ubaydli. Why current publication practices may distort science. *PLoS Med*, 5(10):e201, Oct 2008.

[62] C Reactive Protein Coronary Heart Disease Genetics Collaboration (C. C. G. C) , Frances Wensley, Pei Gao, Stephen Burgess, Stephen Kaptoge, Emanuele Di Angelantonio, Tina Shah, James C. Engert, Robert Clarke, George Davey-Smith, Brge G. Nordestgaard, Danish Saleheen, Nilesh J. Samani, Manjinder Sandhu, Sonia Anand, Mark B. Pepys, Liam Smeeth, John Whittaker, Juan Pablo Casas, Simon G. Thompson, Aroon D. Hingorani, and John Danesh. Association between c reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *BMJ*, 342:d548, 2011.

[63] Jennifer A. Hutcheon, Arnaud Chiolero, and James A. Hanley. Random measurement error and regression dilution bias. *BMJ*, 340:c2289, 2010.

[64] Sander Greenland. Quantifying biases in causal models: classical confounding vs collider-stratification bias. *Epidemiology*, 14(3):300–306, May 2003.

[65] Peter Armitage. Fisher, bradford hill, and randomization. *Int J Epidemiol*, 32(6):925–8; discussion 945–8, Dec 2003.

[66] Cochrane handbook for systematic reviews of interventions. edited by jpt higgins and s green. cochrane book series. available at: http://www.cochrane.org/training/cochrane-handbook. 2012.

[67] A. Hingorani and S. Humphries. Nature's randomised trials. *Lancet*, 366(9501):1906–8, 2005. Hingorani, Aroon Humphries, Steve Comment England Lancet Lancet. 2005 Dec 3;366(9501):1906-8.

[68] D. Oldroyd. Gregor mendel: founding-father of modern genetics? *Endeavour*, 8(1):29–31, 1984.

[69] M. B. Katan. Apolipoprotein e isoforms, serum cholesterol, and cancer. *Lancet*, 1(8479):507–508, Mar 1986.

[70] A. J. McMichael, O. M. Jensen, D. M. Parkin, and D. G. Zaridze. Dietary and endogenous cholesterol and human cancer. *Epidemiol Rev*, 6:192–216, 1984.

[71] Richard Karas Paul Michael Lavigne, Haseeb Jafri. The association between lower levels of low-density lipoprotein cholesterol and cancer predates the diagnosis of cancer by 18 years. *JACC*, 59(13):E1622, 2012.

[72] G. Davey Smith and S. Ebrahim. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures? 330(7499):1076–9–, 2005.

[73] G. Davey Smith and S. Ebrahim. 'mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*, 32(1):1–22, 2003. Davey Smith, George Ebrahim, Shah Lectures England International journal of epidemiology Int J Epidemiol. 2003 Feb;32(1):1-22.

[74] Martijn B. Katan. Commentary: Mendelian randomization, 18 years on. *Int J Epidemiol*, 33(1):10–11, Feb 2004.

[75] George Davey Smith and Shah Ebrahim. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol*, 33(1):30–42, Feb 2004.

[76] Martin D. Tobin, Cosetta Minelli, Paul R. Burton, and John R. Thompson. Commentary: development of mendelian randomization: from hypothesis test to 'mendelian deconfounding'. *Int J Epidemiol*, 33(1):26–29, Feb 2004.

[77] Duncan C. Thomas and David V. Conti. Commentary: the concept of 'mendelian randomization'. *Int J Epidemiol*, 33(1):21–25, Feb 2004.

[78] Paul Brennan. Commentary: Mendelian randomization and gene-environment interaction. *Int J Epidemiol*, 33(1):17–21, Feb 2004.

[79] Keith Wheatley and Richard Gray. Commentary: Mendelian randomization–an update on its use to evaluate allogeneic stem cell transplantation in leukaemia. *Int J Epidemiol*, 33(1):15–17, Feb 2004.

[80] Jrgen K. Willmann, Nicholas van Bruggen, Ludger M. Dinkelborg, and Sanjiv S. Gambhir. Molecular imaging in drug development. *Nat Rev Drug Discov*, 7(7):591–607, Jul 2008.

[81] Michael V. Holmes, Paul Newcombe, Jaroslav A. Hubacek, Reecha Sofat, Sally L. Ricketts, Jackie Cooper, Monique M B. Breteler, Leonelo E. Bautista, Pankaj Sharma, John C. Whittaker, Liam Smeeth, F Gerald R. Fowkes, Ale Algra, Veronika Shmeleva, Zoltan Szolnoki, Mark Roest, Michael Linnebank, Jeppe Zacho, Michael A. Nalls, Andrew B. Singleton, Luigi Ferrucci, John Hardy, Bradford B. Worrall, Stephen S. Rich, Mar Matarin, Paul E. Norman, Leon Flicker, Osvaldo P. Almeida, Frank M. van Bockxmeer, Hiroshi Shimokata, Kay-Tee Khaw, Nicholas J. Wareham, Martin Bobak, Jonathan A C. Sterne, George Davey Smith, Philippa J. Talmud, Cornelia van Duijn, Steve E. Humphries, Jackie F. Price, Shah Ebrahim, Debbie A. Lawlor, Graeme J. Hankey, James F. Meschia, Manjinder S. Sandhu, Aroon D. Hingorani, and Juan P. Casas. Effect modification by population dietary folate on the association between mthfr genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet*, 378(9791):584–594, Aug 2011.

[82] Tom M. Palmer, Debbie A. Lawlor, Roger M. Harbord, Nuala A. Sheehan, Jon H. Tobias, Nicholas J. Timpson, George Davey Smith, and Jonathan A C. Sterne. Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat Methods Med Res*, 21(3):223–242, Jun 2012.

[83] D.I. Swerdlow, M. V. Holmes, K. Kuchenbaeker, J.E. Engmann, T. Shah, R. Sofat, Y Guo, C. Chung, and Peas. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet*, 379(9822):1214–1224, Mar 2012.

[84] D. A. Lawlor, R. M. Harbord, J. A. Sterne, N. Timpson, and G. Davey Smith. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*, 27(8):1133–63, 2008.

[85] M. Marmot and E. Brunner. Cohort profile: the whitehall ii study. *Int J Epidemiol*, 34(2):251–6, 2005.

[86] Tina Shah, Delilah Zabaneh, Tom Gaunt, Daniel I. Swerdlow, Sonia Shah, Philippa J. Talmud, Ian N. Day, John Whittaker, Michael V. Holmes, Reecha Sofat, Steve E. Humphries, Mika Kivimaki, Meena Kumari, Aroon D. Hingorani, and Juan P. Casas. Gene-centric analysis identifies variants associated with interleukin-6 levels and shared pathways with other inflammation markers. *Circ Cardiovasc Genet*, 6(2):163–170, Apr 2013.

[87] Nuala A. Sheehan, Vanessa Didelez, Paul R. Burton, and Martin D. Tobin. Mendelian randomisation and causal inference in observational epidemiology. *PLoS Med*, 5(8):e177, Aug 2008.

[88] Andrew D. Johnson, Robert E. Handsaker, Sara L. Pulit, Marcia M. Nizzari, Christopher J. O'Donnell, and Paul I W. de Bakker. Snap: a web-based tool for identification and annotation of proxy snps using hapmap. *Bioinformatics*, 24(24):2938–2939, Dec 2008.

[89] Robert Lawrence, Aaron G. Day-Williams, Richard Mott, John Broxholme, Lon R. Cardon, and Eleftheria Zeggini. Gliders–a web-based search engine for genome-wide linkage disequilibrium between hapmap snps. *BMC Bioinformatics*, 10:367, 2009.

[90] S. Yusuf, S. Reddy, S. Ounpuu, and S. Anand. Global burden of cardiovascular diseases: Part ii: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Circulation*, 104(23):2855–2864, Dec 2001.

[91] Dai Wang, Yu Sun, Paul Stang, Jesse A. Berlin, Marsha A. Wilcox, and Qingqin Li. Comparison of methods for correcting population stratification in a genome-wide association study of rheumatoid arthritis: principal-component analysis versus multidimensional scaling. *BMC Proc*, 3 Suppl 7:S109, 2009.

[92] Alkes L. Price, Nick J. Patterson, Robert M. Plenge, Michael E. Weinblatt, Nancy A. Shadick, and David Reich. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*, 38(8):904–909, Aug 2006.

[93] Mingyao Li, Muredach P. Reilly, Daniel J. Rader, and Li-San Wang. Correcting population stratification in genetic association studies using a phylogenetic approach. *Bioinformatics*, 26(6):798–806, Mar 2010.

[94] Alkes L. Price, Nick J. Patterson, Robert M. Plenge, Michael E. Weinblatt, Nancy A. Shadick, and David Reich. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*, 38(8):904–909, Aug 2006.

[95] Ikechukwu U. Ogbuanu, Hongmei Zhang, and Wilfried Karmaus. Can we apply the mendelian randomization methodology without considering epigenetic effects? *Emerg Themes Epidemiol*, 6:3, 2009.

[96] G. Gibson and G. Wagner. Canalization in evolutionary genetics: a stabilizing theory? *Bioessays*, 22(4):372–80, 2000. Gibson, G Wagner, G Research Support, Non-U.S. Gov't Review England BioEssays : news and reviews in molecular, cellular and developmental biology Bioessays. 2000 Apr;22(4):372-80.

[97] Daniel S. Budnitz, Maribeth C. Lovegrove, Nadine Shehab, and Chesley L. Richards. Emergency hospitalizations for adverse drug events in older americans. *N Engl J Med*, 365(21):2002–2012, Nov 2011.

[98] Aroon D. Hingorani, Danille A van der Windt, Richard D. Riley, Keith Abrams, Karel G M. Moons, Ewout W. Steyerberg, Sara Schroter, Willi Sauerbrei, Douglas G. Altman, Harry Hemingway, and P. R. O. G. R. E. S. S Group . Prognosis research strategy (progress) 4: stratified medicine research. *BMJ*, 346:e5793, 2013.

[99] Peter Brindle, Jonathan Emberson, Fiona Lampe, Mary Walker, Peter Whincup, Tom Fahey, and Shah Ebrahim. Predictive accuracy of the framingham coronary risk score in british men: prospective cohort study. *BMJ*, 327(7426):1267, Nov 2003.

[100] Statins for the prevention of cardiovascular events http://www.nice.org.uk/nicemedia/pdf/ta094guidance.pdf. *NICE*, 2008.

[101] I. Graham, D. Atar, K. Borch-Johnsen, G. Boysen, G. Burell, R. Cifkova, J. Dallongeville, G. De Backer, S. Ebrahim, B. Gjelsvik, C. Herrmann-Lingen, A. Hoes, S. Humphries, M. Knapton, J. Perk, S. G. Priori, K. Pyorala, Z. Reiner, L. Ruilope, S. Sans-Menendez, W. Scholte op Reimer, P. Weissberg, D. Wood, J. Yarnell, J. L. Zamorano, E. Walma, T. Fitzgerald, M. T. Cooney, A. Dudina, A. Vahanian, J. Camm, R. De Caterina, V. Dean, K. Dickstein, C. Funck-Brentano, G. Filippatos, I. Hellemans, S. D. Kristensen, K. McGregor, U. Sechtem, S. Silber, M. Tendera, P. Widimsky, J. L. Zamorano, I. Hellemans, A. Altiner, E. Bonora, P. N. Durrington, R. Fagard, S. Giampaoli, H. Hemingway, J. Hakansson, S. E. Kjeldsen, M. L. Larsen, G. Mancia, A. J. Manolis, K. Orth-Gomer, T. Pedersen, M. Rayner, L. Ryden, M. Sammut, N. Schneiderman, A. F. Stalenhoef, L. Tokgozoglu, O. Wiklund, and A. Zampelas. European guidelines on cardiovascular disease prevention in clinical practice: executive summary. *Eur Heart J*, 28(19):2375–414, 2007. Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts) Other experts who contributed to parts of the guidelines: European Society of Cardiology (ESC) Committee for Practice Guidelines (CPG) Journal Article Practice Guideline England.

[102] T. A. Pearson, S. N. Blair, S. R. Daniels, R. H. Eckel, J. M. Fair, S. P. Fortmann, B. A. Franklin, L. B. Goldstein, P. Greenland, S. M. Grundy, Y. Hong, N. H. Miller, R. M. Lauer, I. S. Ockene, R. L. Sacco, Jr. Sallis, J. F., Jr. Smith, S. C., N. J. Stone, and K. A. Taubert. Aha guidelines for primary prevention of cardiovascular disease and stroke: 2002 update: Consensus panel guide to comprehensive risk reduction for adult patients without coronary or other atherosclerotic vascular diseases. american heart association science advisory and coordinating committee. *Circulation*, 106(3):388–91, 2002. Consensus Development Conference Guideline Journal Article Practice Guideline Review United States.

[103] Thomas J. Lynch, Daphne W. Bell, Raffaella Sordella, Sarada Gurubhagavatula, Ross A. Okimoto, Brian W. Brannigan, Patricia L. Harris, Sara M. Haserlat, Jeffrey G. Supko, Frank G. Haluska, David N. Louis, David C. Christiani, Jeff Settleman, and Daniel A. Haber. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*, 350(21):2129–2139, May 2004.

[104] J Guillermo Paez, Pasi A. Jnne, Jeffrey C. Lee, Sean Tracy, Heidi Greulich, Stacey Gabriel, Paula Herman, Frederic J. Kaye, Neal Lindeman, Titus J. Boggon, Katsuhiko Naoki, Hidefumi Sasaki, Yoshitaka Fujii, Michael J. Eck, William R. Sellers, Bruce E. Johnson, and Matthew Meyerson. Egfr mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*, 304(5676):1497–1500, Jun 2004.

[105] G. Guyatt, P. Wyer, and J. Ioannidis. *When to believe a subgroup analysis. In: Guyatt, G. Rennie, D. Meade, M.O. Cook, D.J (ed). A Manual for Evidence-Based Clinical Practice*. McGraw-Hill, New York, 2nd edition, 2008.

[106] Janet Wittes. On looking at subgroups. *Circulation*, 119(7):912–915, Feb 2009.

[107] Peter M. Rothwell. Treating individuals 2. subgroup analysis in randomised controlled trials: importance, indications, and interpretation. *Lancet*, 365(9454):176–186, 2005.

[108] A. G. Motulsky. Drug reactions enzymes, and biochemical genetics. *J Am Med Assoc*, 165(7):835–7, 1957. Motulsky, a g Not Available Journal of the American Medical Association J Am Med Assoc. 1957 Oct 19;165(7):835-7.

[109] J. C. Venter, M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt, J. D. Gocayne, P. Amanatides, R. M. Ballew, D. H. Huson, J. R. Wortman, Q. Zhang, C. D. Kodira, X. H. Zheng, L. Chen, M. Skupski, G. Subramanian, P. D. Thomas, J. Zhang, G. L. Gabor Miklos, C. Nelson, S. Broder, A. G. Clark, J. Nadeau, V. A. McKusick, N. Zinder, A. J. Levine, R. J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A. E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T. J. Heiman, M. E. Higgins, R. R. Ji, Z. Ke, K. A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G. V. Merkulov, N. Milshina, H. M. Moore, A. K. Naik, V. A. Narayan, B. Neelam, D. Nusskern, D. B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, et al. The sequence of the human genome. *Science*, 291(5507):1304–51, 2001. Venter, J C Adams, M D Myers, E W Li, P W Mural, R J Sutton, G G Smith, H O Yandell, M Evans, C A Holt, R A Gocayne, J D Amanatides, P Ballew, R M Huson, D H Wortman, J R Zhang, Q Kodira, C D Zheng, X H Chen, L Skupski, M Subramanian, G Thomas, P D Zhang, J Gabor Miklos, G L Nelson, C Broder, S Clark, A G Nadeau, J McKusick, V A Zinder, N Levine, A J Roberts, R J Simon, M Slayman, C Hunkapiller, M Bolanos, R Delcher, A Dew, I Fasulo, D Flanigan, M Florea, L Halpern, A Hannenhalli, S Kravitz, S Levy, S Mobarry, C Reinert, K Remington, K Abu-Threideh, J Beasley, E Biddick, K Bonazzi, V Brandon, R Cargill, M Chandramouliswaran, I Charlab, R Chaturvedi, K Deng, Z Di Francesco, V Dunn, P Eilbeck, K Evangelista, C Gabrielian, A E Gan, W Ge, W Gong, F Gu, Z Guan, P Heiman, T J Higgins, M E Ji, R R Ke, Z Ketchum, K A Lai, Z Lei, Y Li, Z Li, J Liang, Y Lin, X Lu, F Merkulov, G V Milshina, N Moore, H M Naik, A K Narayan, V A Neelam, B Nusskern, D Rusch, D B Salzberg, S Shao, W Shue, B Sun, J Wang, Z Wang, A Wang, X Wang, J Wei, M Wides, R Xiao, C Yan, C Yao, A Ye, J Zhan, M Zhang, W Zhang, H Zhao, Q Zheng, L Zhong, F Zhong, W Zhu, S Zhao, S Gilbert, D Baumhueter, S Spier, G Carter, C Cravchik, A Woodage, T Ali, F An, H Awe, A Baldwin, D Baden, H Barnstead, M Barrow, I Beeson, K Busam, D Carver, A Center, A Cheng, M L Curry, L Danaher, S Davenport, L Desilets, R Dietz, S Dodson, K Doup, L Ferriera, S Garg, N Gluecksmann, A Hart, B Haynes, J Haynes, C Heiner, C Hladun, S Hostin, D Houck, J Howland, T Ibegwam, C Johnson, J Kalush, F Kline, L Koduru, S Love, A Mann, F May, D McCawley, S McIntosh, T McMullen, I Moy, M Moy, L Murphy, B Nelson, K Pfannkoch, C Pratts, E Puri, V Qureshi, H Reardon, M Rodriguez, R Rogers, Y H Romblad, D Ruhfel, B Scott, R Sitter, C Smallwood, M Stewart, E Strong, R Suh, E Thomas, R Tint, N N Tse, S Vech, C Wang, G Wetter, J Williams, S Williams, M Windsor, S Winn-Deen, E Wolfe, K Zaveri, J Zaveri, K Abril, J F Guigo, R Campbell, M J Sjolander, K V

Karlak, B Kejariwal, A Mi, H Lazareva, B Hatton, T Narechania, A Diemer, K Muruganujan, A Guo, N Sato, S Bafna, V Istrail, S Lippert, R Schwartz, R Walenz, B Yooseph, S Allen, D Basu, A Baxendale, J Blick, L Caminha, M Carnes-Stine, J Caulk, P Chiang, Y H Coyne, M Dahlke, C Mays, A Dombroski, M Donnelly, M Ely, D Esparham, S Fosler, C Gire, H Glanowski, S Glasser, K Glodek, A Gorokhov, M Graham, K Gropman, B Harris, M Heil, J Henderson, S Hoover, J Jennings, D Jordan, C Jordan, J Kasha, J Kagan, L Kraft, C Levitsky, A Lewis, M Liu, X Lopez, J Ma, D Majoros, W McDaniel, J Murphy, S Newman, M Nguyen, T Nguyen, N Nodell, M Pan, S Peck, J Peterson, M Rowe, W Sanders, R Scott, J Simpson, M Smith, T Sprague, A Stockwell, T Turner, R Venter, E Wang, M Wen, M Wu, D Wu, M Xia, A Zandieh, A Zhu, X Research Support, Non-U.S. Gov't United States Science (New York, N.Y.) Science. 2001 Feb 16;291(5507):1304-51.

[110] M. V. Holmes, T. Shah, C. Vickery, L. Smeeth, A. D. Hingorani, and J. P. Casas. Fulfilling the promise of personalized medicine? systematic review and field synopsis of pharmacogenetic studies. *PLoS One*, 4(12):e7960, 2009. Holmes, Michael V Shah, Tina Vickery, Christine Smeeth, Liam Hingorani, Aroon D Casas, Juan P 082178/Wellcome Trust/United Kingdom FS 05/125/British Heart Foundation/United Kingdom G0802432/Medical Research Council/United Kingdom Research Support, Non-U.S. Gov't United States PloS one PLoS One. 2009 Dec 2;4(12):e7960.

[111] C. R. Wolf, G. Smith, and R. L. Smith. Science, medicine, and the future: Pharmacogenetics. *Bmj*, 320(7240):987–90, 2000. Wolf, C R Smith, G Smith, R L Research Support, Non-U.S. Gov't Review England BMJ (Clinical research ed.) BMJ. 2000 Apr 8;320(7240):987-90.

[112] U. A. Meyer. Pharmacogenetics and adverse drug reactions. *Lancet*, 356(9242):1667–1671, Nov 2000.

[113] A. D. Roses. Pharmacogenetics and the practice of medicine. *Nature*, 405(6788):857–65, 2000. Journal Article Review England.

[114] B. B. Spear, M. Heath-Chiozzi, and J. Huff. Clinical application of pharmacogenetics. *Trends Mol Med*, 7(5):201–204, May 2001.

[115] Harry Hemingway, Richard D. Riley, and Douglas G. Altman. Ten steps towards improving prognosis research. *BMJ*, 339:b4184, 2009.

[116] S. Colombo, A. Rauch, M. Rotger, J. Fellay, R. Martinez, C. Fux, C. Thurnheer, H. F. Gunthard, D. B. Goldstein, H. Furrer, and A. Telenti. The hcp5 single-nucleotide polymorphism: a simple screening tool for prediction of hypersensitivity reaction to abacavir. *J Infect Dis*, 198(6):864–7, 2008. Swiss HIV Cohort Study Journal Article Research Support, Non-U.S. Gov't United States.

[117] S. Mallal, E. Phillips, G. Carosi, J. M. Molina, C. Workman, J. Tomazic, E. Jagel-Guedes, S. Rugina, O. Kozyrev, J. F. Cid, P. Hay, D. Nolan, S. Hughes, A. Hughes, S. Ryan, N. Fitch, D. Thorborn, and A. Benbow. Hla-b*5701 screening for hypersensitivity to abacavir. *N Engl J Med*, 358(6):568–79, 2008. Mallal, Simon Phillips, Elizabeth Carosi, Giampiero Molina, Jean-Michel Workman, Cassy Tomazic, Janez Jagel-Guedes, Eva Rugina, Sorin Kozyrev, Oleg Cid, Juan Flores Hay, Phillip Nolan, David Hughes, Sara Hughes, Arlene Ryan, Susanna Fitch, Nicholas Thorborn, Daren Benbow, Alastair PREDICT-1 Study Team Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United States The New England journal of medicine N Engl J Med. 2008 Feb 7;358(6):568-79.

[118] E. Link, S. Parish, J. Armitage, L. Bowman, S. Heath, F. Matsuda, I. Gut, M. Lathrop, and R. Collins. Slco1b1 variants and statin-induced myopathy–a genomewide study. *N Engl J Med*,

359(8):789–99, 2008. SEARCH Collaborative Group British Heart Foundation/United Kingdom Medical Research Council/United Kingdom Journal Article Randomized Controlled Trial Research Support, Non-U.S. Gov't United States.

[119] Tong Yin and Toshiyuki Miyata. Pharmacogenomics of clopidogrel: evidence and perspectives. *Thromb Res*, 128(4):307–316, Oct 2011.

[120] Kyle J. Ellis, George A. Stouffer, Howard L. McLeod, and Craig R. Lee. Clopidogrel pharmacogenomics and risk of inadequate platelet inhibition: Us fda recommendations. *Pharmacogenomics*, 10(11):1799–1817, Nov 2009.

[121] Samir B. Damani and Eric J. Topol. Emerging genomic applications in coronary artery disease. *JACC Cardiovasc Interv*, 4(5):473–482, May 2011.

[122] IMSHealth. Top 20 global products 2010. available at:. *IMS Health Midas (Accessed: October 26th, 2011)*, 2010.

[123] A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (caprie). caprie steering committee. *Lancet*, 348(9038):1329–1339, Nov 1996.

[124] S. Yusuf, F. Zhao, S. R. Mehta, S. Chrolavicius, G. Tognoni, and K. K. Fox. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without st-segment elevation. *N Engl J Med*, 345(7):494–502, 2001. Yusuf, S Zhao, F Mehta, S R Chrolavicius, S Tognoni, G Fox, K K Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators Clinical Trial Comparative Study Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United States The New England journal of medicine N Engl J Med. 2001 Aug 16;345(7):494-502.

[125] J. S. Berger, D. L. Bhatt, C. P. Cannon, Z. Chen, L. Jiang, J. B. Jones, S. R. Mehta, M. S. Sabatine, S. R. Steinhubl, E. J. Topol, and P. B. Berger. The relative efficacy and safety of clopidogrel in women and men a sex-specific collaborative meta-analysis. *J Am Coll Cardiol*, 54(21):1935–45, 2009. Berger, Jeffrey S Bhatt, Deepak L Cannon, Christopher P Chen, Zhengming Jiang, Lixin Jones, James B Mehta, Shamir R Sabatine, Marc S Steinhubl, Steven R Topol, Eric J Berger, Peter B Meta-Analysis Review United States Journal of the American College of Cardiology J Am Coll Cardiol. 2009 Nov 17;54(21):1935-45.

[126] P. A. Gurbel, U. S. Tantry, and A. R. Shuldiner. The worry about clopidogrel "nonresponsiveness": identification and treatment in the post-percutaneous coronary intervention patient. *JACC Cardiovasc Interv*, 2(11):1102–4, 2009. Gurbel, Paul A Tantry, Udaya S Shuldiner, Alan R Comment Editorial United States JACC. Cardiovascular interventions JACC Cardiovasc Interv. 2009 Nov;2(11):1102-4.

[127] P. Jremo, T. L. Lindahl, S. G. Fransson, and A. Richter. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med*, 252(3):233–238, Sep 2002.

[128] W. C. Lau, P. A. Gurbel, P. B. Watkins, C. J. Neer, A. S. Hopp, D. G. Carville, K. E. Guyer, A. R. Tait, and E. R. Bates. Contribution of hepatic cytochrome p450 3a4 metabolic activity to the phenomenon of clopidogrel resistance. *Circulation*, 109(2):166–71, 2004. Lau, Wei C Gurbel, Paul A Watkins, Paul B Neer, Charlene J Hopp, Amy S Carville, David G M Guyer, Kirk E Tait, Alan R Bates, Eric R GM 38149/GM/NIGMS NIH HHS/United States GM61971/GM/NIGMS NIH HHS/United States M01-00042/PHS HHS/United States Clinical Trial Research Support,

U.S. Gov't, P.H.S. United States Circulation Circulation. 2004 Jan 20;109(2):166-71. Epub 2004 Jan 5.

[129] Iris Mller, Felicitas Besta, Christian Schulz, Steffen Massberg, Albert Schnig, and Meinrad Gawaz. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost*, 89(5):783–787, May 2003.

[130] Thuy Anh Nguyen, Jean G. Diodati, and Chantal Pharand. Resistance to clopidogrel: a review of the evidence. *J Am Coll Cardiol*, 45(8):1157–1164, Apr 2005.

[131] A. Pena, J. P. Collet, J. S. Hulot, J. Silvain, O. Barthelemy, F. Beygui, C. Funck-Brentano, and G. Montalescot. Can we override clopidogrel resistance? *Circulation*, 119(21):2854–7, 2009. Pena, Ana Collet, Jean-Philippe Hulot, Jean-Sebastien Silvain, Johanne Barthelemy, Olivier Beygui, Farzin Funck-Brentano, Christian Montalescot, Gilles Research Support, Non-U.S. Gov't United States Circulation Circulation. 2009 Jun 2;119(21):2854-7.

[132] S. D. Wiviott and E. M. Antman. Clopidogrel resistance: a new chapter in a fast-moving story. *Circulation*, 109(25):3064–7, 2004. Wiviott, Stephen D Antman, Elliott M Comment Editorial United States Circulation Circulation. 2004 Jun 29;109(25):3064-7.

[133] Fda drug safety communication: Reduced effectiveness of plavix (clopidogrel) in patients who are poor metabolizers of the drug. available at: http://www.fda.gov/drugs/drugsafety/postmarketdrugsafetyinformationforpatientsandproviders/ucm203888.htm (accessed october 26th, 2011). *U.S. Food and Drug Administration*, 2010.

[134] Jr. Holmes, D. R., G. J. Dehmer, S. Kaul, D. Leifer, P. T. O'Gara, and C. M. Stein. Accf/aha clopidogrel clinical alert: approaches to the fda "boxed warning": a report of the american college of cardiology foundation task force on clinical expert consensus documents and the american heart association endorsed by the society for cardiovascular angiography and interventions and the society of thoracic surgeons. *J Am Coll Cardiol*, 56(4):321–41, 2010. Holmes, David R Jr Dehmer, Gregory J Kaul, Sanjay Leifer, Dana O'Gara, Patrick T Stein, C Michael Consensus Development Conference Review United States Journal of the American College of Cardiology J Am Coll Cardiol. 2010 Jul 20;56(4):321-41.

[135] Medical devices. october 2010 510(k) clearances. available at: http://www.fda.gov/medicaldevices/productsandmedicalprocedures/deviceapprovalsandclearances/510kclearances/ (accessed october 26th, 2011). *U.S. Food and Drug Administration*.

[136] G. McMillin. Implementation of cyp2c19 genotyping for clopidogrel. available at: http://www.aruplab.com/aboutarup/events/aacc10/presentations/aacc2010-autogenomics.pdf accessed (october 26th, 2011). *ARUP Laboratories*, 2010.

[137] P. Gladding, M. Webster, I. Zeng, H. Farrell, J. Stewart, P. Ruygrok, J. Ormiston, S. El-Jack, G. Armstrong, P. Kay, D. Scott, A. Gunes, and M. L. Dahl. The pharmacogenetics and pharmacodynamics of clopidogrel response: an analysis from the princ (plavix response in coronary intervention) trial. *JACC Cardiovasc Interv*, 1(6):620–7, 2008. Gladding, Patrick Webster, Mark Zeng, Irene Farrell, Helen Stewart, Jim Ruygrok, Peter Ormiston, John El-Jack, Seif Armstrong, Guy Kay, Patrick Scott, Douglas Gunes, Arzu Dahl, Marja-Liisa Comparative Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United States JACC. Cardiovascular interventions JACC Cardiovasc Interv. 2008 Dec;1(6):620-7.

[138] L. A. Malek, B. Kisiel, M. Spiewak, M. Grabowski, K. J. Filipiak, G. Kostrzewa, Z. Huczek, R. Ploski, and G. Opolski. Coexisting polymorphisms of p2y12 and cyp2c19 genes as a risk factor for persistent platelet activation with clopidogrel. *Circ J*, 72(7):1165–9, 2008. Malek, Lukasz A Kisiel, Bartlomiej Spiewak, Mateusz Grabowski, Marcin Filipiak, Krzysztof J Kostrzewa, Grazyna Huczek, Zenon Ploski, Rafal Opolski, Grzegorz Research Support, Non-U.S. Gov't Japan Circulation journal : official journal of the Japanese Circulation Society Circ J. 2008 Jul;72(7):1165-9.

[139] Kathryn M. Momary, Michael P. Dorsch, and Eric R. Bates. Genetic causes of clopidogrel nonresponsiveness: which ones really count? *Pharmacotherapy*, 30(3):265–274, Mar 2010.

[140] A. R. Shuldiner, J. R. O'Connell, K. P. Bliden, A. Gandhi, K. Ryan, R. B. Horenstein, C. M. Damcott, R. Pakyz, U. S. Tantry, Q. Gibson, T. I. Pollin, W. Post, A. Parsa, B. D. Mitchell, N. Faraday, W. Herzog, and P. A. Gurbel. Association of cytochrome p450 2c19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*, 302(8):849–57, 2009. Shuldiner, Alan R O'Connell, Jeffrey R Bliden, Kevin P Gandhi, Amish Ryan, Kathleen Horenstein, Richard B Damcott, Coleen M Pakyz, Ruth Tantry, Udaya S Gibson, Quince Pollin, Toni I Post, Wendy Parsa, Afshin Mitchell, Braxton D Faraday, Nauder Herzog, William Gurbel, Paul A M01 RR 16500/RR/NCRR NIH HHS/United States P30 DK072488/DK/NIDDK NIH HHS/United States U01 GM074518/GM/NIGMS NIH HHS/United States U01 HL084756/HL/NHLBI NIH HHS/United States Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. United States JAMA : the journal of the American Medical Association JAMA. 2009 Aug 26;302(8):849-57.

[141] Jason D. Roberts, George A. Wells, Michel R. Le May, Marino Labinaz, Chris Glover, Michael Froeschl, Alexander Dick, Jean-Francois Marquis, Edward O'Brien, Sandro Goncalves, Irena Druce, Alexandre Stewart, Michael H. Gollob, and Derek Yf So. Point-of-care genetic testing for personalisation of antiplatelet treatment (rapid gene): a prospective, randomised, proof-of-concept trial. *Lancet*, Mar 2012.

[142] J. Little, JPT. Higgins, and (editors). Huge review handbook (2004) http://www.hugenet.org.uk/resources/handbook.php accessed october 26th 2011.

[143] D. Moher, A. Liberati, J. Tetzlaff, and D. G. Altman. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. *PLoS Med*, 6(7):e1000097, 2009.

[144] S. Sanderson, J. Emery, and J. Higgins. Cyp2c9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a hugenet systematic review and meta-analysis. *Genet Med*, 7(2):97–104, 2005. Sanderson, Simon Emery, Jon Higgins, Julian Meta-Analysis Review United States Genetics in medicine : official journal of the American College of Medical Genetics Genet Med. 2005 Feb;7(2):97-104.

[145] J. L. Mega, J. V. Thakuria, C. P. Cannon, and M. S. Sabatine. Sequence variations in cyp metabolism genes and cardiovascular outcomes following treatment with clopidogrel: insights from the clarity-timi 28 genomic study [abstract]. *J Am Coll Cardiol*, 51:A206, 2008.

[146] J. L. Mega, S. L. Close, S. D. Wiviott, L. Shen, R. D. Hockett, J. T. Brandt, J. R. Walker, E. M. Antman, W. Macias, E. Braunwald, and M. S. Sabatine. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*, 360(4):354–62, 2009. Mega, Jessica L Close, Sandra L Wiviott, Stephen D Shen, Lei Hockett, Richard D Brandt, John T Walker, Joseph R Antman, Elliott M Macias, William Braunwald, Eugene Sabatine, Marc S Research Support, Non-U.S. Gov't United States The New England journal of medicine N Engl J Med. 2009 Jan 22;360(4):354-62. Epub 2008 Dec 22.

[147] J. P. Higgins, S. G. Thompson, J. J. Deeks, and D. G. Altman. Measuring inconsistency in meta-analyses. *BMJ*, 327(7414):557–60, 2003. Higgins, Julian P T Thompson, Simon G Deeks, Jonathan J Altman, Douglas G Research Support, Non-U.S. Gov't Review England BMJ (Clinical research ed.) BMJ. 2003 Sep 6;327(7414):557-60.

[148] D. G. Altman and J. M. Bland. Interaction revisited: the difference between two estimates. *BMJ*, 326(7382):219, 2003. Altman, Douglas G Bland, J Martin Review England BMJ (Clinical research ed.) BMJ. 2003 Jan 25;326(7382):219.

[149] Roger M. Harbord, Matthias Egger, and Jonathan A C. Sterne. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Stat Med*, 25(20):3443–3457, Oct 2006.

[150] S. Duval and R. Tweedie. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*, 56(2):455–63, 2000. Duval, S Tweedie, R United states Biometrics Biometrics. 2000 Jun;56(2):455-63.

[151] D.L. Bhatt, K. L. Simonsen, E. S. Emison, K. A. A. Fox, P. G. Steg, G. Montalescot, N. Bhatka, W. Hacke, M. Flather, P. Cacoub, M. A. Creager, P. B. Berger, S. R. Steinhubl, G. Murugesan, K. Kottke-Marchant, A. M. Lincoff, and E. J. Topol. Charisma genomics substudy: evaluation of the cyp2c19 polymorphism in a prospective, randomized, placebo-controlled trial of chronic clopidogrel use for primary and secondary prevention. powerpoint presentation available at: http://www.clinicaltrialresults.org/slides/charisma *Conference: Transcatheter Cardiovascular Therapeutics (http://www.tctconference.com/2009)*, 2009 Accessed 28th October 2011.

[152] J. L. Anderson, C. P. Mower, B. D. Horne, J. B. Muhlestein, J. J. Park, T. L. Blair, and J. F. Carlquist. Carriage of the cyp2c19*2 allele increases one-year risk of myocardial infarction among recipients of drug-eluting stents treated with clopidogrel [abstract]. *J Am Coll Cardiol*, 53(10):A27 (2903–8), 2009.

[153] H. J. Bouman, E. Schomig, J. W. van Werkum, J. Velder, C. M. Hackeng, C. Hirschhauser, C. Waldmann, H. G. Schmalz, J. M. Ten Berg, and D. Taubert. Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med*, 17(1):110–6, 2010. Journal article Nature medicine Nat Med. 2010 Dec 19.

[154] G. Campo, G. Parrinello, P. Ferraresi, B. Lunghi, M. Tebaldi, M. Miccoli, J. Marchesini, F. Bernardi, R. Ferrari, and M. Valgimigli. Prospective evaluation of on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention relationship with gene polymorphisms and clinical outcome. *J Am Coll Cardiol*, 57(25):2474–83, 2011. Campo, Gianluca Parrinello, Giovanni Ferraresi, Paolo Lunghi, Barbara Tebaldi, Matteo Miccoli, Matteo Marchesini, Jlenia Bernardi, Francesco Ferrari, Roberto Valgimigli, Marco United States Journal of the American College of Cardiology J Am Coll Cardiol. 2011 Jun 21;57(25):2474-83.

[155] J. P. Collet, J. S. Hulot, A. Pena, E. Villard, J. B. Esteve, J. Silvain, L. Payot, D. Brugier, G. Cayla, F. Beygui, G. Bensimon, C. Funck-Brentano, and G. Montalescot. Cytochrome p450 2c19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet*, 373(9660):309–17, 2009. Collet, Jean-Philippe Hulot, Jean-Sebastien Pena, Anna Villard, Eric Esteve, Jean-Baptiste Silvain, Johanne Payot, Laurent Brugier, Delphine Cayla, Guillaume Beygui, Farzin Bensimon, Gilbert Funck-Brentano, Christian Montalescot, Gilles Clinical Trial Research Support, Non-U.S. Gov't England Lancet Lancet. 2009 Jan 24;373(9660):309-17. Epub 2008 Dec 26.

[156] B. Giusti, A. M. Gori, R. Marcucci, C. Saracini, I. Sestini, R. Paniccia, P. Buonamici, D. Antoniucci, R. Abbate, and G. F. Gensini. Relation of cytochrome p450 2c19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. *Am J Cardiol*, 103(6):806–11, 2009. Giusti, Betti Gori, Anna Maria Marcucci, Rossella Saracini, Claudia Sestini, Ilaria Paniccia, Rita Buonamici, Piergiovanni Antoniucci, David Abbate, Rosanna Gensini, Gian Franco Research Support, Non-U.S. Gov't United States The American journal of cardiology Am J Cardiol. 2009 Mar 15;103(6):806-11. Epub 2009 Jan 24.

[157] A. M. Harmsze, V. A. N. Werkum JW, P. C. Souverein, N. J. Breet, H. J. Bouman, C. M. Hackeng, H. J. Ruven, J. M. Ten Berg, O. H. Klungel, D. E. Boer A, and V. H. Deneer. Combined influence of proton-pump inhibitors, calcium-channel blockers and cyp2c19*2 on on-treatment platelet reactivity and on the occurrence of atherothrombotic events after percutaneous coronary intervention. *J Thromb Haemost*, 9(10):1892–1901, 2011. Journal article Journal of thrombosis and haemostasis : JTH J Thromb Haemost. 2011 Oct;9(10):1892-1901. doi: 10.1111/j.1538-7836.2011.04483.x.

[158] Y.H. Jeong, U.T. Tantry, T.J. Jung, Y.H. Park, I.S. Kim, K.A. Bliden, and P.A. Gurbel. Impact of the cytochrome p450 2c19*3 polymorphism on platelet reactivity and adverse clinical events in patients with acute myocardial infarction. *European Heart Journal*, 32(S1):313–631; Abstract: 3102 doi: 10.1093/eurheartj/ehr323, 2011.

[159] A. M. Harmsze, J. W. van Werkum, J. M. Ten Berg, B. Zwart, H. J. Bouman, N. J. Breet, A. W. van 't Hof, H. J. Ruven, C. M. Hackeng, O. H. Klungel, A. de Boer, and V. H. Deneer. Cyp2c19*2 and cyp2c9*3 alleles are associated with stent thrombosis: a case-control study. *Eur Heart J*, 31(24):3046–53, 2010. Journal article.

[160] A. Komarov, O. Shakhmatova, A. Donnikov, T. Ilyushchenko, G. Dzhalilova, and E. Panchenko. Carrying of p450 2c19*2 polymorphism and use of proton pump inhibitors increase risk of adverse outcomes after elective pci in russian patients with cad. *European Journal of Cardiovascular Prevention & Rehabilitation*, 18:S100–S125; Abstract: P491 doi:10.1177/1741826711407011, 2011.

[161] L. A. Malek, J. Przyluski, M. Spiewak, M. Klopotowski, G. Kostrzewa, M. Kruk, R. Ploski, A. Witkowski, and W. Ruzyllo. Cytochrome p450 2c19 polymorphism, suboptimal reperfusion and all-cause mortality in patients with acute myocardial infarction. *Cardiology*, 117(2):81–7, 2010. Malek, Lukasz A Przyluski, Jakub Spiewak, Mateusz Klopotowski, Mariusz Kostrzewa, Grazyna Kruk, Mariusz Ploski, Rafal Witkowski, Adam Ruzyllo, Witold Research Support, Non-U.S. Gov't Switzerland Cardiology Cardiology. 2010;117(2):81-7. Epub 2010 Oct 6.

[162] I. Y. Oh, K. W. Park, S. H. Kang, J. J. Park, S. H. Na, H. J. Kang, B. K. Koo, Y. H. Jeong, J. Y. Hwang, C. H. Kwak, Y. Park, S. J. Hwang, Y. G. Ko, D. J. Shin, Y. Jang, and H. S. Kim. Association of cytochrome p450 2c19*2 polymorphism with clopidogrel response variability and cardiovascular events in koreans treated with drug-eluting stents. *Heart*, 2011; doi:10.1136/hrt.2011.227272. Journal article Heart (British Cardiac Society) Heart. 2011 Jun 23.

[163] T. Ono, K. Kaikita, S. Hokimoto, S. Iwashita, K. Yamamoto, Y. Miyazaki, E. Horio, K. Sato, K. Tsujita, T. Abe, M. Deguchi, S. Tayama, H. Sumida, S. Sugiyama, H. Yamabe, S. Nakamura, K. Nakagawa, and H. Ogawa. Determination of cut-off levels for on-clopidogrel platelet aggregation based on functional cyp2c19 gene variants in patients undergoing elective percutaneous coronary intervention. *Thromb Res*, page doi:10.1016/j.thromres.2011.07.028, 2011. Journal article Thrombosis research Thromb Res. 2011 Aug 20.

[164] G. Par, S. R. Mehta, and J. W. Eikelboom. Cyp2c19 genotype and outcomes of clopidogrel treatment: Author response. *New England Journal of Medicine*, 364(5):481–482, 2011.

[165] T. Sawada, T. Shinke, J. Shite, T. Honjo, Y. Haraguchi, R. Nishio, M. Shinohara, R. Toh, T. Ishida, H. Kawamori, A. Kozuki, T. Inoue, H. Hariki, and K. I. Hirata. Impact of cytochrome p450 2c19*2 polymorphism on intra-stent thrombus after drug-eluting stent implantation in japanese patients receiving clopidogrel. *Circ J*, 75(1):99–105, 2010. Journal article Circulation journal : official journal of the Japanese Circulation Society Circ J. 2010 Nov 16.

[166] D. Sibbing, W. Koch, D. Gebhard, T. Schuster, S. Braun, J. Stegherr, T. Morath, A. Schomig, N. von Beckerath, and A. Kastrati. Cytochrome 2c19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation*, 121(4):512–8, 2010. Sibbing, Dirk Koch, Werner Gebhard, Daniela Schuster, Tibor Braun, Siegmund Stegherr, Julia Morath, Tanja Schomig, Albert von Beckerath, Nicolas Kastrati, Adnan Research Support, Non-U.S. Gov't United States Circulation Circulation. 2010 Feb 2;121(4):512-8. Epub 2010 Jan 18.

[167] D. Sibbing, W. Koch, S. Massberg, R. A. Byrne, J. Mehilli, S. Schulz, K. Mayer, I. Bernlochner, A. Schomig, and A. Kastrati. No association of paraoxonase-1 q192r genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting. *Eur Heart J*, 32(13):1605–13, 2011. Sibbing, Dirk Koch, Werner Massberg, Steffen Byrne, Robert A Mehilli, Julinda Schulz, Stefanie Mayer, Katharina Bernlochner, Isabell Schomig, Albert Kastrati, Adnan Research Support, Non-U.S. Gov't England European heart journal Eur Heart J. 2011 Jul;32(13):1605-13. Epub 2011 Apr 28.

[168] D. Sibbing, J. Stegherr, W. Latz, W. Koch, J. Mehilli, K. Dorrler, T. Morath, A. Schomig, A. Kastrati, and N. von Beckerath. Cytochrome p450 2c19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J*, 30(8):916–22, 2009. Sibbing, Dirk Stegherr, Julia Latz, Wolfgang Koch, Werner Mehilli, Julinda Dorrler, Katharina Morath, Tanja Schomig, Albert Kastrati, Adnan von Beckerath, Nicolas England European heart journal Eur Heart J. 2009 Apr;30(8):916-22. Epub 2009 Feb 4.

[169] T. Simon, C. Verstuyft, M. Mary-Krause, L. Quteineh, E. Drouet, N. Meneveau, P. G. Steg, J. Ferrieres, N. Danchin, and L. Becquemont. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*, 360(4):363–75, 2009. French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators Journal Article Research Support, Non-U.S. Gov't United States.

[170] A. Tello-Montoliu, E. Jover, F. Marin, A. Bernal, M.L. Lozano, B. Sanchez-Vega, F. Pastor-Perez, J.A. Hurtado, M. Valdes, and J. Rivera. Influence of cyp2c19 polymorphisms in on-treatment platelet reactivity and prognosis in an unselected population of non st elevation acute coronary syndrome. *Eur Heart J*, 32(S1):1–312. P1652 doi:10.1093/eurheartj/ehr322, 2011.

[171] K. A. Tiroch, D. Sibbing, W. Koch, T. Roosen-Runge, J. Mehilli, A. Schomig, and A. Kastrati. Protective effect of the cyp2c19 *17 polymorphism with increased activation of clopidogrel on cardiovascular events. *Am Heart J*, 160(3):506–12, 2010. Tiroch, Klaus A Sibbing, Dirk Koch, Werner Roosen-Runge, Tobias Mehilli, Julinda Schomig, Albert Kastrati, Adnan United States American heart journal Am Heart J. 2010 Sep;160(3):506-12.

[172] D. Trenk, W. Hochholzer, M. F. Fromm, L. E. Chialda, A. Pahl, C. M. Valina, C. Stratz, P. Schmiebusch, H. P. Bestehorn, H. J. Buttner, and F. J. Neumann. Cytochrome p450 2c19

681g¿a polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol*, 51(20):1925–34, 2008. Trenk, Dietmar Hochholzer, Willibald Fromm, Martin F Chialda, Ligia-Emilia Pahl, Andreas Valina, Christian M Stratz, Christian Schmiebusch, Peter Bestehorn, Hans-Peter Buttner, Heinz Joachim Neumann, Franz-Josef Research Support, Non-U.S. Gov't United States Journal of the American College of Cardiology J Am Coll Cardiol. 2008 May 20;51(20):1925-34.

[173] L. Wallentin, S. James, R. F. Storey, M. Armstrong, B. J. Barratt, J. Horrow, S. Husted, H. Katus, P. G. Steg, S. H. Shah, and R. C. Becker. Effect of cyp2c19 and abcb1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the plato trial. *Lancet*, 376(9749):1320–8, 2010. Wallentin, Lars James, Stefan Storey, Robert F Armstrong, Martin Barratt, Bryan J Horrow, Jay Husted, Steen Katus, Hugo Steg, P Gabriel Shah, Svati H Becker, Richard C PLATO investigators Comparative Study Research Support, Non-U.S. Gov't England Lancet Lancet. 2010 Oct 16;376(9749):1320-8.

[174] A. Worrall, A. Armesilla, M. Norell, S. Khogali, M. Cusack, A. Smallwood, and J. Cotton. The presence of the cyp p450 c19*2 allele is associated with impaired response to clopidogrel as measured by the verifynow p2y12 near-patient testing device in pateints undergoing coronary angiography [abstract]. *Eur Heart J*, 30(Suppl1):327 (P2089), 2009.

[175] K. Yamamoto, S. Hokimoto, T. Chitose, K. Morita, T. Ono, K. Kaikita, K. Tsujita, T. Abe, M. Deguchi, H. Miyagawa, J. Saruwatari, H. Sumida, S. Sugiyama, K. Nakagawa, and H. Ogawa. Impact of cyp2c19 polymorphism on residual platelet reactivity in patients with coronary heart disease during antiplatelet therapy. *J Cardiol*, 57(2):194–201, 2011. Yamamoto, Koichiro Hokimoto, Seiji Chitose, Tadasuke Morita, Kazunori Ono, Takamichi Kaikita, Koichi Tsujita, Kenichi Abe, Tomohide Deguchi, Mariko Miyagawa, Haruna Saruwatari, Junji Sumida, Hitoshi Sugiyama, Seigo Nakagawa, Kazuko Ogawa, Hisao Research Support, Non-U.S. Gov't Netherlands Journal of cardiology J Cardiol. 2011 Mar;57(2):194-201. Epub 2010 Dec 17.

[176] S. D. Yan, M. Chen, Q. Li, X. J. Liu, Y. Peng, H. Chai, Y. N. Xu, J. F. Wei, and D. J. Huang. The value of combining cyp2c19*2 polymorphism with classic risk factors in prediction of clinical prognosis in acute coronary syndrome patients. *Cardiology*, 119(1):15–20, 2011. Yan, Shao-Di Chen, Mao Li, Qiao Liu, Xiao-Jing Peng, Yong Chai, Hua Xu, Yuan-Ning Wei, Jia-Fu Huang, De-Jia Research Support, Non-U.S. Gov't Switzerland Cardiology Cardiology. 2011;119(1):15-20. Epub 2011 Jul 16.

[177] J. Yuan, X.F. Tang, Y.J. Yang, and R.L. Gao. Association between cytochrome p450 2c19 681g¿a polymorphisms and risk of cardiovascular events in coronary heart disease with clopidogrel in chinese. *Eur Heart J*, 32(S1):1–312. P1548 doi:10.1093/eurheartj/ehr322, 2011.

[178] M. Ingelman-Sundberg, A.K. Daly, and D. W. Nebert. Home page of the human cytochrome p450 (cyp) allele nomenclature committee. (available at: http://www.cypalleles.ki.se). Updated Sep 2008.

[179] International hapmap project http://www.hapmap.org/ (accessed october 26th, 2011).

[180] J. L. Mega, S. L. Close, S. D. Wiviott, L. Shen, R. D. Hockett, J. T. Brandt, J. R. Walker, E. M. Antman, W. L. Macias, E. Braunwald, and M. S. Sabatine. Cytochrome p450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation*, 119(19):2553–60, 2009. Mega, Jessica L Close, Sandra L

Wiviott, Stephen D Shen, Lei Hockett, Richard D Brandt, John T Walker, Joseph R Antman, Elliott M Macias, William L Braunwald, Eugene Sabatine, Marc S Comparative Study Research Support, Non-U.S. Gov't United States Circulation Circulation. 2009 May 19;119(19):2553-60. Epub 2009 May 4.

[181] C. Frere, T. Cuisset, P. E. Morange, J. Quilici, L. Camoin-Jau, N. Saut, D. Faille, M. Lambert, I. Juhan-Vague, J. L. Bonnet, and M. C. Alessi. Effect of cytochrome p450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. *Am J Cardiol*, 101(8):1088–93, 2008. Frere, Corinne Cuisset, Thomas Morange, Pierre-Emmanuel Quilici, Jacques Camoin-Jau, Laurence Saut, Noemie Faille, Dorothee Lambert, Marc Juhan-Vague, Irene Bonnet, Jean-Louis Alessi, Marie-Christine United States The American journal of cardiology Am J Cardiol. 2008 Apr 15;101(8):1088-93. Epub 2008 Feb 6.

[182] B. Giusti, A. M. Gori, R. Marcucci, C. Saracini, I. Sestini, R. Paniccia, S. Valente, D. Antoniucci, R. Abbate, and G. F. Gensini. Cytochrome p450 2c19 loss-of-function polymorphism, but not cyp3a4 ivs10 + 12g/a and p2y12 t744c polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. *Pharmacogenet Genomics*, 17(12):1057–64, 2007. Giusti, Betti Gori, Anna Maria Marcucci, Rossella Saracini, Claudia Sestini, Ilaria Paniccia, Rita Valente, Serafina Antoniucci, Davide Abbate, Rosanna Gensini, Gian Franco Research Support, Non-U.S. Gov't United States Pharmacogenetics and genomics Pharmacogenet Genomics. 2007 Dec;17(12):1057-64.

[183] JEREMIAH P. DEPTA and DEEPAK L. BHATT. Omeprazole and clopidogrel: Should clinicians be worried? *Cleveland Clinic Journal of Medicine*, 77(2):113–116, 2010.

[184] S. Yusuf, J. Wittes, J. Probstfield, and H. A. Tyroler. Analysis and interpretation of treatment effects in subgroups of patients in randomized clinical trials. *JAMA*, 266(1):93–8, 1991. Yusuf, S Wittes, J Probstfield, J Tyroler, H A Comparative Study United states JAMA : the journal of the American Medical Association JAMA. 1991 Jul 3;266(1):93-8.

[185] Daniel W. Nebert and Timothy P. Dalton. The role of cytochrome p450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer*, 6(12):947–960, 2006. 10.1038/nrc2015.

[186] H. Schunkert, I. R. Konig, S. Kathiresan, M. P. Reilly, T. L. Assimes, H. Holm, M. Preuss, A. F. Stewart, M. Barbalic, C. Gieger, D. Absher, Z. Aherrahrou, H. Allayee, D. Altshuler, S. S. Anand, K. Andersen, J. L. Anderson, D. Ardissino, S. G. Ball, A. J. Balmforth, T. A. Barnes, D. M. Becker, L. C. Becker, K. Berger, J. C. Bis, S. M. Boekholdt, E. Boerwinkle, P. S. Braund, M. J. Brown, M. S. Burnett, I. Buysschaert, J. F. Carlquist, L. Chen, S. Cichon, V. Codd, R. W. Davies, G. Dedoussis, A. Dehghan, S. Demissie, J. M. Devaney, P. Diemert, R. Do, A. Doering, S. Eifert, N. E. Mokhtari, S. G. Ellis, R. Elosua, J. C. Engert, S. E. Epstein, U. de Faire, M. Fischer, A. R. Folsom, J. Freyer, B. Gigante, D. Girelli, S. Gretarsdottir, V. Gudnason, J. R. Gulcher, E. Halperin, N. Hammond, S. L. Hazen, A. Hofman, B. D. Horne, T. Illig, C. Iribarren, G. T. Jones, J. W. Jukema, M. A. Kaiser, L. M. Kaplan, J. J. Kastelein, K. T. Khaw, J. W. Knowles, G. Kolovou, A. Kong, R. Laaksonen, D. Lambrechts, K. Leander, G. Lettre, M. Li, W. Lieb, C. Loley, A. J. Lotery, P. M. Mannucci, S. Maouche, N. Martinelli, P. P. McKeown, C. Meisinger, T. Meitinger, O. Melander, P. A. Merlini, V. Mooser, T. Morgan, T. W. Muhleisen, J. B. Muhlestein, T. Munzel, K. Musunuru, J. Nahrstaedt, C. P. Nelson, M. M. Nothen, O. Olivieri, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet*, 43(4):333–8, 2011. Schunkert, Heribert Konig, Inke R Kathiresan, Sekar Reilly, Muredach

P Assimes, Themistocles L Holm, Hilma Preuss, Michael Stewart, Alexandre F R Barbalic, Maja Gieger, Christian Absher, Devin Aherrahrou, Zouhair Allayee, Hooman Altshuler, David Anand, Sonia S Andersen, Karl Anderson, Jeffrey L Ardissino, Diego Ball, Stephen G Balmforth, Anthony J Barnes, Timothy A Becker, Diane M Becker, Lewis C Berger, Klaus Bis, Joshua C Boekholdt, S Matthijs Boerwinkle, Eric Braund, Peter S Brown, Morris J Burnett, Mary Susan Buysschaert, Ian Cardiogenics Carlquist, John F Chen, Li Cichon, Sven Codd, Veryan Davies, Robert W Dedoussis, George Dehghan, Abbas Demissie, Serkalem Devaney, Joseph M Diemert, Patrick Do, Ron Doering, Angela Eifert, Sandra Mokhtari, Nour Eddine El Ellis, Stephen G Elosua, Roberto Engert, James C Epstein, Stephen E de Faire, Ulf Fischer, Marcus Folsom, Aaron R Freyer, Jennifer Gigante, Bruna Girelli, Domenico Gretarsdottir, Solveig Gudnason, Vilmundur Gulcher, Jeffrey R Halperin, Eran Hammond, Naomi Hazen, Stanley L Hofman, Albert Horne, Benjamin D Illig, Thomas Iribarren, Carlos Jones, Gregory T Jukema, J Wouter Kaiser, Michael A Kaplan, Lee M Kastelein, John J P Khaw, Kay-Tee Knowles, Joshua W Kolovou, Genovefa Kong, Augustine Laaksonen, Reijo Lambrechts, Diether Leander, Karin Lettre, Guillaume Li, Mingyao Lieb, Wolfgang Loley, Christina Lotery, Andrew J Mannucci, Pier M Maouche, Seraya Martinelli, Nicola McKeown, Pascal P Meisinger, Christa Meitinger, Thomas Melander, Olle Merlini, Pier Angelica Mooser, Vincent Morgan, Thomas Muhleisen, Thomas W Muhlestein, Joseph B Munzel, Thomas Musunuru, Kiran Nahrstaedt, Janja Nelson, Christopher P Nothen, Markus M Olivieri, Oliviero Patel, Riyaz S Patterson, Chris C Peters, Annette Peyvandi, Flora Qu, Liming Quyyumi, Arshed A Rader, Daniel J Rallidis, Loukianos S Rice, Catherine Rosendaal, Frits R Rubin, Diana Salomaa, Veikko Sampietro, M Lourdes Sandhu, Manj S Schadt, Eric Schafer, Arne Schillert, Arne Schreiber, Stefan Schrezenmeir, Jurgen Schwartz, Stephen M Siscovick, David S Sivananthan, Mohan Sivapalaratnam, Suthesh Smith, Albert Smith, Tamara B Snoep, Jaapjan D Soranzo, Nicole Spertus, John A Stark, Klaus Stirrups, Kathy Stoll, Monika Tang, W H Wilson Tennstedt, Stephanie Thorgeirsson, Gudmundur Thorleifsson, Gudmar Tomaszewski, Maciej Uitterlinden, Andre G van Rij, Andre M Voight, Benjamin F Wareham, Nick J Wells, George A Wichmann, H-Erich Wild, Philipp S Willenborg, Christina Witteman, Jaqueline C M Wright, Benjamin J Ye, Shu Zeller, Tanja Ziegler, Andreas Cambien, Francois Goodall, Alison H Cupples, L Adrienne Quertermous, Thomas Marz, Winfried Hengstenberg, Christian Blankenberg, Stefan Ouwehand, Willem H Hall, Alistair S Deloukas, Panos Thompson, John R Stefansson, Kari Roberts, Robert Thorsteinsdottir, Unnur O'Donnell, Christopher J McPherson, Ruth Erdmann, Jeanette CARDIoGRAM Consortium Samani, Nilesh J HL087647/HL/NHLBI NIH HHS/United States R01HL089650-02/HL/NHLBI NIH HHS/United States Meta-Analysis Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't United States Nature genetics Nat Genet. 2011 Mar 6;43(4):333-8.

[187] C. Newton-Cheh, T. Johnson, V. Gateva, M. D. Tobin, M. Bochud, L. Coin, S. S. Najjar, J. H. Zhao, S. C. Heath, S. Eyheramendy, K. Papadakis, B. F. Voight, L. J. Scott, F. Zhang, M. Farrall, T. Tanaka, C. Wallace, J. C. Chambers, K. T. Khaw, P. Nilsson, P. van der Harst, S. Polidoro, D. E. Grobbee, N. C. Onland-Moret, M. L. Bots, L. V. Wain, K. S. Elliott, A. Teumer, J. Luan, G. Lucas, J. Kuusisto, P. R. Burton, D. Hadley, W. L. McArdle, M. Brown, A. Dominiczak, S. J. Newhouse, N. J. Samani, J. Webster, E. Zeggini, J. S. Beckmann, S. Bergmann, N. Lim, K. Song, P. Vollenweider, G. Waeber, D. M. Waterworth, X. Yuan, L. Groop, M. Orho-Melander, A. Allione, A. Di Gregorio, S. Guarrera, S. Panico, F. Ricceri, V. Romanazzi, C. Sacerdote, P. Vineis, I. Barroso, M. S. Sandhu, R. N. Luben, G. J. Crawford, P. Jousilahti, M. Perola, M. Boehnke, L. L. Bonnycastle, F. S. Collins, A. U. Jackson, K. L. Mohlke, H. M. Stringham, T. T. Valle, C. J. Willer, R. N. Bergman, M. A. Morken, A. Doring, C. Gieger, T. Illig, T. Meitinger, E. Org, A. Pfeufer, H. E. Wichmann, S. Kathiresan, J. Marrugat, C. J. O'Donnell, S. M. Schwartz,

D. S. Siscovick, I. Subirana, N. B. Freimer, A. L. Hartikainen, M. I. McCarthy, P. F. O'Reilly, L. Peltonen, A. Pouta, P. E. de Jong, H. Snieder, W. H. van Gilst, R. Clarke, A. Goel, A. Hamsten, J. F. Peden, et al. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet*, 41(6):666–76, 2009. Wellcome Trust Case Control Consortium Journal article.

[188] D. Levy, G. B. Ehret, K. Rice, G. C. Verwoert, L. J. Launer, A. Dehghan, N. L. Glazer, A. C. Morrison, A. D. Johnson, T. Aspelund, Y. Aulchenko, T. Lumley, A. Kottgen, R. S. Vasan, F. Rivadeneira, G. Eiriksdottir, X. Guo, D. E. Arking, G. F. Mitchell, F. U. Mattace-Raso, A. V. Smith, K. Taylor, R. B. Scharpf, S. J. Hwang, E. J. Sijbrands, J. Bis, T. B. Harris, S. K. Ganesh, C. J. O'Donnell, A. Hofman, J. I. Rotter, J. Coresh, E. J. Benjamin, A. G. Uitterlinden, G. Heiss, C. S. Fox, J. C. Witteman, E. Boerwinkle, T. J. Wang, V. Gudnason, M. G. Larson, A. Chakravarti, B. M. Psaty, and C. M. van Duijn. Genome-wide association study of blood pressure and hypertension. *Nat Genet*, 41(6):677–87, 2009. Levy, Daniel Ehret, Georg B Rice, Kenneth Verwoert, Germaine C Launer, Lenore J Dehghan, Abbas Glazer, Nicole L Morrison, Alanna C Johnson, Andrew D Aspelund, Thor Aulchenko, Yurii Lumley, Thomas Kottgen, Anna Vasan, Ramachandran S Rivadeneira, Fernando Eiriksdottir, Gudny Guo, Xiuqing Arking, Dan E Mitchell, Gary F Mattace-Raso, Francesco U S Smith, Albert V Taylor, Kent Scharpf, Robert B Hwang, Shih-Jen Sijbrands, Eric J G Bis, Joshua Harris, Tamara B Ganesh, Santhi K O'Donnell, Christopher J Hofman, Albert Rotter, Jerome I Coresh, Josef Benjamin, Emelia J Uitterlinden, Andre G Heiss, Gerardo Fox, Caroline S Witteman, Jacqueline C M Boerwinkle, Eric Wang, Thomas J Gudnason, Vilmundur Larson, Martin G Chakravarti, Aravinda Psaty, Bruce M van Duijn, Cornelia M HHSN268200625226C/PHS HHS/United States N01 AG012100/AG/NIA NIH HHS/United States N01 HC-15103/HC/NHLBI NIH HHS/United States N01 HC-55222/HC/NHLBI NIH HHS/United States N01 HC015103/HC/NHLBI NIH HHS/United States N01 HC025195/HC/NHLBI NIH HHS/United States N01 HC035129/HC/NHLBI NIH HHS/United States N01 HC045133/HC/NHLBI NIH HHS/United States N01 HC055015/HC/NHLBI NIH HHS/United States N01 HC055016/HC/NHLBI NIH HHS/United States N01 HC055018/HC/NHLBI NIH HHS/United States N01 HC055019/HC/NHLBI NIH HHS/United States N01 HC055020/HC/NHLBI NIH HHS/United States N01 HC055021/HC/NHLBI NIH HHS/United States N01 HC055022/HC/NHLBI NIH HHS/United States N01 HC055222/HC/NHLBI NIH HHS/United States N01 HC075150/HC/NHLBI NIH HHS/United States N01 HC085079/HC/NHLBI NIH HHS/United States N01 HC085086/HC/NHLBI NIH HHS/United States N01-AG-12100/AG/NIA NIH HHS/United States N01-HC-25195/HC/NHLBI NIH HHS/United States N01-HC-35129/HC/NHLBI NIH HHS/United States N01-HC-45133/HC/NHLBI NIH HHS/United States N01-HC-55015/HC/NHLBI NIH HHS/United States N01-HC-55016/HC/NHLBI NIH HHS/United States N01-HC-55018/HC/NHLBI NIH HHS/United States N01-HC-55019/HC/NHLBI NIH HHS/United States N01-HC-55020/HC/NHLBI NIH HHS/United States N01-HC-55021/HC/NHLBI NIH HHS/United States N01-HC-55022/HC/NHLBI NIH HHS/United States N01-HC-75150/HC/NHLBI NIH HHS/United States N01-HC-85079/HC/NHLBI NIH HHS/United States N01-HC-85080/HC/NHLBI NIH HHS/United States N01-HC-85081/HC/NHLBI NIH HHS/United States N01-HC-85082/HC/NHLBI NIH HHS/United States N01-HC-85083/HC/NHLBI NIH HHS/United States N01-HC-85084/HC/NHLBI NIH HHS/United States N01-HC-85085/HC/NHLBI NIH HHS/United States N01-HC-85086/HC/NHLBI NIH HHS/United States N02 HL64278/HL/NHLBI NIH HHS/United States N02-HL-6-4278/HL/NHLBI NIH HHS/United States P30 DK063491-019004/DK/NIDDK NIH HHS/United States P30 DK063491-029004/DK/NIDDK NIH HHS/United States P30 DK063491-039004/DK/NIDDK NIH HHS/United States P30 DK063491-049004/DK/NIDDK NIH HHS/United States

R01 HL059367-02/HL/NHLBI NIH HHS/United States R01 HL086694-01A1/HL/NHLBI NIH HHS/United States R01 HL087641-01/HL/NHLBI NIH HHS/United States R01 HL087652/HL/NHLBI NIH HHS/United States R01 HL087652-01/HL/NHLBI NIH HHS/United States R01HL086694/HL/NHLBI NIH HHS/United States R01HL087641/HL/NHLBI NIH HHS/United States R01HL59367/HL/NHLBI NIH HHS/United States R37 HL051021-06/HL/NHLBI NIH HHS/United States R37 HL051021-10/HL/NHLBI NIH HHS/United States R37 HL051021-11/HL/NHLBI NIH HHS/United States R37 HL051021-12/HL/NHLBI NIH HHS/United States R37 HL051021-13/HL/NHLBI NIH HHS/United States R37 HL051021-14/HL/NHLBI NIH HHS/United States R37 HL051021-15/HL/NHLBI NIH HHS/United States R37HL051021/HL/NHLBI NIH HHS/United States U01 HG004402-01/HG/NHGRI NIH HHS/United States U01 HL080295/HL/NHLBI NIH HHS/United States U01 HL080295-01/HL/NHLBI NIH HHS/United States U01HG004402/HG/NHGRI NIH HHS/United States U10 HL054512-05/HL/NHLBI NIH HHS/United States U10HL054512/HL/NHLBI NIH HHS/United States UL1 RR025005-01/RR/NCRR NIH HHS/United States UL1RR025005/RR/NCRR NIH HHS/United States Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't United States Nature genetics Nat Genet. 2009 Jun;41(6):677-87. Epub 2009 May 10.

[189] Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet*, 42(5):441–7, 2010. Tobacco and Genetics Consortium K07 CA118412/CA/NCI NIH HHS/United States K07 CA118412-04/CA/NCI NIH HHS/United States R01 CA082659-13/CA/NCI NIH HHS/United States R01 DA012844-11/DA/NIDA NIH HHS/United States Meta-Analysis Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't United States Nature genetics Nat Genet. 2010 May;42(5):441-7. Epub 2010 Apr 25.

[190] J. Ahn, K. Yu, R. Stolzenberg-Solomon, K. C. Simon, M. L. McCullough, L. Gallicchio, E. J. Jacobs, A. Ascherio, K. Helzlsouer, K. B. Jacobs, Q. Li, S. J. Weinstein, M. Purdue, J. Virtamo, R. Horst, W. Wheeler, S. Chanock, D. J. Hunter, R. B. Hayes, P. Kraft, and D. Albanes. Genome-wide association study of circulating vitamin d levels. *Hum Mol Genet*, 19(13):2739–45, 2010. Ahn, Jiyoung Yu, Kai Stolzenberg-Solomon, Rachael Simon, K Claire McCullough, Marjorie L Gallicchio, Lisa Jacobs, Eric J Ascherio, Alberto Helzlsouer, Kathy Jacobs, Kevin B Li, Qizhai Weinstein, Stephanie J Purdue, Mark Virtamo, Jarmo Horst, Ronald Wheeler, William Chanock, Stephen Hunter, David J Hayes, Richard B Kraft, Peter Albanes, Demetrius 5U01HG004399-2/HG/NHGRI NIH HHS/United States HHSN261201000006C/PHS HHS/United States N01-CN-45165/CN/NCI NIH HHS/United States N01-CO-12400/CO/NCI NIH HHS/United States N01-RC- 45035/RC/CCR/United States N01-RC-37004/RC/CCR/United States P01CA055075/CA/NCI NIH HHS/United States P01CA087969/CA/NCI NIH HHS/United States T32 ES016645-01/ES/NIEHS NIH HHS/United States U01 CA098710/CA/NCI NIH HHS/United States Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, U.S. Gov't, P.H.S. England Human molecular genetics Hum Mol Genet. 2010 Jul 1;19(13):2739-45. Epub 2010 Apr 23.

[191] C. Bertrand-Thiebault, H. Berrahmoune, A. Thompson, B. Marie, S. Droesch, G. Siest, D. Foernzler, and S. Visvikis-Siest. Genetic polymorphism of cyp2c19 gene in the stanislas cohort. a link with inflammation. *Ann Hum Genet*, 72(Pt 2):178–83, 2008. Bertrand-Thiebault, C Berrahmoune, H Thompson, A Marie, B Droesch, S Siest, G Foernzler, D Visvikis-Siest, S Comparative Study Research Support, Non-U.S. Gov't England Annals of human genetics Ann Hum Genet. 2008 Mar;72(Pt 2):178-83. Epub 2008 Jan 20.

[192] Marie-Claude Blatter Garin, Xenia Moren, and Richard W. James. Paraoxonase-1 and serum concentrations of hdl-cholesterol and apoa-i. *J Lipid Res*, 47(3):515–520, Mar 2006.

[193] Y. Song, M. J. Stampfer, and S. Liu. Meta-analysis: apolipoprotein e genotypes and risk for coronary heart disease. *Ann Intern Med*, 141(2):137–47, 2004. Song, Yiqing Stampfer, Meir J Liu, Simin DK62290/DK/NIDDK NIH HHS/United States HL26490/HL/NHLBI NIH HHS/United States Meta-Analysis Research Support, U.S. Gov't, P.H.S. United States Annals of internal medicine Ann Intern Med. 2004 Jul 20;141(2):137-47.

[194] G. Montalescot, G. Sideris, C. Meuleman, C. Bal-dit Sollier, N. Lellouche, P. G. Steg, M. Slama, O. Milleron, J. P. Collet, P. Henry, F. Beygui, and L. Drouet. A randomized comparison of high clopidogrel loading doses in patients with non-st-segment elevation acute coronary syndromes: the albion (assessment of the best loading dose of clopidogrel to blunt platelet activation, inflammation and ongoing necrosis) trial. *J Am Coll Cardiol*, 48(5):931–8, 2006. Montalescot, Gilles Sideris, Georges Meuleman, Catherine Bal-dit-Sollier, Claire Lellouche, Nicolas Steg, Ph Gabriel Slama, Michel Milleron, Olivier Collet, Jean-Philippe Henry, Patrick Beygui, Farzin Drouet, Ludovic ALBION Trial Investigators Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United States Journal of the American College of Cardiology J Am Coll Cardiol. 2006 Sep 5;48(5):931-8. Epub 2006 Aug 17.

[195] M. Abuzahra, M. Pillai, A. Caldera, W. B. Hartley, R. Gonzalez, J. Bobek, H. Dokainish, and N. Lakkis. Comparison of higher clopidogrel loading and maintenance dose to standard dose on platelet function and outcomes after percutaneous coronary intervention using drug-eluting stents. *Am J Cardiol*, 102(4):401–3, 2008. Abuzahra, Mohammed Pillai, Manu Caldera, Angel Hartley, W Bryan Gonzalez, Rafael Bobek, Jaromir Dokainish, Hisham Lakkis, Nasser Comparative Study Randomized Controlled Trial United States The American journal of cardiology Am J Cardiol. 2008 Aug 15;102(4):401-3. Epub 2008 May 28.

[196] G. Patti, G. Colonna, V. Pasceri, L. L. Pepe, A. Montinaro, and G. Di Sciascio. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: results from the armyda-2 (antiplatelet therapy for reduction of myocardial damage during angioplasty) study. *Circulation*, 111(16):2099–106, 2005. Patti, Giuseppe Colonna, Giuseppe Pasceri, Vincenzo Pepe, Leonardo Lassandro Montinaro, Antonio Di Sciascio, Germano Randomized Controlled Trial United States Circulation Circulation. 2005 Apr 26;111(16):2099-106. Epub 2005 Mar 6.

[197] A. Fernandez, M. S. Aboodi, K. Milewski, J. A. Delgado, A. Rodriguez, and J. F. Granada. Comparison of adverse cardiovascular events and bleeding complications of loading dose of clopidogrel 300 mg versus 600 mg in stable patients undergoing elective percutaneous intervention (from the cadice study). *Am J Cardiol*, 107(1):6–9, 2011. Fernandez, Andres Aboodi, Michael S Milewski, Krzysztof Delgado, Juan A Rodriguez, Arturo Granada, Juan F Randomized Controlled Trial United States The American journal of cardiology Am J Cardiol. 2011 Jan;107(1):6-9.

[198] T. Cuisset, C. Frere, J. Quilici, P. E. Morange, L. Nait-Saidi, J. Carvajal, A. Lehmann, M. Lambert, J. L. Bonnet, and M. C. Alessi. Benefit of a 600-mg loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non-st-segment elevation acute coronary syndrome undergoing coronary stenting. *J Am Coll Cardiol*, 48(7):1339–45, 2006. Cuisset, Thomas Frere, Corinne Quilici, Jacques Morange, Pierre-Emmanuel Nait-Saidi, Lyassine Carvajal, Joseph Lehmann, Agnes Lambert, Marc Bonnet, Jean-Louis Alessi, Marie-Christine Randomized Controlled Trial Research Support, Non-U.S. Gov't United States Journal of the American College of Cardiology J Am Coll Cardiol. 2006 Oct 3;48(7):1339-45. Epub 2006 Sep 12.

[199] G. Yong, J. Rankin, L. Ferguson, J. Thom, J. French, D. Brieger, D. P. Chew, R. Dick, D. Eccleston, B. Hockings, D. Walters, A. Whelan, and J. W. Eikelboom. Randomized trial comparing 600- with 300-mg loading dose of clopidogrel in patients with non-st elevation acute coronary syndrome undergoing percutaneous coronary intervention: results of the platelet responsiveness to aspirin and clopidogrel and troponin increment after coronary intervention in acute coronary lesions (practical) trial. *Am Heart J*, 157(1):60 e1–9, 2009. Yong, Gerald Rankin, Jamie Ferguson, Louise Thom, Jim French, John Brieger, David Chew, Derek P Dick, Ron Eccleston, David Hockings, Bernard Walters, Darren Whelan, Alan Eikelboom, John W Comparative Study Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United States American heart journal Am Heart J. 2009 Jan;157(1):60.e1-9.

[200] S. R. Mehta, J. F. Tanguay, J. W. Eikelboom, S. S. Jolly, C. D. Joyner, C. B. Granger, D. P. Faxon, H. J. Rupprecht, A. Budaj, A. Avezum, P. Widimsky, P. G. Steg, J. P. Bassand, G. Montalescot, C. Macaya, G. Di Pasquale, K. Niemela, A. E. Ajani, H. D. White, S. Chrolavicius, P. Gao, K. A. Fox, and S. Yusuf. Double-dose versus standard-dose clopidogrel and high-dose versus low-dose aspirin in individuals undergoing percutaneous coronary intervention for acute coronary syndromes (current-oasis 7): a randomised factorial trial. *Lancet*, 376(9748):1233–43, 2010. Mehta, Shamir R Tanguay, Jean-Francois Eikelboom, John W Jolly, Sanjit S Joyner, Campbell D Granger, Christopher B Faxon, David P Rupprecht, Hans-Jurgen Budaj, Andrzej Avezum, Alvaro Widimsky, Petr Steg, Philippe Gabriel Bassand, Jean-Pierre Montalescot, Gilles Macaya, Carlos Di Pasquale, Giuseppe Niemela, Kari Ajani, Andrew E White, Harvey D Chrolavicius, Susan Gao, Peggy Fox, Keith A A Yusuf, Salim CURRENT-OASIS 7 trial investigators Comparative Study Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't England Lancet Lancet. 2010 Oct 9;376(9748):1233-43.

[201] Cyp2c19 allele nomenclature. available at: http://www.cypalleles.ki.se/cyp2c19.htm accessed october 26th, 2011.

[202] J. D. Robarge, L. Li, Z. Desta, A. Nguyen, and D. A. Flockhart. The star-allele nomenclature: retooling for translational genomics. *Clin Pharmacol Ther*, 82(3):244–8, 2007. Robarge, J D Li, L Desta, Z Nguyen, A Flockhart, D A United States Clinical pharmacology and therapeutics Clin Pharmacol Ther. 2007 Sep;82(3):244-8.

[203] Kim D. Pruitt, Tatiana Tatusova, and Donna R. Maglott. Ncbi reference sequence (refseq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res*, 33(Database issue):D501–D504, Jan 2005.

[204] J. P. Ioannidis, T. A. Trikalinos, and M. J. Khoury. Implications of small effect sizes of individual genetic variants on the design and interpretation of genetic association studies of complex diseases. *Am J Epidemiol*, 164(7):609–14, 2006. Journal Article United States.

[205] J. P. Ioannidis, T. A. Trikalinos, E. E. Ntzani, and D. G. Contopoulos-Ioannidis. Genetic associations in large versus small studies: an empirical assessment. *Lancet*, 361(9357):567–71, 2003. Journal Article Meta-Analysis Research Support, Non-U.S. Gov't Review England.

[206] Jean-Sebastien Hulot, Jean-Philippe Collet, Johanne Silvain, Ana Pena, Anne Bellemain-Appaix, Olivier Barthelemy, Guillaume Cayla, Farzin Beygui, and Gilles Montalescot. Cardiovascular risk in clopidogrel-treated patients according to cytochrome p450 2c19*2 loss-of-function allele or proton pump inhibitor coadministration: A systematic meta-analysis. *J Am Coll Cardiol*, 56(2):134–143, 2010.

[207] J. L. Mega, T. Simon, J. P. Collet, J. L. Anderson, E. M. Antman, K. Bliden, C. P. Cannon, N. Danchin, B. Giusti, P. Gurbel, B. D. Horne, J. S. Hulot, A. Kastrati, G. Montalescot, F. J. Neumann, L. Shen, D. Sibbing, P. G. Steg, D. Trenk, S. D. Wiviott, and M. S. Sabatine. Reduced-function cyp2c19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for pci: a meta-analysis. *JAMA*, 304(16):1821–30, 2010. Mega, Jessica L Simon, Tabassome Collet, Jean-Philippe Anderson, Jeffrey L Antman, Elliott M Bliden, Kevin Cannon, Christopher P Danchin, Nicolas Giusti, Betti Gurbel, Paul Horne, Benjamin D Hulot, Jean-Sebastian Kastrati, Adnan Montalescot, Gilles Neumann, Franz-Josef Shen, Lei Sibbing, Dirk Steg, P Gabriel Trenk, Dietmar Wiviott, Stephen D Sabatine, Marc S K99/R00 HL098461-01/HL/NHLBI NIH HHS/United States Meta-Analysis Research Support, N.I.H., Extramural United States JAMA : the journal of the American Medical Association JAMA. 2010 Oct 27;304(16):1821-30.

[208] F. Sofi, B. Giusti, R. Marcucci, A. M. Gori, R. Abbate, and G. F. Gensini. Cytochrome p450 2c19[ast]2 polymorphism and cardiovascular recurrences in patients taking clopidogrel: a meta-analysis. *Pharmacogenomics J*, 2010.

[209] T. Bauer. Impact of cyp2c19 variant genotypes on clinical efficacy of the antiplatelet therapy with clopidogrel: systematic review and meta-analysis. *BMJ*, 2011.

[210] Matthew J. Price, Peter B. Berger, Paul S. Teirstein, Jean-Franois Tanguay, Dominick J. Angiolillo, Douglas Spriggs, Sanjeev Puri, Mark Robbins, Kirk N. Garratt, Olivier F. Bertrand, Michael E. Stillabower, Michael E. Stillablower, Joseph R. Aragon, David E. Kandzari, Curtiss T. Stinis, Michael S. Lee, Steven V. Manoukian, Christopher P. Cannon, Nicholas J. Schork, Eric J. Topol, and G. R. A. V. I. T. A. S Investigators . Standard- vs high-dose clopidogrel based on platelet function testing after percutaneous coronary intervention: the gravitas randomized trial. *JAMA*, 305(11):1097–1105, Mar 2011.

[211] Dietmar Trenk, Gregg W. Stone, Meinrad Gawaz, Adnan Kastrati, Dominick J. Angiolillo, Ulrike Mller, Gert Richardt, Joseph A. Jakubowski, and Franz-Josef Neumann. A randomized trial of prasugrel versus clopidogrel in patients with high platelet reactivity on clopidogrel after elective percutaneous coronary intervention with implantation of drug-eluting stents: results of the trigger-pci (testing platelet reactivity in patients undergoing elective stent placement on clopidogrel to guide alternative therapy with prasugrel) study. *J Am Coll Cardiol*, 59(24):2159–2164, Jun 2012.

[212] Jean-Philippe Collet, Thomas Cuisset, Grgoire Rang, Guillaume Cayla, Simon Elhadad, Christophe Pouillot, Patrick Henry, Pascal Motreff, Didier Carri, Ziad Boueri, Loic Belle, Eric Van Belle, Hlne Rousseau, Pierre Aubry, Jacques Monsgu, Pierre Sabouret, Stephen A. O'Connor, Jrmie Abtan, Mathieu Kerneis, Christophe Saint-Etienne, Olivier Barthlmy, Farzin Beygui, Johanne Silvain, Eric Vicaut, Gilles Montalescot, and A. R. C. T. I. C Investigators . Bedside monitoring to adjust antiplatelet therapy for coronary stenting. *N Engl J Med*, 367(22):2100–2109, Nov 2012.

[213] Michael V. Holmes, Pablo Perel, Tina Shah, Aroon D. Hingorani, and Juan P. Casas. Cyp2c19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA*, 306(24):2704–2714, Dec 2011.

[214] Study: Plavix warning may not be necessary one doctor explains why and what to do if you take the drug. available at: http://am.blogs.cnn.com/2011/12/28/study-plavix-warning-may-not-be-necessary-one-doctor-explains-why-and-what-to-do-if-you-take-the-drug/. *CNN*, 2011.

[215] L Nainggolan. Analysis slams use of clopidogrel loss-of-function gene test; proponents fire back. available at: http://www.theheart.org/article/1334629.do. *theheart.org*, 2011.

[216] E Dolgin. Doubts about usefulness of gene testing for antiplatelet drug prompt video rebuttal. availeble at: http://blogs.nature.com/spoonful/2011/12/doubts-about-usefulness-of-gene-testing-for-antiplatelet-drug-prompt-video-rebuttal.html. *Nature Blog Spoonful of Medicine*, 2011.

[217] J. A. Johnson, D. M. Roden, L. J. Lesko, E. Ashley, T. E. Klein, and A. R. Shuldiner. Clopidogrel: a case for indication-specific pharmacogenetics. *Clin Pharmacol Ther*, 91(5):774–776, May 2012.

[218] Jessica L Mega, Eric J Topol, and Marc S Sabatine. Cyp2c19 genotype and cardiovascular events. *JAMA: the journal of the American Medical Association*, 307(14):1482–1483, 2012.

[219] Jurrin M. Ten Berg and Vera H. Deneer. Antiplatelet therapy: Does cyp2c19 genotype affect clinical outcome? *Nat Rev Cardiol*, 9(4):192–194, 2012.

[220] A. L. Beitelshees and H. L. McLeod. Clopidogrel pharmacogenetics: Promising steps towards patient care? *Arteriosclerosis, Thrombosis, and Vascular Biology*, 26(8):1681–1683, 2006.

[221] Paddy M. Barrett and Eric J. Topol. Pharmacogenetics: Point-of-care genetic testing–a new frontier explored. *Nat Rev Cardiol*, 9(6):315–316, Jun 2012.

[222] Teirstein P.S. Price M. P Roden D. Montalescot G. Topol, E. J. An important miscue in clopidogrel pharmacogenomics. avaiable at: http://www.theheart.org/columns/topolog/miscue-in-clopidogrel-pharmacogenomics.do. *theheart.org*, 2011.

[223] J. L. Mega, E. J. Topol, and M. S. Sabatine. Cyp2c19 genotype and cardiovascular events. letter. *JAMA*, 307:1482, 2012.

[224] A. R. Shuldiner, M. R. Vesely, and A. Fisch. Cyp2c19 genotype and cardiovascular events. letter. *JAMA*, 307:1482, 2012.

[225] M. V. Holmes, A. D. Hingorani, and J.P. Casas. Cyp2c19 genotype and cardiovascular events. authors reply. *JAMA*, 307:1484, 2012.

[226] S. J. Connolly, J. Pogue, R. G. Hart, S. H. Hohnloser, M. Pfeffer, S. Chrolavicius, and S. Yusuf. Effect of clopidogrel added to aspirin in patients with atrial fibrillation. *N Engl J Med*, 360(20):2066–78, 2009. ACTIVE Investigators Connolly, Stuart J Pogue, Janice Hart, Robert G Hohnloser, Stefan H Pfeffer, Marc Chrolavicius, Susan Yusuf, Salim Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't United States The New England journal of medicine N Engl J Med. 2009 May 14;360(20):2066-78. Epub 2009 Mar 31.

[227] Peter P. Tth. Atherosclerosis: The underlying disease. *The Journal of Family Practice*, 58:S19–S25, 2009.

[228] Gran K. Hansson and Peter Libby. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol*, 6(7):508–519, Jul 2006.

[229] Peter Libby. Inflammation and cardiovascular disease mechanisms. *Am J Clin Nutr*, 83(2):456S–460S, Feb 2006.

[230] R. Ross. Atherosclerosis–an inflammatory disease. *N Engl J Med*, 340(2):115–126, Jan 1999.

[231] Peter Libby, Paul M. Ridker, and Attilio Maseri. Inflammation and atherosclerosis. *Circulation*, 105(9):1135–1143, Mar 2002.

[232] HC McGill, Jr, C. A. McMahan, E. E. Herderick, G. T. Malcom, R. E. Tracy, and J. P. Strong. Origin of atherosclerosis in childhood and adolescence. *Am J Clin Nutr*, 72(5 Suppl):1307S–1315S, Nov 2000.

[233] Lina Badimn, Gemma Vilahur, and Teresa Padr. Lipoproteins, platelets and atherothrombosis. *Rev Esp Cardiol*, 62(10):1161–1178, Oct 2009.

[234] V. Pasceri and E. T. Yeh. A tale of two diseases: atherosclerosis and rheumatoid arthritis. *Circulation*, 100(21):2124–2126, Nov 1999.

[235] Cecilia P. Chung, Ingrid Avalos, Paolo Raggi, and C Michael Stein. Atherosclerosis and inflammation: insights from rheumatoid arthritis. *Clin Rheumatol*, 26(8):1228–1233, Aug 2007.

[236] Maurizio S. Tonetti, Francesco D'Aiuto, Luigi Nibali, Ann Donald, Clare Storry, Mohamed Parkar, Jean Suvan, Aroon D. Hingorani, Patrick Vallance, and John Deanfield. Treatment of periodontitis and endothelial function. *N Engl J Med*, 356(9):911–920, Mar 2007.

[237] Liam Smeeth, Sara L. Thomas, Andrew J. Hall, Richard Hubbard, Paddy Farrington, and Patrick Vallance. Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med*, 351(25):2611–2618, Dec 2004.

[238] Liam Smeeth, Juan P. Casas, and Aroon D. Hingorani. The role of infection in cardiovascular disease: more support but many questions remain. *Eur Heart J*, 28(10):1178–1179, May 2007.

[239] Charlotte Warren-Gash, Liam Smeeth, and Andrew C. Hayward. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. *Lancet Infect Dis*, 9(10):601–610, Oct 2009.

[240] Thomas A. Pearson, George A. Mensah, R Wayne Alexander, Jeffrey L. Anderson, Richard O Cannon, 3rd, Michael Criqui, Yazid Y. Fadl, Stephen P. Fortmann, Yuling Hong, Gary L. Myers, Nader Rifai, Sidney C Smith, Jr, Kathryn Taubert, Russell P. Tracy, Frank Vinicor, Centers for Disease Control , Prevention, and American Heart Association . Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the centers for disease control and prevention and the american heart association. *Circulation*, 107(3):499–511, Jan 2003.

[241] P. M. Ridker, C. H. Hennekens, J. E. Buring, and N. Rifai. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*, 342(12):836–843, Mar 2000.

[242] P. C. Heinrich, J. V. Castell, and T. Andus. Interleukin-6 and the acute phase response. *Biochem J*, 265(3):621–636, Feb 1990.

[243] John Danesh, Stephen Kaptoge, Andrea G. Mann, Nadeem Sarwar, Angela Wood, Sara B. Angleman, Frances Wensley, Julian P T. Higgins, Lucy Lennon, Gudny Eiriksdottir, Ann Rumley, Peter H. Whincup, Gordon D O. Lowe, and Vilmundur Gudnason. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med*, 5(4):e78, Apr 2008.

[244] Charles N. Serhan, Nan Chiang, and Thomas E. Van Dyke. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol*, 8(5):349–361, May 2008.

[245] Colin D. Funk. Leukotriene modifiers as potential therapeutics for cardiovascular disease. *Nat Rev Drug Discov*, 4(8):664–672, Aug 2005.

[246] S. E. Dahln, P. Hedqvist, S. Hammarstrm, and B. Samuelsson. Leukotrienes are potent constrictors of human bronchi. *Nature*, 288(5790):484–486, Dec 1980.

[247] S. E. Dahln, J. Bjrk, P. Hedqvist, K. E. Arfors, S. Hammarstrm, J. A. Lindgren, and B. Samuelsson. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci U S A*, 78(6):3887–3891, Jun 1981.

[248] K. B. Glaser, D. Mobilio, J. Y. Chang, and N. Senko. Phospholipase a2 enzymes: regulation and inhibition. *Trends Pharmacol Sci*, 14(3):92–98, Mar 1993.

[249] Yoshiko Ogawa and J Andrew Grant. Mediators of anaphylaxis. *Immunol Allergy Clin North Am*, 27(2):249–60, vii, May 2007.

[250] M. J. Berridge and C. W. Taylor. Inositol trisphosphate and calcium signaling. *Cold Spring Harb Symp Quant Biol*, 53 Pt 2:927–933, 1988.

[251] G. M. Jenkins and M. A. Frohman. Phospholipase d: a lipid centric review. *Cell Mol Life Sci*, 62(19-20):2305–2316, Oct 2005.

[252] Mark McDermott, Michael J O. Wakelam, and Andrew J. Morris. Phospholipase d. *Biochem Cell Biol*, 82(1):225–253, Feb 2004.

[253] E. A. Dennis. Phospholipase a2 in eicosanoid generation. *Am J Respir Crit Care Med*, 161(2 Pt 2):S32–S35, Feb 2000.

[254] E. A. Dennis. Diversity of group types, regulation, and function of phospholipase a2. *J Biol Chem*, 269(18):13057–13060, May 1994.

[255] Robert S. Rosenson and Eva Hurt-Camejo. Phospholipase a2 enzymes and the risk of atherosclerosis. *Eur Heart J*, 33(23):2899–2909, Dec 2012.

[256] Robert S. Rosenson. Phospholipase a2 inhibition and atherosclerotic vascular disease: prospects for targeting secretory and lipoprotein-associated phospholipase a2 enzymes. *Curr Opin Lipidol*, 21(6):473–480, Dec 2010.

[257] Frederick C. de Beer and Nancy R. Webb. Inflammation and atherosclerosis: Group iia and group v spla2 are not redundant. *Arterioscler Thromb Vasc Biol*, 26(7):1421–1422, Jul 2006.

[258] Makoto Murakami, Yoshitaka Taketomi, Hiroyasu Sato, and Kei Yamamoto. Secreted phospholipase a2 revisited. *J Biochem*, 150(3):233–255, Sep 2011.

[259] Z. Mallat, G. Lambeau, and A. Tedgui. Lipoprotein-associated and secreted phospholipases a in cardiovascular disease: roles as biological effectors and biomarkers. *Circulation*, 122(21):2183–200, 2010.

[260] Z. Mallat, P. G. Steg, J. Benessiano, M. L. Tanguy, K. A. Fox, J. P. Collet, O. H. Dabbous, P. Henry, K. F. Carruthers, A. Dauphin, C. S. Arguelles, J. Masliah, B. Hugel, G. Montalescot, J. M. Freyssinet, B. Asselain, and A. Tedgui. Circulating secretory phospholipase a2 activity predicts recurrent events in patients with severe acute coronary syndromes. *J Am Coll Cardiol*, 46(7):1249–57, 2005.

[261] Z. Huang, P. Payette, K. Abdullah, W. A. Cromlish, and B. P. Kennedy. Functional identification of the active-site nucleophile of the human 85-kda cytosolic phospholipase a2. *Biochemistry*, 35(12):3712–3721, Mar 1996.

[262] J. D. Clark, A. R. Schievella, E. A. Nalefski, and L. L. Lin. Cytosolic phospholipase a2. *J Lipid Mediat Cell Signal*, 12(2-3):83–117, Oct 1995.

[263] Joerg Herrmann, Dallit Mannheim, Christine Wohlert, Daniele Versari, Fredric B. Meyer, Joseph P. McConnell, Mario Gssl, Lilach O. Lerman, and Amir Lerman. Expression of lipoprotein-associated phospholipase a(2) in carotid artery plaques predicts long-term cardiac outcome. *Eur Heart J*, 30(23):2930–2938, Dec 2009.

[264] Dallit Mannheim, Joerg Herrmann, Daniele Versari, Mario Gssl, Fredric B. Meyer, Joseph P. McConnell, Lilach O. Lerman, and Amir Lerman. Enhanced expression of lp-pla2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. *Stroke*, 39(5):1448–1455, May 2008.

[265] Lp-P. L. A(2) Studies Collaboration , Alexander Thompson, Pei Gao, Lia Orfei, Sarah Watson, Emanuele Di Angelantonio, Stephen Kaptoge, Christie Ballantyne, Christopher P. Cannon, Michael Criqui, Mary Cushman, Albert Hofman, Chris Packard, Simon G. Thompson, Rory Collins, and John Danesh. Lipoprotein-associated phospholipase a(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet*, 375(9725):1536–1544, May 2010.

[266] M. L. Booker. Lp-pla2 inhibitors (glaxosmithkline). *IDrugs*, 4(10):1173–1177, Oct 2001.

[267] Michelle L. O'Donoghue, Eugene Braunwald, Harvey D. White, Patrick Serruys, Ph Gabriel Steg, Judith Hochman, Aldo P. Maggioni, Christoph Bode, Douglas Weaver, Joel L. Johnson, Greg Cicconetti, Mary Ann Lukas, Elizabeth Tarka, and Christopher P. Cannon. Study design and rationale for the stabilization of plaques using darapladib-thrombolysis in myocardial infarction (solid-timi 52) trial in patients after an acute coronary syndrome. *Am Heart J*, 162(4):613–619.e1, Oct 2011.

[268] Harvey White, Claes Held, Ralph Stewart, David Watson, Robert Harrington, Andrzej Budaj, Ph Gabriel Steg, Christopher P. Cannon, Susan Krug-Gourley, Janet Wittes, Trupti Trivedi, Elizabeth Tarka, and Lars Wallentin. Study design and rationale for the clinical outcomes of the stability trial (stabilization of atherosclerotic plaque by initiation of darapladib therapy) comparing darapladib versus placebo in patients with coronary heart disease. *Am Heart J*, 160(4):655–661, Oct 2010.

[269] Juan P. Casas, Ewa Ninio, Andrie Panayiotou, Jutta Palmen, Jackie A. Cooper, Sally L. Ricketts, Reecha Sofat, Andrew N. Nicolaides, James P. Corsetti, F Gerry R. Fowkes, Ioanna Tzoulaki, Meena Kumari, Eric J. Brunner, Mika Kivimaki, Michael G. Marmot, Michael M. Hoffmann, Karl Winkler, Winfred Mrz, Shu Ye, Heide A. Stirnadel, S Matthijs Boekholdt, Kay-Tee Khaw, Steve E. Humphries, Manjinder S. Sandhu, Aroon D. Hingorani, and Philippa J. Talmud. Pla2g7

genotype, lipoprotein-associated phospholipase a2 activity, and coronary heart disease risk in 10 494 cases and 15 624 controls of european ancestry. *Circulation*, 121(21):2284–2293, Jun 2010.

[270] Sreekanth Racherla and Rohit Arora. Utility of lp-pla2 in lipid-lowering therapy. *Am J Ther*, 19(2):115–120, Mar 2012.

[271] F. Radvanyi, L. Jordan, F. Russo-Marie, and C. Bon. A sensitive and continuous fluorometric assay for phospholipase a2 using pyrene-labeled phospholipids in the presence of serum albumin. *Anal Biochem*, 177(1):103–109, Feb 1989.

[272] P. Pernas, J. Masliah, J. L. Olivier, C. Salvat, T. Rybkine, and G. Bereziat. Type ii phospholipase a2 recombinant overexpression enhances stimulated arachidonic acid release. *Biochem Biophys Res Commun*, 178(3):1298–1305, Aug 1991.

[273] G. J. Wolbink, C. Schalkwijk, J. W. Baars, J. Wagstaff, H. van den Bosch, and C. E. Hack. Therapy with interleukin-2 induces the systemic release of phospholipase-a2. *Cancer Immunol Immunother*, 41(5):287–292, Nov 1995.

[274] M. A. Gijn and C. C. Leslie. Regulation of arachidonic acid release and cytosolic phospholipase a2 activation. *J Leukoc Biol*, 65(3):330–336, Mar 1999.

[275] A. J. Aarsman, F. W. Neys, H. A. van der Helm, F. A. Kuypers, and H. van den Bosch. Sera of patients suffering from inflammatory diseases contain group iia but not group v phospholipase a(2). *Biochim Biophys Acta*, 1502(2):257–263, Oct 2000.

[276] M M J. Nielen, D. van Schaardenburg, H. W. Reesink, J W R. Twisk, R. J. van de Stadt, I. E. van der Horst-Bruinsma, M H M T. de Koning, M. R. Habibuw, and B A C. Dijkmans. Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis. *Ann Rheum Dis*, 65(4):535–537, Apr 2006.

[277] Remco Nijmeijer, Wim K. Lagrand, Alexi Baidoshvili, Yvonne T P. Lubbers, Wim Th Hermens, Chris J L M. Meijer, Cees A. Visser, C Erik Hack, and Hans W M. Niessen. Secretory type ii phospholipase a(2) binds to ischemic myocardium during myocardial infarction in humans. *Cardiovasc Res*, 53(1):138–146, Jan 2002.

[278] I. Lauritzen, C. Heurteaux, and M. Lazdunski. Expression of group ii phospholipase a2 in rat brain after severe forebrain ischemia and in endotoxic shock. *Brain Res*, 651(1-2):353–356, Jul 1994.

[279] Grace Y. Sun, Jianfeng Xu, Michael D. Jensen, and Agnes Simonyi. Phospholipase a2 in the central nervous system: implications for neurodegenerative diseases. *J Lipid Res*, 45(2):205–213, Feb 2004.

[280] Karim M. Mansour, Frans A. Kuypers, Tammy N. Wang, Annabeth M. Miller, Sandra K. Larkin, and Claudia R. Morris. Secretory phospholipase a2: a marker of infection in febrile children presenting to a pediatric ed. *Am J Emerg Med*, 29(9):1163–1168, Nov 2011.

[281] J. A. Green, G. M. Smith, R. Buchta, R. Lee, K. Y. Ho, I. A. Rajkovic, and K. F. Scott. Circulating phospholipase a2 activity associated with sepsis and septic shock is indistinguishable from that associated with rheumatoid arthritis. *Inflammation*, 15(5):355–367, Oct 1991.

[282] Michael C. Seeds, Bonnie L. Grier, Bruce N. Suckling, Anca M. Safta, David L. Long, B Moseley Waite, Peter E. Morris, and R Duncan Hite. Secretory phospholipase a2-mediated depletion of phosphatidylglycerol in early acute respiratory distress syndrome. *Am J Med Sci*, 343(6):446–451, Jun 2012.

[283] G. J. Riggins, S. Markowitz, J. K. Wilson, B. Vogelstein, and K. W. Kinzler. Absence of secretory phospholipase a2 gene alterations in human colorectal cancer. *Cancer Res*, 55(22):5184–5186, Nov 1995.

[284] I. P. Tomlinson, N. E. Beck, K. Neale, and W. F. Bodmer. Variants at the secretory phospholipase a2 (pla2g2a) locus: analysis of associations with familial adenomatous polyposis and sporadic colorectal tumours. *Ann Hum Genet*, 60(Pt 5):369–376, Sep 1996.

[285] M. K. Lin, V. Farewell, P. Vadas, A. A. Bookman, E. C. Keystone, and W. Pruzanski. Secretory phospholipase a2 as an index of disease activity in rheumatoid arthritis. prospective double blind study of 212 patients. *J Rheumatol*, 23(7):1162–1166, Jul 1996.

[286] O. S. Jamal, P. G. Conaghan, A. M. Cunningham, P. M. Brooks, V. F. Munro, and K. F. Scott. Increased expression of human type iia secretory phospholipase a2 antigen in arthritic synovium. *Ann Rheum Dis*, 57(9):550–558, Sep 1998.

[287] Seiko Masuda, Makoto Murakami, Kazuo Komiyama, Motoko Ishihara, Yukio Ishikawa, Toshiharu Ishii, and Ichiro Kudo. Various secretory phospholipase a2 enzymes are expressed in rheumatoid arthritis and augment prostaglandin production in cultured synovial cells. *FEBS J*, 272(3):655–672, Feb 2005.

[288] T. J. Nevalainen, M. M. Haapamki, and J. M. Grnroos. Roles of secretory phospholipases a(2) in inflammatory diseases and trauma. *Biochim Biophys Acta*, 1488(1-2):83–90, Oct 2000.

[289] V. J. Laine, D. S. Grass, and T. J. Nevalainen. Protection by group ii phospholipase a2 against staphylococcus aureus. *J Immunol*, 162(12):7402–7408, Jun 1999.

[290] V. J. Laine, D. S. Grass, and T. J. Nevalainen. Resistance of transgenic mice expressing human group ii phospholipase a2 to escherichia coli infection. *Infect Immun*, 68(1):87–92, Jan 2000.

[291] Juha O. Grnroos, Veli J O. Laine, and Timo J. Nevalainen. Bactericidal group iia phospholipase a2 in serum of patients with bacterial infections. *J Infect Dis*, 185(12):1767–1772, Jun 2002.

[292] J. O. Grnroos, V. J. Laine, M. J. Janssen, M. R. Egmond, and T. J. Nevalainen. Bactericidal properties of group iia and group v phospholipases a2. *J Immunol*, 166(6):4029–4034, Mar 2001.

[293] Mario Menschikowski, Albert Hagelgans, and Gabriele Siegert. Secretory phospholipase a2 of group iia: is it an offensive or a defensive player during atherosclerosis and other inflammatory diseases? *Prostaglandins Other Lipid Mediat*, 79(1-2):1–33, Mar 2006.

[294] Jessica A. Yu, David Mauchley, Howard Li, Xianzhong Meng, Raphael A. Nemenoff, David A. Fullerton, and Michael J. Weyant. Knockdown of secretory phospholipase a2 iia reduces lung cancer growth in vitro and in vivo. *J Thorac Cardiovasc Surg*, 144(5):1185–1191, Nov 2012.

[295] Jessica A. Yu, Shana Kalatardi, John Dohse, Miral R. Sadaria, Xianzhong Meng, David A. Fullerton, and Michael J. Weyant. Group iia spla2 inhibition attenuates nf-b activity and promotes apoptosis of lung cancer cells. *Anticancer Res*, 32(9):3601–3607, Sep 2012.

[296] Dominique Wendum, Magali Svrcek, Valrie Rigau, Pierre-Yves Bolle, Nicole Sebbagh, Rolland Parc, Jolle Masliah, Germain Trugnan, and Jean-Franois Fljou. Cox-2, inflammatory secreted pla2, and cytoplasmic pla2 protein expression in small bowel adenocarcinomas compared with colorectal adenocarcinomas. *Mod Pathol*, 16(2):130–136, Feb 2003.

[297] Leslie Oleksowicz, Yin Liu, R Bruce Bracken, Krishnanath Gaitonde, Barbara Burke, Paul Succop, Linda Levin, Zhongyun Dong, and Shan Lu. Secretory phospholipase a2-iia is a target gene of the her/her2-elicited pathway and a potential plasma biomarker for poor prognosis of prostate cancer. *Prostate*, 72(10):1140–1149, Jul 2012.

[298] Zhongyun Dong, Yin Liu, Kieran F. Scott, Linda Levin, Krishnanath Gaitonde, R Bruce Bracken, Barbara Burke, Qihui Jim Zhai, Jiang Wang, Leslie Oleksowicz, and Shan Lu. Secretory phospholipase a2-iia is involved in prostate cancer progression and may potentially serve as a biomarker for prostate cancer. *Carcinogenesis*, 31(11):1948–1955, Nov 2010.

[299] Franois Curtin and Pierre Schulz. Assessing the benefit: risk ratio of a drug–randomized and naturalistic evidence. *Dialogues Clin Neurosci*, 13(2):183–190, 2011.

[300] P. S. Sanmuganathan, P. Ghahramani, P. R. Jackson, E. J. Wallis, and L. E. Ramsay. Aspirin for primary prevention of coronary heart disease: safety and absolute benefit related to coronary risk derived from meta-analysis of randomised trials. *Heart*, 85(3):265–271, Mar 2001.

[301] Paul M. Ridker, Aruna Pradhan, Jean G. MacFadyen, Peter Libby, and Robert J. Glynn. Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: an analysis from the jupiter trial. *Lancet*, 380(9841):565–571, Aug 2012.

[302] James H. Dwyer, Hooman Allayee, Kathleen M. Dwyer, Jing Fan, Huiyun Wu, Rebecca Mar, Aldons J. Lusis, and Margarete Mehrabian. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med*, 350(1):29–37, Jan 2004.

[303] John E. Deanfield, Julian P. Halcox, and Ton J. Rabelink. Endothelial function and dysfunction: testing and clinical relevance. *Circulation*, 115(10):1285–1295, Mar 2007.

[304] Giovambattista Desideri and Claudio Ferri. Endothelial activation. sliding door to atherosclerosis. *Curr Pharm Des*, 11(17):2163–2175, 2005.

[305] Fernando Ribeiro, Alberto J. Alves, Madalena Teixeira, Vasco Ribeiro, Jos A. Duarte, and Jos Oliveira. Endothelial function and atherosclerosis: circulatory markers with clinical usefulness. *Rev Port Cardiol*, 28(10):1121–1151, Oct 2009.

[306] D. Steinberg. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem*, 272(34):20963–20966, Aug 1997.

[307] M. Y. Chang, S. Potter-Perigo, T. N. Wight, and A. Chait. Oxidized ldl bind to nonproteoglycan components of smooth muscle extracellular matrices. *J Lipid Res*, 42(5):824–833, May 2001.

[308] Noriko Satoh, Hiromichi Wada, Koh Ono, Hajime Yamakage, Kazunori Yamada, Tameo Nakano, Masakazu Hattori, Akira Shimatsu, Hideshi Kuzuya, and Koji Hasegawa. Small dense ldl-cholesterol relative to ldl-cholesterol is a strong independent determinant of hypoadiponectinemia in metabolic syndrome. *Circ J*, 72(6):932–939, Jun 2008.

[309] J. W. Semple and J. Freedman. Cellular immune mechanisms in chronic autoimmune thrombocytopenic purpura (atp). *Autoimmunity*, 13(4):311–319, 1992.

[310] U. J. Tietge, C. Maugeais, W. Cain, D. Grass, J. M. Glick, F. C. de Beer, and D. J. Rader. Overexpression of secretory phospholipase a(2) causes rapid catabolism and altered tissue uptake of high density lipoprotein cholesteryl ester and apolipoprotein a-i. *J Biol Chem*, 275(14):10077–10084, Apr 2000.

[311] Uwe J F. Tietge, Domenico Pratico, Tao Ding, Colin D. Funk, Reeni B. Hildebrand, Theo Van Berkel, and Miranda Van Eck. Macrophage-specific expression of group iia spla2 results in accelerated atherogenesis by increasing oxidative stress. *J Lipid Res*, 46(8):1604–1614, Aug 2005.

[312] Y. V. Bobryshev, J. A. Crozier, R. S. Lord, D. Tran, O. S. Jamal, H. N. Prsson, and K. F. Scott. Expression of secretory group ii phospholipase a2 by cd1a positive cells-in human atherosclerotic plaques. *Atherosclerosis*, 127(2):283–285, Dec 1996.

[313] M. Romano, E. Romano, S. Bjrkerud, and E. Hurt-Camejo. Ultrastructural localization of secretory type ii phospholipase a2 in atherosclerotic and nonatherosclerotic regions of human arteries. *Arterioscler Thromb Vasc Biol*, 18(4):519–525, Apr 1998.

[314] S. M. Boekholdt, T. T. Keller, N. J. Wareham, R. Luben, S. A. Bingham, N. E. Day, M. S. Sandhu, J. W. Jukema, J. J. Kastelein, C. E. Hack, and K. T. Khaw. Serum levels of type ii secretory phospholipase a2 and the risk of future coronary artery disease in apparently healthy men and women: the epic-norfolk prospective population study. *Arterioscler Thromb Vasc Biol*, 25(4):839–46, 2005.

[315] Z. Mallat, J. Benessiano, T. Simon, S. Ederhy, C. Sebella-Arguelles, A. Cohen, V. Huart, N. J. Wareham, R. Luben, K. T. Khaw, A. Tedgui, and S. M. Boekholdt. Circulating secretory phospholipase a2 activity and risk of incident coronary events in healthy men and women: the epic-norfolk study. *Arterioscler Thromb Vasc Biol*, 27(5):1177–83, 2007.

[316] K. Kugiyama, Y. Ota, K. Takazoe, Y. Moriyama, H. Kawano, Y. Miyao, T. Sakamoto, H. Soejima, H. Ogawa, H. Doi, S. Sugiyama, and H. Yasue. Circulating levels of secretory type ii phospholipase a(2) predict coronary events in patients with coronary artery disease. *Circulation*, 100(12):1280–4, 1999.

[317] P. Y. Liu, Y. H. Li, W. C. Tsai, T. H. Chao, L. M. Tsai, H. L. Wu, and J. H. Chen. Prognostic value and the changes of plasma levels of secretory type ii phospholipase a2 in patients with coronary artery disease undergoing percutaneous coronary intervention. *Eur Heart J*, 24(20):1824–32, 2003.

[318] W. Koenig, C. Y. Vossen, Z. Mallat, H. Brenner, J. Benessiano, and D. Rothenbacher. Association between type ii secretory phospholipase a2 plasma concentrations and activity and cardiovascular events in patients with coronary heart disease. *Eur Heart J*, 30(22):2742–8, 2009.

[319] K. Kugiyama, Y. Ota, S. Sugiyama, H. Kawano, H. Doi, H. Soejima, S. Miyamoto, H. Ogawa, K. Takazoe, and H. Yasue. Prognostic value of plasma levels of secretory type ii phospholipase a2 in patients with unstable angina pectoris. *Am J Cardiol*, 86(7):718–22, 2000.

[320] Lars Lind, Tabassome Simon, Lars Johansson, Salma Kotti, Tomas Hansen, Jacques Machecourt, Ewa Ninio, Alain Tedgui, Nicolas Danchin, Hkan Ahlstrm, and Ziad Mallat. Circulating levels of secretory- and lipoprotein-associated phospholipase a2 activities: relation to atherosclerotic plaques and future all-cause mortality. *Eur Heart J*, 33(23):2946–2954, Dec 2012.

[321] Paul M. Ridker. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. *J Am Coll Cardiol*, 49(21):2129–2138, May 2007.

[322] S. A. Julious and M. A. Mullee. Confounding and simpson's paradox. *BMJ*, 309(6967):1480–1481, Dec 1994.

[323] Benoit J. Arsenault, S Matthijs Boekholdt, and John J P. Kastelein. Varespladib: targeting the inflammatory face of atherosclerosis. *Eur Heart J*, 32(8):923–926, Apr 2011.

[324] Grard Lambeau and Michael H. Gelb. Biochemistry and physiology of mammalian secreted phospholipases a2. *Annu Rev Biochem*, 77:495–520, 2008.

[325] R. W. Schevitz, N. J. Bach, D. G. Carlson, N. Y. Chirgadze, D. K. Clawson, R. D. Dillard, S. E. Draheim, L. W. Hartley, N. D. Jones, and E. D. Mihelich. Structure-based design of the first potent and selective inhibitor of human non-pancreatic secretory phospholipase a2. *Nat Struct Biol*, 2(6):458–465, Jun 1995.

[326] R. D. Dillard, N. J. Bach, S. E. Draheim, D. R. Berry, D. G. Carlson, N. Y. Chirgadze, D. K. Clawson, L. W. Hartley, L. M. Johnson, N. D. Jones, E. R. McKinney, E. D. Mihelich, J. L. Olkowski, R. W. Schevitz, A. C. Smith, D. W. Snyder, C. D. Sommers, and J. P. Wery. Indole inhibitors of human nonpancreatic secretory phospholipase a2. 1. indole-3-acetamides. *J Med Chem*, 39(26):5119–5136, Dec 1996.

[327] E. D. Mihelich and R. W. Schevitz. Structure-based design of a new class of anti-inflammatory drugs: secretory phospholipase a(2) inhibitors, spi. *Biochim Biophys Acta*, 1441(2-3):223–228, Nov 1999.

[328] M. C. White and J. McHowat. The therapeutic potential of phospholipase a2 inhibitors in cardiovascular disease. *Cardiovasc Hematol Agents Med Chem*, 5(1):91–95, Jan 2007.

[329] D. W. Snyder, N. J. Bach, R. D. Dillard, S. E. Draheim, D. G. Carlson, N. Fox, N. W. Roehm, C. T. Armstrong, C. H. Chang, L. W. Hartley, L. M. Johnson, C. R. Roman, A. C. Smith, M. Song, and J. H. Fleisch. Pharmacology of ly315920/s-5920, [[3-(aminooxoacetyl)-2-ethyl-1- (phenylmethyl)-1h-indol-4-yl]oxy] acetate, a potent and selective secretory phospholipase a2 inhibitor: A new class of anti-inflammatory drugs, spi. *J Pharmacol Exp Ther*, 288(3):1117–24, 1999. Snyder, D W Bach, N J Dillard, R D Draheim, S E Carlson, D G Fox, N Roehm, N W Armstrong, C T Chang, C H Hartley, L W Johnson, L M Roman, C R Smith, A C Song, M Fleisch, J H Research Support, Non-U.S. Gov't United states The Journal of pharmacology and experimental therapeutics J Pharmacol Exp Ther. 1999 Mar;288(3):1117-24.

[330] Heather Fraser, Colin Hislop, Robert M. Christie, Heather L. Rick, Charles A. Reidy, Michael L. Chouinard, Patrick I. Eacho, Kenneth E. Gould, and Joaquim Trias. Varespladib (a-002), a secretory phospholipase a2 inhibitor, reduces atherosclerosis and aneurysm formation in apoe-/- mice. *J Cardiovasc Pharmacol*, 53(1):60–65, Jan 2009.

[331] J. L. Sebaugh. Guidelines for accurate ec50/ic50 estimation. *Pharm Stat*, 10(2):128–134, 2011.

[332] Jose O. Leite, Ushma Vaishnav, Michael Puglisi, Heather Fraser, Joaquim Trias, and Maria Luz Fernandez. A-002 (varespladib), a phospholipase a2 inhibitor, reduces atherosclerosis in guinea pigs. *BMC Cardiovasc Disord*, 9:7, 2009.

[333] R. S. Rosenson, C. Hislop, M. Elliott, Y. Stasiv, M. Goulder, and D. Waters. Effects of varespladib methyl on biomarkers and major cardiovascular events in acute coronary syndrome patients. *J Am Coll Cardiol*, 56(14):1079–88, 2010.

[334] R. S. Rosenson, C. Hislop, D. McConnell, M. Elliott, Y. Stasiv, N. Wang, and D. D. Waters. Effects of 1-h-indole-3-glyoxamide (a-002) on concentration of secretory phospholipase a2 (plasma study): a phase ii double-blind, randomised, placebo-controlled trial. *Lancet*, 373(9664):649–58, 2009.

[335] R. S. Rosenson, M. Elliott, Y. Stasiv, and C. Hislop. Randomized trial of an inhibitor of secretory phospholipase a2 on atherogenic lipoprotein subclasses in statin-treated patients with coronary heart disease. *Eur Heart J*, 32(8):999–1005, 2011.

[336] V. Dzavik, S. Lavi, K. Thorpe, P. M. Yip, S. Plante, D. Ing, C. B. Overgaard, M. D. Osten, J. Lan, K. Robbins, S. E. Miner, E. M. Horlick, and W. J. Cantor. The spla2 inhibition to decrease enzyme release after percutaneous coronary intervention (spider-pci) trial. *Circulation*, 122(23):2411–8, 2010.

[337] S. J. Nicholls, M. A. Cavender, J. J. Kastelein, G. Schwartz, D. D. Waters, R. S. Rosenson, D. Bash, and C. Hislop. Inhibition of secretory phospholipase a(2) in patients with acute coronary syndromes: Rationale and design of the vascular inflammation suppression to treat acute coronary syndrome for 16 weeks (vista-16) trial. *Cardiovasc Drugs Ther*, 2011. Journal article Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy Cardiovasc Drugs Ther. 2011 Nov 24.

[338] John Reckless. Addressing residual cardiovascular risk: what does the future hold? *The British Journal of Cardiology*, 19:s1–s16, 2012.

[339] P. T. Wootton, F. Drenos, J. A. Cooper, S. R. Thompson, J. W. Stephens, E. Hurt-Camejo, O. Wiklund, S. E. Humphries, and P. J. Talmud. Tagging-snp haplotype analysis of the secretory pla2iia gene pla2g2a shows strong association with serum levels of spla2iia: results from the udacs study. *Hum Mol Genet*, 15(2):355–61, 2006.

[340] Holly J. Exeter, Lasse Folkersen, Jutta Palmen, Anders Franco-Cereceda, Jackie A. Cooper, Anastasia Z. Kalea, Ferdinand Van't Hooft, Per Eriksson, Steve E. Humphries, and Philippa J. Talmud. Functional analysis of two pla2g2a variants associated with secretory phospholipase a2-iia levels. *PLoS One*, 7(7):e41139, 2012.

[341] N. Day, S. Oakes, R. Luben, K. T. Khaw, S. Bingham, A. Welch, and N. Wareham. Epic-norfolk: study design and characteristics of the cohort. european prospective investigation of cancer. *Br J Cancer*, 80 Suppl 1:95–103, 1999. Day, N Oakes, S Luben, R Khaw, K T Bingham, S Welch, A Wareham, N Research Support, Non-U.S. Gov't Scotland British journal of cancer Br J Cancer. 1999 Jul;80 Suppl 1:95-103.

[342] S. S. Dhamrait, J. W. Stephens, J. A. Cooper, J. Acharya, A. R. Mani, K. Moore, G. J. Miller, S. E. Humphries, S. J. Hurel, and H. E. Montgomery. Cardiovascular risk in healthy men and markers of oxidative stress in diabetic men are associated with common variation in the gene for uncoupling protein 2. *Eur Heart J*, 25(6):468–75, 2004.

[343] J. P. Cambou, T. Simon, G. Mulak, V. Bataille, and N. Danchin. The french registry of acute st elevation or non-st-elevation myocardial infarction (fast-mi): study design and baseline characteristics. *Arch Mal Coeur Vaiss*, 100(6-7):524–34, 2007.

[344] Rationale and design of the grace (global registry of acute coronary events) project: a multinational registry of patients hospitalized with acute coronary syndromes. *Am Heart J*, 141(2):190–9, 2001.

[345] C. B. Granger, R. J. Goldberg, O. Dabbous, K. S. Pieper, K. A. Eagle, C. P. Cannon, F. Van De Werf, A. Avezum, S. G. Goodman, M. D. Flather, and K. A. Fox. Predictors of hospital mortality in the global registry of acute coronary events. *Arch Intern Med*, 163(19):2345–53, 2003. Granger, Christopher B Goldberg, Robert J Dabbous, Omar Pieper, Karen S Eagle, Kim A Cannon, Christopher P Van De Werf, Frans Avezum, Alvaro Goodman, Shaun G Flather, Marcus D Fox, Keith A A Global Registry of Acute Coronary Events Investigators Research Support, Non-U.S. Gov't United States Archives of internal medicine Arch Intern Med. 2003 Oct 27;163(19):2345-53.

[346] G. G. Schwartz, A. G. Olsson, M. D. Ezekowitz, P. Ganz, M. F. Oliver, D. Waters, A. Zeiher, B. R. Chaitman, S. Leslie, T. Stern, and Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (M. I. R. A. C. L) Study Investigators . Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the miracl study: a randomized controlled trial. *JAMA*, 285(13):1711–1718, Apr 2001.

[347] D. Rothenbacher, W. Koenig, and H. Brenner. Comparison of n-terminal pro-b-natriuretic peptide, c-reactive protein, and creatinine clearance for prognosis in patients with known coronary heart disease. *Arch Intern Med*, 166(22):2455–60, 2006. Rothenbacher, Dietrich Koenig, Wolfgang Brenner, Hermann Comparative Study United States Archives of internal medicine Arch Intern Med. 2006 Dec 11-25;166(22):2455-60.

[348] Timo J. Nevalainen, Leena I. Eerola, Esa Rintala, V Jukka O. Laine, Grard Lambeau, and Michael H. Gelb. Time-resolved fluoroimmunoassays of the complete set of secreted phospholipases a2 in human serum. *Biochim Biophys Acta*, 1733(2-3):210–223, Apr 2005.

[349] Philip Barter. Effects of inflammation on high-density lipoproteins. *Arterioscler Thromb Vasc Biol*, 22(7):1062–1063, Jul 2002.

[350] N. T. Mulvihill and J. B. Foley. Inflammation in acute coronary syndromes. *Heart*, 87(3):201–204, Mar 2002.

[351] Cheryl R. Clark, Brent Coull, Lisa F. Berkman, Julie E. Buring, and Paul M. Ridker. Geographic variation in cardiovascular inflammation among healthy women in the women's health study. *PLoS One*, 6(11):e27468, 2011.

[352] Katariina Orni and Petri T. Kovanen. Lipoprotein modification by secretory phospholipase a(2) enzymes contributes to the initiation and progression of atherosclerosis. *Curr Opin Lipidol*, 20(5):421–427, Oct 2009.

[353] Danella M. Hafeman and Sharon Schwartz. Opening the black box: a motivation for the assessment of mediation. *Int J Epidemiol*, 38(3):838–845, Jun 2009.

[354] Ping-Yen Liu, Yi-Heng Li, Wei-Chuan Tsai, Ting-Hsing Chao, Liang-Miin Tsai, Hua-Lin Wu, and Jyh-Hong Chen. Prognostic value and the changes of plasma levels of secretory type ii phospholipase a2 in patients with coronary artery disease undergoing percutaneous coronary intervention. *Eur Heart J*, 24(20):1824–1832, Oct 2003.

[355] P. A. Kyzas, D. Denaxa-Kyza, and J. P. Ioannidis. Almost all articles on cancer prognostic markers report statistically significant results. *Eur J Cancer*, 43(17):2559–79, 2007. Journal Article Meta-Analysis Review England 1990).

[356] Robert F. Bonvini, Taoufik Hendiri, and Edoardo Camenzind. Inflammatory response post-myocardial infarction and reperfusion: a new therapeutic target? *European Heart Journal Supplements*, 7(suppl I):I27–I36, 2005.

[357] Daniel G. Hackam and Sonia S. Anand. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. *JAMA*, 290(7):932–940, Aug 2003.

[358] M. Murakami, T. Kambe, S. Shimbara, K. Higashino, K. Hanasaki, H. Arita, M. Horiguchi, M. Arita, H. Arai, K. Inoue, and I. Kudo. Different functional aspects of the group ii subfamily (types iia and v) and type x secretory phospholipase a(2)s in regulating arachidonic acid release and prostaglandin generation. implications of cyclooxygenase-2 induction and phospholipid scramblase-mediated cellular membrane perturbation. *J Biol Chem*, 274(44):31435–31444, Oct 1999.

[359] Vianda S. Stel, Friedo W. Dekker, Carmine Zoccali, and Kitty J. Jager. Instrumental variable analysis. *Nephrol Dial Transplant*, Jul 2012.

[360] P. C. Simons, A. Algra, M. F. van de Laak, D. E. Grobbee, and Y. van der Graaf. Second manifestations of arterial disease (smart) study: rationale and design. *Eur J Epidemiol*, 15(9):773–81, 1999.

[361] M. L. Sampietro, D. Pons, P. de Knijff, P. E. Slagboom, A. Zwinderman, and J. W. Jukema. A genome wide association analysis in the gender study. *Neth Heart J*, 17(6):262–4, 2009. Sampietro, M L Pons, D de Knijff, P Slagboom, P E Zwinderman, A Jukema, J W Netherlands Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation Neth Heart J. 2009 Jun;17(6):262-4.

[362] L. Folkersen, F. van't Hooft, E. Chernogubova, H. E. Agardh, G. K. Hansson, U. Hedin, J. Liska, A. C. Syvanen, G. Paulsson-Berne, A. Franco-Cereceda, A. Hamsten, A. Gabrielsen, and P. Eriksson. Association of genetic risk variants with expression of proximal genes identifies novel susceptibility genes for cardiovascular disease. *Circ Cardiovasc Genet*, 3(4):365–73, 2010.

[363] Sung Kee Ryu, Ziad Mallat, Joelle Benessiano, Alain Tedgui, Anders G. Olsson, Weihang Bao, Gregory G. Schwartz, Sotirios Tsimikas, and Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering (M. I. R. A. C. L) Trial Investigators . Phospholipase a2 enzymes, high-dose atorvastatin, and prediction of ischemic events after acute coronary syndromes. *Circulation*, 125(6):757–766, Feb 2012.

[364] Juan P. Casas, Tina Shah, Jackie Cooper, Emma Hawe, Alex D. McMahon, Dairena Gaffney, Christopher J. Packard, Denis S. O'Reilly, Irene Juhan-Vague, John S. Yudkin, Elena Tremoli, Maurizio Margaglione, Giovanni Di Minno, Anders Hamsten, Teake Kooistra, Jeffrey W. Stephens, Steven J. Hurel, Shona Livingstone, Helen M. Colhoun, George J. Miller, Leonelo E. Bautista, Tom Meade, Naveed Sattar, Steve E. Humphries, and Aroon D. Hingorani. Insight into the nature of the crp-coronary event association using mendelian randomization. *Int J Epidemiol*, 35(4):922–931, Aug 2006.

[365] R. A. Irizarry, B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis, U. Scherf, and T. P. Speed. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, 4(2):249–64, 2003. Irizarry, Rafael A Hobbs, Bridget Collin, Francois Beazer-Barclay, Yasmin D Antonellis, Kristen J Scherf, Uwe Speed, Terence P U01 HL66583/HL/NHLBI NIH HHS/United States Research Support, U.S. Gov't, P.H.S. England Biostatistics (Oxford, England) Biostatistics. 2003 Apr;4(2):249-64.

[366] R. C. Gentleman, V. J. Carey, D. M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A. J. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J. Y. Yang, and J. Zhang. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*, 5(10):R80, 2004. Gentleman, Robert C Carey, Vincent J Bates, Douglas M Bolstad, Ben Dettling, Marcel Dudoit, Sandrine Ellis, Byron Gautier, Laurent Ge, Yongchao Gentry, Jeff Hornik, Kurt Hothorn, Torsten Huber, Wolfgang Iacus, Stefano Irizarry, Rafael Leisch, Friedrich Li, Cheng Maechler, Martin Rossini, Anthony J Sawitzki, Gunther Smith, Colin Smyth, Gordon Tierney, Luke Yang, Jean Y H Zhang, Jianhua England Genome biology Genome Biol. 2004;5(10):R80. Epub 2004 Sep 15.

[367] L. Folkersen, D. Diez, C. E. Wheelock, J. Z. Haeggstrom, S. Goto, P. Eriksson, and A. Gabrielsen. Generegionscan: a bioconductor package for probe-level analysis of specific, small regions of the genome. *Bioinformatics*, 25(15):1978–9, 2009. Folkersen, Lasse Diez, Diego Wheelock, Craig E Haeggstrom, Jesper Z Goto, Susumu Eriksson, Per Gabrielsen, Anders Research Support, Non-U.S. Gov't England Bioinformatics (Oxford, England) Bioinformatics. 2009 Aug 1;25(15):1978-9. Epub 2009 Apr 27.

[368] F. Innocenti, G. M. Cooper, I. B. Stanaway, E. R. Gamazon, J. D. Smith, S. Mirkov, J. Ramirez, W. Liu, Y. S. Lin, C. Moloney, S. F. Aldred, N. D. Trinklein, E. Schuetz, D. A. Nickerson, K. E. Thummel, M. J. Rieder, A. E. Rettie, M. J. Ratain, N. J. Cox, and C. D. Brown. Identification, replication, and functional fine-mapping of expression quantitative trait loci in primary human liver tissue. *PLoS Genet*, 7(5):e1002078, 2011. Innocenti, Federico Cooper, Gregory M Stanaway, Ian B Gamazon, Eric R Smith, Joshua D Mirkov, Snezana Ramirez, Jacqueline Liu, Wanqing Lin, Yvonne S Moloney, Cliona Aldred, Shelly Force Trinklein, Nathan D Schuetz, Erin Nickerson, Deborah A Thummel, Ken E Rieder, Mark J Rettie, Allan E Ratain, Mark J Cox, Nancy J Brown, Christopher D 1KL2RR025015/RR/NCRR NIH HHS/United States K07CA140390-01/CA/NCI NIH HHS/United States N01-DK-7-0004/HHSN267200700004C/DK/NIDDK NIH HHS/United States NS053646/NS/NINDS NIH HHS/United States P01 GM32165/GM/NIGMS NIH HHS/United States R01GM094418/GM/NIGMS NIH HHS/United States R21DK081157-01A2/DK/NIDDK NIH HHS/United States U01 GM61393/GM/NIGMS NIH HHS/United States U01 HL66682/HL/NHLBI NIH HHS/United States Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't United States PLoS genetics PLoS Genet. 2011 May;7(5):e1002078. Epub 2011 May 26.

[369] D. C. Thomas, D. A. Lawlor, and J. R. Thompson. Re: Estimation of bias in nongenetic observational studies using "mendelian triangulation" by bautista et al. 17(7):511–3–, 2007.

[370] Joshua D Angrist and Alan B. Krueger. Split-sample instrumental variables estimates of the return to schooling. *Journal of Business & Economic Statistics*, 13(2):225–35, 1995.

[371] John R. Thompson, Cosetta Minelli, Keith R. Abrams, Martin D. Tobin, and Richard D. Riley. Meta-analysis of genetic studies using mendelian randomization–a multivariate approach. *Stat Med*, 24(14):2241–2254, Jul 2005.

[372] Atsushi Inoue and Gary Solon. Two-sample instrumental variables estimators. *The Review of Economics and Statistics*, 92(3):557–561, 2010.

[373] U.S. National Library of Medicine. Medical subject headings (available at: http://www.nlm.nih.gov/mesh/meshhome.html); accessed april 12th 2012.

[374] Robert S. Rosenson, Heather Fraser, Joaquim Trias, and Colin Hislop. Varespladib methyl in cardiovascular disease. *Expert Opin Investig Drugs*, 19(10):1245–1255, Oct 2010.

[375] R. S. Rosenson. After francis: next steps in the clinical evaluation of varespladib methyl. *Future Cardiol*, 7(1):11–8, 2011.

[376] R. M. Harbord and J. P. T. Higgins. Meta-regression in stata. *Stata Journal*, 8(4):493–519(27), 2008.

[377] International Consortium for Blood Pressure Genome-Wide Association Studies , Georg B. Ehret, Patricia B. Munroe, Kenneth M. Rice, Murielle Bochud, Andrew D. Johnson, Daniel I. Chasman, Albert V. Smith, Martin D. Tobin, Germaine C. Verwoert, Shih-Jen Hwang, Vasyl Pihur, Peter Vollenweider, Paul F. O'Reilly, Najaf Amin, Jennifer L. Bragg-Gresham, Alexander Teumer, Nicole L. Glazer, Lenore Launer, Jing Hua Zhao, Yurii Aulchenko, Simon Heath, Siim Sber, Afshin Parsa, Jian'an Luan, Pankaj Arora, Abbas Dehghan, Feng Zhang, Gavin Lucas, Andrew A. Hicks, Anne U. Jackson, John F. Peden, Toshiko Tanaka, Sarah H. Wild, Igor Rudan, Wilmar Igl, Yuri Milaneschi, Alex N. Parker, Cristiano Fava, John C. Chambers, Ervin R. Fox, Meena Kumari, Min Jin Go, Pim van der Harst, Wen Hong Linda Kao, Marketa Sjgren, D. G. Vinay, Myriam Alexander, Yasuharu Tabara, Sue Shaw-Hawkins, Peter H. Whincup, Yongmei Liu, Gang Shi, Johanna Kuusisto, Bamidele Tayo, Mark Seielstad, Xueling Sim, Khanh-Dung Hoang Nguyen, Terho Lehtimki, Giuseppe Matullo, Ying Wu, Tom R. Gaunt, N Charlotte Onland-Moret, Matthew N. Cooper, Carl G P. Platou, Elin Org, Rebecca Hardy, Santosh Dahgam, Jutta Palmen, Veronique Vitart, Peter S. Braund, Tatiana Kuznetsova, Cuno S P M. Uiterwaal, Adebowale Adeyemo, Walter Palmas, Harry Campbell, Barbara Ludwig, Maciej Tomaszewski, Ioanna Tzoulaki, Nicholette D. Palmer, C. A. R. D. IoG. R. A. M consortium , C. K. D. Gen Consortium , Kidney-Gen Consortium , EchoGen consortium , C. H. A. R. G. E-H. F consortium , Thor Aspelund, Melissa Garcia, Yen-Pei C. Chang, Jeffrey R. O'Connell, Nanette I. Steinle, Diederick E. Grobbee, Dan E. Arking, Sharon L. Kardia, Alanna C. Morrison, Dena Hernandez, Samer Najjar, Wendy L. McArdle, David Hadley, Morris J. Brown, John M. Connell, Aroon D. Hingorani, Ian N M. Day, Debbie A. Lawlor, John P. Beilby, Robert W. Lawrence, Robert Clarke, Jemma C. Hopewell, Halit Ongen, Albert W. Dreisbach, Yali Li, J Hunter Young, Joshua C. Bis, Mika Khnen, Jorma Viikari, Linda S. Adair, Nanette R. Lee, Ming-Huei Chen, Matthias Olden, Cristian Pattaro, Judith A Hoffman Bolton, Anna Kttgen, Sven Bergmann, Vincent Mooser, Nish Chaturvedi, Timothy M. Frayling, Muhammad Islam, Tazeen H. Jafar, Jeanette Erdmann, Smita R. Kulkarni, Stefan R. Bornstein, Jrgen Grssler, Leif Groop, Benjamin F. Voight, Johannes Kettunen, Philip Howard, Andrew Taylor, Simonetta Guarrera, Fulvio Ricceri, Valur Emilsson, Andrew Plump, Ins Barroso, Kay-Tee Khaw, Alan B. Weder, Steven C. Hunt, Yan V. Sun, Richard N. Bergman, Francis S. Collins, Lori L. Bonnycastle, Laura J. Scott, Heather M. Stringham, Leena Peltonen, Markus Perola, Erkki Vartiainen, Stefan-Martin Brand, Jan A. Staessen, Thomas J. Wang, Paul R. Burton, Maria Soler Artigas, Yanbin Dong, Harold Snieder, Xiaoling Wang, Haidong Zhu, Kurt K. Lohman, Megan E. Rudock, Susan R. Heckbert, Nicholas L. Smith, Kerri L. Wiggins, Ayo Doumatey, Daniel Shriner, Gudrun Veldre, Margus Viigimaa, Sanjay Kinra, Dorairaj Prabhakaran, Vikal Tripathy, Carl D. Langefeld, Annika Rosengren, Dag S. Thelle, Anna Maria Corsi, Andrew Singleton, Terrence Forrester, Gina Hilton, Colin A. McKenzie, Tunde Salako, Naoharu Iwai, Yoshikuni Kita, Toshio Ogihara, Takayoshi Ohkubo, Tomonori Okamura, Hirotsugu Ueshima, Satoshi Umemura, Susana Eyheramendy, Thomas Meitinger, H-Erich Wichmann, Yoon Shin Cho, Hyung-Lae Kim, Jong-Young Lee, James Scott, Joban S. Sehmi, Weihua Zhang, Bo Hedblad, Peter Nilsson, George Davey Smith, Andrew Wong, Narisu Narisu, Alena Stankov, Leslie J. Raffel, Jie Yao, Sekar Kathiresan, Christopher J. O'Donnell, Stephen M. Schwartz, M Arfan Ikram,

WT Longstreth, Jr, Thomas H. Mosley, Sudha Seshadri, Nick R G. Shrine, Louise V. Wain, Mario A. Morken, Amy J. Swift, Jaana Laitinen, Inga Prokopenko, Paavo Zitting, Jackie A. Cooper, Steve E. Humphries, John Danesh, Asif Rasheed, Anuj Goel, Anders Hamsten, Hugh Watkins, Stephan J L. Bakker, Wiek H. van Gilst, Charles S. Janipalli, K Radha Mani, Chittaranjan S. Yajnik, Albert Hofman, Francesco U S. Mattace-Raso, Ben A. Oostra, Ayse Demirkan, Aaron Isaacs, Fernando Rivadeneira, Edward G. Lakatta, Marco Orru, Angelo Scuteri, Mika Ala-Korpela, Antti J. Kangas, Leo-Pekka Lyytikinen, Pasi Soininen, Taru Tukiainen, Peter Wrtz, Rick Twee-Hee Ong, Marcus Drr, Heyo K. Kroemer, Uwe Vlker, Henry Vlzke, Pilar Galan, Serge Hercberg, Mark Lathrop, Diana Zelenika, Panos Deloukas, Massimo Mangino, Tim D. Spector, Guangju Zhai, James F. Meschia, Michael A. Nalls, Pankaj Sharma, Janos Terzic, M V Kranthi Kumar, Matthew Denniff, Ewa Zukowska-Szczechowska, Lynne E. Wagenknecht, F Gerald R. Fowkes, Fadi J. Charchar, Peter E H. Schwarz, Caroline Hayward, Xiuqing Guo, Charles Rotimi, Michiel L. Bots, Eva Brand, Nilesh J. Samani, Ozren Polasek, Philippa J. Talmud, Fredrik Nyberg, Diana Kuh, Maris Laan, Kristian Hveem, Lyle J. Palmer, Yvonne T. van der Schouw, Juan P. Casas, Karen L. Mohlke, Paolo Vineis, Olli Raitakari, Santhi. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*, 478(7367):103–109, Oct 2011.

[378] Tanya M. Teslovich, Kiran Musunuru, Albert V. Smith, Andrew C. Edmondson, Ioannis M. Stylianou, Masahiro Koseki, James P. Pirruccello, Samuli Ripatti, Daniel I. Chasman, Cristen J. Willer, Christopher T. Johansen, Sigrid W. Fouchier, Aaron Isaacs, Gina M. Peloso, Maja Barbalic, Sally L. Ricketts, Joshua C. Bis, Yurii S. Aulchenko, Gudmar Thorleifsson, Mary F. Feitosa, John Chambers, Marju Orho-Melander, Olle Melander, Toby Johnson, Xiaohui Li, Xiuqing Guo, Mingyao Li, Yoon Shin Cho, Min Jin Go, Young Jin Kim, Jong-Young Lee, Taesung Park, Kyunga Kim, Xueling Sim, Rick Twee-Hee Ong, Damien C. Croteau-Chonka, Leslie A. Lange, Joshua D. Smith, Kijoung Song, Jing Hua Zhao, Xin Yuan, Jian'an Luan, Claudia Lamina, Andreas Ziegler, Weihua Zhang, Robert Y L. Zee, Alan F. Wright, Jacqueline C M. Witteman, James F. Wilson, Gonneke Willemsen, H-Erich Wichmann, John B. Whitfield, Dawn M. Waterworth, Nicholas J. Wareham, Grard Waeber, Peter Vollenweider, Benjamin F. Voight, Veronique Vitart, Andre G. Uitterlinden, Manuela Uda, Jaakko Tuomilehto, John R. Thompson, Toshiko Tanaka, Ida Surakka, Heather M. Stringham, Tim D. Spector, Nicole Soranzo, Johannes H. Smit, Juha Sinisalo, Kaisa Silander, Eric J G. Sijbrands, Angelo Scuteri, James Scott, David Schlessinger, Serena Sanna, Veikko Salomaa, Juha Saharinen, Chiara Sabatti, Aimo Ruokonen, Igor Rudan, Lynda M. Rose, Robert Roberts, Mark Rieder, Bruce M. Psaty, Peter P. Pramstaller, Irene Pichler, Markus Perola, Brenda W J H. Penninx, Nancy L. Pedersen, Cristian Pattaro, Alex N. Parker, Guillaume Pare, Ben A. Oostra, Christopher J. O'Donnell, Markku S. Nieminen, Deborah A. Nickerson, Grant W. Montgomery, Thomas Meitinger, Ruth McPherson, Mark I. McCarthy, Wendy McArdle, David Masson, Nicholas G. Martin, Fabio Marroni, Massimo Mangino, Patrik K E. Magnusson, Gavin Lucas, Robert Luben, Ruth J F. Loos, Marja-Liisa Lokki, Guillaume Lettre, Claudia Langenberg, Lenore J. Launer, Edward G. Lakatta, Reijo Laaksonen, Kirsten O. Kyvik, Florian Kronenberg, Inke R. Knig, Kay-Tee Khaw, Jaakko Kaprio, Lee M. Kaplan, Asa Johansson, Marjo-Riitta Jarvelin, A Cecile J W. Janssens, Erik Ingelsson, Wilmar Igl, G. Kees Hovingh, Jouke-Jan Hottenga, Albert Hofman, Andrew A. Hicks, Christian Hengstenberg, Iris M. Heid, Caroline Hayward, Aki S. Havulinna, Nicholas D. Hastie, Tamara B. Harris, Talin Haritunians, Alistair S. Hall, Ulf Gyllensten, Candace Guiducci, Leif C. Groop, Elena Gonzalez, Christian Gieger, Nelson B. Freimer, Luigi Ferrucci, Jeanette Erdmann, Paul Elliott, Kenechi G. Ejebe, Angela Dring, Anna F. Dominiczak, Serkalem Demissie, Panagiotis Deloukas, Eco J C. de Geus, Ulf de Faire, Gabriel Crawford, Francis S. Collins, Yii-der I. Chen, Mark J. Caulfield, Harry Campbell, Noel P. Burtt,

Lori L. Bonnycastle, Dorret I. Boomsma, S Matthijs Boekholdt, Richard N. Bergman, Ins Barroso, Stefania Bandinelli, Christie M. Ballantyne, Themistocles L. Assimes, Thomas Quertermous, David Altshuler, Mark Seielstad, Tien Y. Wong, E-Shyong Tai, Alan B. Feranil, Christopher W. Kuzawa, Linda S. Adair, Herman A Taylor, Jr, Ingrid B. Borecki, Stacey B. Gabriel, James G. Wilson, Hilma Holm, Unnur Thorsteinsdottir, Vilmundur Gudnason, Ronald M. Krauss, Karen L. Mohlke, Jose M. Ordovas, Patricia B. Munroe, Jaspal S. Kooner, Alan R. Tall, Robert A. Hegele, John J P. Kastelein, Eric E. Schadt, Jerome I. Rotter, Eric Boerwinkle, David P. Strachan, Vincent Mooser, Kari Stefansson, Muredach P. Reilly, Nilesh J. Samani, Heribert Schunkert, L Adrienne Cupples, Manjinder S. Sandhu, Paul M. Ridker, Daniel J. Rader, Cornelia M. van Duijn, Leena Peltonen, Gonalo R. Abecasis, Michael Boehnke, and Sekar Kathiresan. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*, 466(7307):707–713, Aug 2010.

[379] Stephen Burgess, Simon G. Thompson, and C. R. P C. H. D Genetics Collaboration . Avoiding bias from weak instruments in mendelian randomization studies. *Int J Epidemiol*, 40(3):755–764, Jun 2011.

[380] C. S. Holladay, R. M. Wright, and B. L. Spangelo. Arachidonic acid stimulates interleukin-6 release from rat peritoneal macrophages in vitro: evidence for a prostacyclin-dependent mechanism. *Prostaglandins Leukot Essent Fatty Acids*, 49(6):915–922, Dec 1993.

[381] Anthera halts vista-16 clinical study due to lack of efficacy following recommendation by the independent data safety monitoring board. *Available at: http://investor.anthera.com/releasedetail.cfm?ReleaseID=656340.*

[382] Stephen J. Nicholls, John J P. Kastelein, Gregory G. Schwartz, Dianna Bash, Robert S. Rosenson, Matthew A. Cavender, Danielle M. Brennan, Wolfgang Koenig, J Wouter Jukema, Vijay Nambi, R Scott Wright, Venu Menon, A Michael Lincoff, Steven E. Nissen, and for the V. I. S. T. A-16 Investigators . Varespladib and cardiovascular events in patients with an acute coronary syndrome: The vista-16 randomized clinical trial. *JAMA*, Nov 2013.

[383] Lutz P. Breitling, Wolfgang Koenig, Marcus Fischer, Ziad Mallat, Christian Hengstenberg, Dietrich Rothenbacher, and Hermann Brenner. Type ii secretory phospholipase a2 and prognosis in patients with stable coronary heart disease: mendelian randomization study. *PLoS One*, 6(7):e22318, 2011.

[384] Stuart G. Baker, Barnett S. Kramer, and Donald Corle. The fallacy of enrolling only high-risk subjects in cancer prevention trials: is there a "free lunch"? *BMC Med Res Methodol*, 4:24, Oct 2004.

[385] Cosetta Minelli, John R. Thompson, Martin D. Tobin, and Keith R. Abrams. An integrated approach to the meta-analysis of genetic association studies using mendelian randomization. *Am J Epidemiol*, 160(5):445–452, Sep 2004.

[386] H. Fraser, C. Hislop, R. M. Christie, H. L. Rick, C. A. Reidy, M. L. Chouinard, P. I. Eacho, K. E. Gould, and J. Trias. Varespladib (a-002), a secretory phospholipase a2 inhibitor, reduces atherosclerosis and aneurysm formation in apoe-/- mice. *J Cardiovasc Pharmacol*, 53(1):60–5, 2009.

[387] J. D. Bradley, A. A. Dmitrienko, A. J. Kivitz, O. S. Gluck, A. L. Weaver, C. Wiesenhutter, S. L. Myers, and G. D. Sides. A randomized, double-blinded, placebo-controlled clinical trial of ly333013, a selective inhibitor of group ii secretory phospholipase a2, in the treatment of rheumatoid arthritis. *J Rheumatol*, 32(3):417–23, 2005.

[388] Rob C. Oslund, Nathan Cermak, and Michael H. Gelb. Highly specific and broadly potent inhibitors of mammalian secreted phospholipases a2. *J Med Chem*, 51(15):4708–4714, Aug 2008.

[389] J. Rehm, R. Room, M. Monteiro, G. Gmel, K. Graham, N. Rehn, C. T. Sempos, and D. Jernigan. Alcohol as a risk factor for global burden of disease. 9(4):157–64–, 2003.

[390] J. Rehm, A. V. Samokhvalov, M. G. Neuman, R. Room, C. Parry, K. Lonnroth, J. Patra, V. Poznyak, and S. Popova. The association between alcohol use, alcohol use disorders and tuberculosis (tb). a systematic review. 9(1):450–, 2009.

[391] Stephen S. Lim, Theo Vos, Abraham D. Flaxman, Goodarz Danaei, Kenji Shibuya, Heather Adair-Rohani, Markus Amann, H Ross Anderson, Kathryn G. Andrews, Martin Aryee, Charles Atkinson, Loraine J. Bacchus, Adil N. Bahalim, Kalpana Balakrishnan, John Balmes, Suzanne Barker-Collo, Amanda Baxter, Michelle L. Bell, Jed D. Blore, Fiona Blyth, Carissa Bonner, Guilherme Borges, Rupert Bourne, Michel Boussinesq, Michael Brauer, Peter Brooks, Nigel G. Bruce, Bert Brunekreef, Claire Bryan-Hancock, Chiara Bucello, Rachelle Buchbinder, Fiona Bull, Richard T. Burnett, Tim E. Byers, Bianca Calabria, Jonathan Carapetis, Emily Carnahan, Zoe Chafe, Fiona Charlson, Honglei Chen, Jian Shen Chen, Andrew Tai-Ann Cheng, Jennifer Christine Child, Aaron Cohen, K Ellicott Colson, Benjamin C. Cowie, Sarah Darby, Susan Darling, Adrian Davis, Louisa Degenhardt, Frank Dentener, Don C. Des Jarlais, Karen Devries, Mukesh Dherani, Eric L. Ding, E Ray Dorsey, Tim Driscoll, Karen Edmond, Suad Eltahir Ali, Rebecca E. Engell, Patricia J. Erwin, Saman Fahimi, Gail Falder, Farshad Farzadfar, Alize Ferrari, Mariel M. Finucane, Seth Flaxman, Francis Gerry R. Fowkes, Greg Freedman, Michael K. Freeman, Emmanuela Gakidou, Santu Ghosh, Edward Giovannucci, Gerhard Gmel, Kathryn Graham, Rebecca Grainger, Bridget Grant, David Gunnell, Hialy R. Gutierrez, Wayne Hall, Hans W. Hoek, Anthony Hogan, H Dean Hosgood, 3rd, Damian Hoy, Howard Hu, Bryan J. Hubbell, Sally J. Hutchings, Sydney E. Ibeanusi, Gemma L. Jacklyn, Rashmi Jasrasaria, Jost B. Jonas, Haidong Kan, John A. Kanis, Nicholas Kassebaum, Norito Kawakami, Young-Ho Khang, Shahab Khatibzadeh, Jon-Paul Khoo, Cindy Kok, Francine Laden, Ratilal Lalloo, Qing Lan, Tim Lathlean, Janet L. Leasher, James Leigh, Yang Li, John Kent Lin, Steven E. Lipshultz, Stephanie London, Rafael Lozano, Yuan Lu, Joelle Mak, Reza Malekzadeh, Leslie Mallinger, Wagner Marcenes, Lyn March, Robin Marks, Randall Martin, Paul McGale, John McGrath, Sumi Mehta, George A. Mensah, Tony R. Merriman, Renata Micha, Catherine Michaud, Vinod Mishra, Khayriyyah Mohd Hanafiah, Ali A. Mokdad, Lidia Morawska, Dariush Mozaffarian, Tasha Murphy, Mohsen Naghavi, Bruce Neal, Paul K. Nelson, Joan Miquel Nolla, Rosana Norman, Casey Olives, Saad B. Omer, Jessica Orchard, Richard Osborne, Bart Ostro, Andrew Page, Kiran D. Pandey, Charles D H. Parry, Erin Passmore, Jayadeep Patra, Neil Pearce, Pamela M. Pelizzari, Max Petzold, Michael R. Phillips, Dan Pope, C Arden Pope, 3rd, John Powles, Mayuree Rao, Homie Razavi, Eva A. Rehfuess, Jrgen T. Rehm, Beate Ritz, Frederick P. Rivara, Thomas Roberts, Carolyn Robinson, Jose A. Rodriguez-Portales, Isabelle Romieu, Robin Room, Lisa C. Rosenfeld, Ananya Roy, Lesley Rushton, Joshua A. Salomon, Uchechukwu Sampson, Lidia Sanchez-Riera, Ella Sanman, Amir Sapkota, Soraya Seedat, Peilin Shi, Kevin Shield, Rupak Shivakoti, Gitanjali M. Singh, David A. Sleet, Emma Smith, Kirk R. Smith, Nicolas J C. Stapelberg, Kyle Steenland, Heidi Stckl, Lars Jacob Stovner, Kurt Straif, Lahn Straney, George D. Thurston, Jimmy H. Tran, Rita Van Dingenen, Aaron van Donkelaar, J Lennert Veerman, Lakshmi Vijayakumar, Robert Weintraub, Myrna M. Weissman, Richard A. White, Harvey Whiteford, Steven T. Wiersma, James D. Wilkinson, Hywel C. Williams, Warwick Williams, Nicholas Wilson, Anthony D. Woolf, Paul Yip, Jan M. Zielinski, Alan D. Lopez, Christopher J L. Murray, Majid Ezzati, Mohammad A. AlMazroa, and Ziad A. Memish. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk

factor clusters in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. *Lancet*, 380(9859):2224–2260, Dec 2012.

[392] M. S. Freiberg and J. H. Samet. Alcohol and coronary heart disease: the answer awaits a randomized controlled trial. 112(10):1379–81–, 2005.

[393] J. H. O'Keefe, K. A. Bybee, and C. J. Lavie. Alcohol and cardiovascular health: the razor-sharp double-edged sword. 50(11):1009–14–, 2007.

[394] K. J. Mukamal. A 42-year-old man considering whether to drink alcohol for his health. 303(20):2065–73–, 2010.

[395] G. Corrao, L. Rubbiati, V. Bagnardi, A. Zambon, and K. Poikolainen. Alcohol and coronary heart disease: a meta-analysis. 95(10):1505–23–, 2000.

[396] A. G. Shaper, G. Wannamethee, and M. Walker. Alcohol and mortality in british men: explaining the u-shaped curve. 2(8623):1267–73–, 1988.

[397] E.B. Rimm and C. Moats. Alcohol and coronary heart disease: Drinking patterns and mediators of effect. 17(5):S3 – S7–, 2007.

[398] R. A. Kloner and S. H. Rezkalla. To drink or not to drink? that is the question. 116(11):1306–17–, 2007.

[399] J. H. Stein, J. G. Keevil, D. A. Wiebe, S. Aeschlimann, and J. D. Folts. Purple grape juice improves endothelial function and reduces the susceptibility of ldl cholesterol to oxidation in patients with coronary artery disease. 100(10):1050–5–, 1999.

[400] D. P. Agarwal. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. 37(5):409–15–, 2002.

[401] E. B. Rimm, P. Williams, K. Fosher, M. Criqui, and M. J. Stampfer. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. 319(7224):1523–8–, 1999.

[402] M. A. DeWood, J. Spores, R. Notske, L. T. Mouser, R. Burroughs, M. S. Golden, and H. T. Lang. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med*, 303(16):897–902, Oct 1980.

[403] Claus Thamer, Michael Haap, Andreas Fritsche, Hans Haering, and Michael Stumvoll. Relationship between moderate alcohol consumption and adiponectin and insulin sensitivity in a large heterogeneous population. *Diabetes Care*, 27(5):1240, May 2004.

[404] T S Mohamed Saleem and S Darbar Basha. Red wine: A drink to your heart. *J Cardiovasc Dis Res*, 1(4):171–176, Oct 2010.

[405] Red-wine researcher charged with photo-editing fraud. available at http://www.theheart.org/article/1339923.do. *theheart.org*.

[406] Emerging Risk Factors Collaboration , Emanuele Di Angelantonio, Nadeem Sarwar, Philip Perry, Stephen Kaptoge, Kausik K. Ray, Alexander Thompson, Angela M. Wood, Sarah Lewington, Naveed Sattar, Chris J. Packard, Rory Collins, Simon G. Thompson, and John Danesh. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*, 302(18):1993–2000, Nov 2009.

[407] J. M. Gaziano, J. E. Buring, J. L. Breslow, S. Z. Goldhaber, B. Rosner, M. VanDenburgh, W. Willett, and C. H. Hennekens. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. 329(25):1829–34–, 1993.

[408] Benjamin F. Voight, Gina M. Peloso, Marju Orho-Melander, Ruth Frikke-Schmidt, Maja Barbalic, Majken K. Jensen, George Hindy, Hilma Hlm, Eric L. Ding, Toby Johnson, Heribert Schunkert, Nilesh J. Samani, Robert Clarke, Jemma C. Hopewell, John F. Thompson, Mingyao Li, Gudmar Thorleifsson, Christopher Newton-Cheh, Kiran Musunuru, James P. Pirruccello, Danish Saleheen, Li Chen, Alexandre Fr Stewart, Arne Schillert, Unnur Thorsteinsdottir, Gudmundur Thorgeirsson, Sonia Anand, James C. Engert, Thomas Morgan, John Spertus, Monika Stoll, Klaus Berger, Nicola Martinelli, Domenico Girelli, Pascal P. McKeown, Christopher C. Patterson, Stephen E. Epstein, Joseph Devaney, Mary-Susan Burnett, Vincent Mooser, Samuli Ripatti, Ida Surakka, Markku S. Nieminen, Juha Sinisalo, Marja-Liisa Lokki, Markus Perola, Aki Havulinna, Ulf de Faire, Bruna Gigante, Erik Ingelsson, Tanja Zeller, Philipp Wild, Paul I W. de Bakker, Olaf H. Klungel, Anke-Hilse Maitland-van der Zee, Bas J M. Peters, Anthonius de Boer, Diederick E. Grobbee, Pieter W. Kamphuisen, Vera H M. Deneer, Clara C. Elbers, N Charlotte Onland-Moret, Marten H. Hofker, Cisca Wijmenga, Wm Monique Verschuren, Jolanda Ma Boer, Yvonne T. van der Schouw, Asif Rasheed, Philippe Frossard, Serkalem Demissie, Cristen Willer, Ron Do, Jose M. Ordovas, Gonalo R. Abecasis, Michael Boehnke, Karen L. Mohlke, Mark J. Daly, Candace Guiducci, Nol P. Burtt, Aarti Surti, Elena Gonzalez, Shaun Purcell, Stacey Gabriel, Jaume Marrugat, John Peden, Jeanette Erdmann, Patrick Diemert, Christina Willenborg, Inke R. Knig, Marcus Fischer, Christian Hengstenberg, Andreas Ziegler, Ian Buysschaert, Diether Lambrechts, Frans Van de Werf, Keith A. Fox, Nour Eddine El Mokhtari, Diana Rubin, Jrgen Schrezenmeir, Stefan Schreiber, Arne Schfer, John Danesh, Stefan Blankenberg, Robert Roberts, Ruth McPherson, Hugh Watkins, Alistair S. Hall, Kim Overvad, Eric Rimm, Eric Boerwinkle, Anne Tybjaerg-Hansen, L Adrienne Cupples, Muredach P. Reilly, Olle Melander, Pier M. Mannucci, Diego Ardissino, David Siscovick, Roberto Elosua, Kari Stefansson, Christopher J. O'Donnell, Veikko Salomaa, Daniel J. Rader, Leena Peltonen, Stephen M. Schwartz, David Altshuler, and Sekar Kathiresan. Plasma hdl cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*, May 2012.

[409] Gregory G. Schwartz, Anders G. Olsson, Markus Abt, Christie M. Ballantyne, Philip J. Barter, Jochen Brumm, Bernard R. Chaitman, Ingar M. Holme, David Kallend, Lawrence A. Leiter, Eran Leitersdorf, John J V. McMurray, Hardi Mundl, Stephen J. Nicholls, Prediman K. Shah, Jean-Claude Tardif, R Scott Wright, and dal-O. U. T. C. O. M. E. S Investigators . Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med*, 367(22):2089–2099, Nov 2012.

[410] L. I. Mennen, B. Balkau, S. Vol, E. Cacs, and E. Eschwge. Fibrinogen: a possible link between alcohol consumption and cardiovascular disease? desir study group. *Arterioscler Thromb Vasc Biol*, 19(4):887–892, Apr 1999.

[411] G. Davey Smith, R. Harbord, and S. Ebrahim. Fibrinogen, c-reactive protein and coronary heart disease: does mendelian randomization suggest the associations are non-causal? *QJM*, 97(3):163–166, Mar 2004.

[412] U. A. Ajani, C. H. Hennekens, A. Spelsberg, and J. E. Manson. Alcohol consumption and risk of type 2 diabetes mellitus among us male physicians. *Arch Intern Med*, 160(7):1025–1030, Apr 2000.

[413] Aafje Sierksma, Hamina Patel, Noriyuki Ouchi, Shinji Kihara, Tohru Funahashi, Robert J. Heine, Diederick E. Grobbee, Cornelis Kluft, and Henk F J. Hendriks. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. *Diabetes Care*, 27(1):184–189, Jan 2004.

[414] Linda Englund Ogge, Gerhard Brohall, Carl Johan Behre, Caroline Schmidt, and Bjrn Fagerberg. Alcohol consumption in relation to metabolic regulation, inflammation, and adiponectin in 64-year-old caucasian women: a population-based study with a focus on impaired glucose regulation. *Diabetes Care*, 29(4):908–913, Apr 2006.

[415] J W J. Beulens, L J C. van Loon, F. J. Kok, M. Pelsers, T. Bobbert, J. Spranger, A. Helander, and H F J. Hendriks. The effect of moderate alcohol consumption on adiponectin oligomers and muscle oxidative capacity: a human intervention study. *Diabetologia*, 50(7):1388–1392, Jul 2007.

[416] Shanshan Li, Hyun Joon Shin, Eric L. Ding, and Rob M. van Dam. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA*, 302(2):179–188, Jul 2009.

[417] Frank B. Hu, Meir J. Stampfer, Steven M. Haffner, Caren G. Solomon, Walter C. Willett, and JoAnn E. Manson. Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care*, 25(7):1129–1134, Jul 2002.

[418] J. S. Gavaler, K. Love, D. Van Thiel, S. Farholt, C. Gluud, E. Monteiro, A. Galvao-Teles, T. C. Ortega, and V. Cuervas-Mons. An international study of the relationship between alcohol consumption and postmenopausal estradiol levels. *Alcohol Alcohol Suppl*, 1:327–330, 1991.

[419] P. Jousilahti, E. Vartiainen, J. Tuomilehto, and P. Puska. Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in finland. *Circulation*, 99(9):1165–1172, Mar 1999.

[420] H. D. Sesso, N. R. Cook, J. E. Buring, J. E. Manson, and J. M. Gaziano. Alcohol consumption and the risk of hypertension in women and men. 51(4):1080–7–, 2008.

[421] B. Taylor, H. M. Irving, D. Baliunas, M. Roerecke, J. Patra, S. Mohapatra, and J. Rehm. Alcohol and hypertension: gender differences in dose-response relationships determined through systematic review and meta-analysis. 104(12):1981–90–, 2009.

[422] M. G. Marmot, P. Elliott, M. J. Shipley, A. R. Dyer, H. Ueshima, D. G. Beevers, R. Stamler, H. Kesteloot, G. Rose, and J. Stamler. Alcohol and blood pressure: the intersalt study. 308(6939):1263–7–, 1994.

[423] Science and technology select committee. inquiry on alcohol guidelines. royal college of physician's written evidence. september 2011. *Royal College of Physicians*.

[424] Sensible drinking - the report of an inter-departmental working grou. *Department of Health*, 1995.

[425] G. Edwards. Sensible drinking. *BMJ*, 312(7022):1, Jan 1996.

[426] Science and technology committee - eleventh report. alcohol guidelines. 2011. available at: http://www.publications.parliament.uk/pa/cm201012/cmselect/cmsctech/1536/153602.htm.

[427] Secretary of State for Health. Government response to the house of commons science and technology committee report of session 201012: Alcohol guidelines. 2012.

[428] J. Rehm, C. Mathers, S. Popova, M. Thavorncharoensap, Y. Teerawattananon, and J. Patra. Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. 373(9682):2223–33–, 2009.

[429] F. D. Fuchs and L. E. Chambless. Is the cardioprotective effect of alcohol real? 41(6):399–402–, 2007.

[430] Cordelia E M. Coltart and Ian T. Gilmore. Minimum alcohol pricing in england. *BMJ*, 342:d1063, 2011.

[431] Melanie Nichols, Peter Scarborough, Steven Allender, and Mike Rayner. What is the optimal level of population alcohol consumption for chronic disease prevention in england? modelling the impact of changes in average consumption levels. *BMJ Open*, 2(3), 2012.

[432] M. A. Bellis, K. Hughes, P. Cook, and M. Morleo. Off measure: How we underestimate the amount we drink. avaiable at: http://www.alcoholconcern.org.uk/publications/policy-reports/off-measure. *Alcohol Concern*, 2009.

[433] Gerhard Gmel and Jurgen Rehm. Measuring alcohol consumption. 31(3):467–540–, 2004.

[434] M. Marmot and E. Brunner. Alcohol and cardiovascular disease: the status of the u shaped curve. 303(6802):565–8–, 1991.

[435] Alcohol and mortality: the myth of the u-shaped curve. *Lancet*, 2(8623):1292–1293, Dec 1988.

[436] D. E. Falk, H. Y. Yi, and S. Hiller-Sturmhofel. An epidemiologic analysis of co-occurring alcohol and tobacco use and disorders: findings from the national epidemiologic survey on alcohol and related conditions. 29(3):162–71–, 2006.

[437] R. W. Wilsnack, N. D. Vogeltanz, S. C. Wilsnack, T. R. Harris, S. Ahlstrom, S. Bondy, L. Csemy, R. Ferrence, J. Ferris, J. Fleming, K. Graham, T. Greenfield, L. Guyon, E. Haavio-Mannila, F. Kellner, R. Knibbe, L. Kubicka, M. Loukomskaia, H. Mustonen, L. Nadeau, A. Narusk, R. Neve, G. Rahav, F. Spak, M. Teichman, K. Trocki, I. Webster, and S. Weiss. Gender differences in alcohol consumption and adverse drinking consequences: cross-cultural patterns. 95(2):251–65–, 2000.

[438] C. Power, B. Rodgers, and S. Hope. Heavy alcohol consumption and marital status: disentangling the relationship in a national study of young adults. 94(10):1477–87–, 1999.

[439] J. De Leon, D. M. Rendon, E. Baca-Garcia, F. Aizpuru, A. Gonzalez-Pinto, C. Anitua, and F. J. Diaz. Association between smoking and alcohol use in the general population: stable and unstable odds ratios across two years in two different countries. 42(3):252–7–, 2007.

[440] T. M. Caldwell, B. Rodgers, C. Clark, B. J. Jefferis, S. A. Stansfeld, and C. Power. Lifecourse socioeconomic predictors of midlife drinking patterns, problems and abstention: findings from the 1958 british birth cohort study. 95(3):269–78–, 2008.

[441] L. Chenet, D. Leon, M. McKee, and S. Vassin. Deaths from alcohol and violence in moscow: socio-economic determinants. 14(1):19–37–, 1998.

[442] H. Darling, A. I. Reeder, R. McGee, and S. Williams. Brief report: Disposable income, and spending on fast food, alcohol, cigarettes, and gambling by new zealand secondary school students. 29(5):837–43–, 2006.

[443] C. Power, B. Rodgers, and S. Hope. U-shaped relation for alcohol consumption and health in early adulthood and implications for mortality. *Lancet*, 352(9131):877, Sep 1998.

[444] Suthat Liangpunsakul, David W. Crabb, and Rong Qi. Relationship among alcohol intake, body fat, and physical activity: a population-based study. *Ann Epidemiol*, 20(9):670–675, Sep 2010.

[445] M. Droomers, C. T. Schrijvers, K. Stronks, D. van de Mheen, and J. P. Mackenbach. Educational differences in excessive alcohol consumption: the role of psychosocial and material stressors. 29(1):1–10–, 1999.

[446] S. Casswell, M. Pledger, and R. Hooper. Socioeconomic status and drinking patterns in young adults. 98(5):601–10–, 2003.

[447] U. Janlert and A. Hammarstrom. Alcohol consumption among unemployed youths: results from a prospective study. 87(5):703–14–, 1992.

[448] Nicholas J S. Christenfeld, Richard P. Sloan, Douglas Carroll, and Sander Greenland. Risk factors, confounding, and the illusion of statistical control. *Psychosom Med*, 66(6):868–875, 2004.

[449] Amy Berrington de Gonzalez, Patricia Hartge, James R. Cerhan, Alan J. Flint, Lindsay Hannan, Robert J. MacInnis, Steven C. Moore, Geoffrey S. Tobias, Hoda Anton-Culver, Laura Beane Freeman, W Lawrence Beeson, Sandra L. Clipp, Dallas R. English, Aaron R. Folsom, D Michal Freedman, Graham Giles, Niclas Hakansson, Katherine D. Henderson, Judith Hoffman-Bolton, Jane A. Hoppin, Karen L. Koenig, I-Min Lee, Martha S. Linet, Yikyung Park, Gaia Pocobelli, Arthur Schatzkin, Howard D. Sesso, Elisabete Weiderpass, Bradley J. Willcox, Alicja Wolk, Anne Zeleniuch-Jacquotte, Walter C. Willett, and Michael J. Thun. Body-mass index and mortality among 1.46 million white adults. *N Engl J Med*, 363(23):2211–2219, Dec 2010.

[450] James H. O'Keefe and Carl J. Lavie. Run for your life ... at a comfortable speed and not too far. *Heart*, Nov 2012.

[451] Francesco P. Cappuccio, Lanfranco D'Elia, Pasquale Strazzullo, and Michelle A. Miller. Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. *Sleep*, 33(5):585–592, May 2010.

[452] Francesco P. Cappuccio, Daniel Cooper, Lanfranco D'Elia, Pasquale Strazzullo, and Michelle A. Miller. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur Heart J*, 32(12):1484–1492, Jun 2011.

[453] Peter M. Nilsson. The j-shaped curve in secondary prevention: shall clinical practice change? *Hypertension*, 59(1):8–9, Jan 2012.

[454] B. Rockhill. Commentary: The message is rarely simple: the j-curve and beyond. *Int J Epidemiol*, 34(1):44–45, Feb 2005.

[455] Jean Adams and Martin White. When the population approach to prevention puts the health of individuals at risk. *Int J Epidemiol*, 34(1):40–43, Feb 2005.

[456] S. Andrasson. Alcohol and j-shaped curves. *Alcohol Clin Exp Res*, 22(7 Suppl):359S–364S, Oct 1998.

[457] Robert A. Kloner and Shereif H. Rezkalla. To drink or not to drink? that is the question. *Circulation*, 116(11):1306–1317, Sep 2007.

[458] Florent Boutitie, Franois Gueyffier, Stuart Pocock, Robert Fagard, Jean Pierre Boissel, and I. N. D. A. N. A Project Steering Committee. I. Ndividual Data A. Nalysis of Antihypertensive intervention . J-shaped relationship between blood pressure and mortality in hypertensive patients: new insights from a meta-analysis of individual-patient data. *Ann Intern Med*, 136(6):438–448, Mar 2002.

[459] Z. Vok, M. L. Bots, A. Hofman, P. J. Koudstaal, J. C. Witteman, and M. M. Breteler. J-shaped relation between blood pressure and stroke in treated hypertensives. *Hypertension*, 34(6):1181–1185, Dec 1999.

[460] S. Lewington, G. Whitlock, R. Clarke, P. Sherliker, J. Emberson, J. Halsey, N. Qizilbash, R. Peto, and R. Collins. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. 370(9602):1829–39–, 2007.

[461] Susan E. Brien, Paul E. Ronksley, Barbara J. Turner, Kenneth J. Mukamal, and William A. Ghali. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. *BMJ*, 342:d636, 2011.

[462] J. A. Sterne, D. Gavaghan, and M. Egger. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol*, 53(11):1119–1129, Nov 2000.

[463] Isabelle Boutron, Candice Estellat, Lydia Guittet, Agnes Dechartres, David L. Sackett, Asbjrn Hrbjartsson, and Philippe Ravaud. Methods of blinding in reports of randomized controlled trials assessing pharmacologic treatments: a systematic review. *PLoS Med*, 3(10):e425, Oct 2006.

[464] Glenn D. Walters. The heritability of alcohol abuse and dependence: a meta-analysis of behavior genetic research. *Am J Drug Alcohol Abuse*, 28(3):557–584, 2002.

[465] H. Wesley Perkins. Religious traditions, parents, and peers as determinants of alcohol and drug use among college students. *Review of Religious Research*, 27(1):15–31, September 1985.

[466] Paula Dias, Andreia Oliveira, and Carla Lopes. Social and behavioural determinants of alcohol consumption. *Ann Hum Biol*, 38(3):337–344, May 2011.

[467] Howard J. Edenberg. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Res Health*, 30(1):5–13, 2007.

[468] J. P. Zacny. Behavioral aspects of alcohol-tobacco interactions. 8:205–19–, 1990.

[469] A. R. Zeiner, A. Paredes, and H. D. Christensen. The role of acetaldehyde in mediating reactivity to an acute dose of ethanol among different racial groups. *Alcohol Clin Exp Res*, 3(1):11–18, Jan 1979.

[470] D. P. Agarwal. Genetic polymorphisms of alcohol metabolizing enzymes. *Pathol Biol (Paris)*, 49(9):703–709, Nov 2001.

[471] S. Macgregor, P. A. Lind, K. K. Bucholz, N. K. Hansell, P. A. Madden, M. M. Richter, G. W. Montgomery, N. G. Martin, A. C. Heath, and J. B. Whitfield. Associations of adh and aldh2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. 18(3):580–93–, 2009.

[472] L. J. Bierut, A. M. Goate, N. Breslau, E. O. Johnson, S. Bertelsen, L. Fox, A. Agrawal, K. K. Bucholz, R. Grucza, V. Hesselbrock, J. Kramer, S. Kuperman, J. Nurnberger, B. Porjesz, N. L. Saccone, M. Schuckit, J. Tischfield, J. C. Wang, T. Foroud, J. P. Rice, and H. J. Edenberg. Adh1b is associated with alcohol dependence and alcohol consumption in populations of european and african ancestry. *Mol Psychiatry*, 17(4):445–450, Apr 2012.

[473] T. K. Li, W. F. Bosron, W. P. Dafeldecker, L. G. Lange, and B. L. Vallee. Isolation of pi-alcohol dehydrogenase of human liver: is it a determinant of alcoholism? *Proc Natl Acad Sci U S A*, 74(10):4378–4381, Oct 1977.

[474] E. Borras, C. Coutelle, A. Rosell, F. Fernandez-Muixi, M. Broch, B. Crosas, L. Hjelmqvist, A. Lorenzo, C. Gutierrez, M. Santos, M. Szczepanek, M. Heilig, P. Quattrocchi, J. Farres, F. Vidal, C. Richart, T. Mach, J. Bogdal, H. Jornvall, H. K. Seitz, P. Couzigou, and X. Pares. Genetic polymorphism of alcohol dehydrogenase in europeans: the adh2*2 allele decreases the risk for alcoholism and is associated with adh3*1. 31(4):984–9–, 2000.

[475] H. Li, N. Mukherjee, U. Soundararajan, Z. Tarnok, C. Barta, S. Khaliq, A. Mohyuddin, S. L. Kajuna, S. Q. Mehdi, J. R. Kidd, and K. K. Kidd. Geographically separate increases in the frequency of the derived adh1b*47his allele in eastern and western asia. 81(4):842–6–, 2007.

[476] S. J. Yin, W. F. Bosron, L. J. Magnes, and T. K. Li. Human liver alcohol dehydrogenase: purification and kinetic characterization of the beta 2 beta 2, beta 2 beta 1, alpha beta 2, and beta 2 gamma 1 "oriental" isoenzymes. *Biochemistry*, 23(24):5847–5853, Nov 1984.

[477] J. C. Burnell, L. G. Carr, F. E. Dwulet, H. J. Edenberg, T. K. Li, and W. F. Bosron. The human beta 3 alcohol dehydrogenase subunit differs from beta 1 by a cys for arg-369 substitution which decreases nad(h) binding. *Biochem Biophys Res Commun*, 146(3):1127–1133, Aug 1987.

[478] K. Y. Seng, L M G. Limenta, D. Heng, and E J D. Lee. Population pharmacokinetics and pharmacogenetics of alcohol in chinese and indians in singapore. *J Clin Pharm Ther*, 38(2):141–149, Apr 2013.

[479] L. G. Carr, T. Foroud, T. Stewart, P. Castelluccio, H. J. Edenberg, and T. K. Li. Influence of adh1b polymorphism on alcohol use and its subjective effects in a jewish population. 112(2):138–43–, 2002.

[480] A. Lorenzo, T. Auguet, F. Vidal, M. Broch, M. Olona, C. Gutierrez, M. Lopez-Dupla, J. J. Sirvent, J. C. Quer, M. Santos, and C. Richart. Polymorphisms of alcohol-metabolizing enzymes and the risk for alcoholism and alcoholic liver disease in caucasian spanish women. 84(2):195–200–, 2006.

[481] S. E. Luczak, S. J. Glatt, and T. J. Wall. Meta-analyses of aldh2 and adh1b with alcohol dependence in asians. 132(4):607–21–, 2006.

[482] J. S. Tolstrup, B. G. Nordestgaard, S. Rasmussen, A. Tybjaerg-Hansen, and M. Gronbaek. Alcoholism and alcohol drinking habits predicted from alcohol dehydrogenase genes. 8(3):220–7–, 2008.

[483] L. Zuccolo, N. Fitz-Simon, R. Gray, S. M. Ring, K. Sayal, G. Davey Smith, and S. J. Lewis. A non-synonymous variant in adh1b is strongly associated with prenatal alcohol use in a european sample of pregnant women. pages –, 2009.

[484] Dawei Li, Hongyu Zhao, and Joel Gelernter. Strong association of the alcohol dehydrogenase 1b gene (adh1b) with alcohol dependence and alcohol-induced medical diseases. *Biol Psychiatry*, 70(6):504–512, Sep 2011.

[485] Debbie A. Lawlor, Brge G. Nordestgaard, Marianne Benn, Luisa Zuccolo, Anne Tybjaerg-Hansen, and George Davey Smith. Exploring causal associations between alcohol and coronary heart disease risk factors: findings from a mendelian randomization study in the copenhagen general population study. *Eur Heart J*, 34(32):2519–2528, Aug 2013.

[486] Nathalie Druesne-Pecollo, Bertrand Tehard, Yann Mallet, Mariette Gerber, Teresa Norat, Serge Hercberg, and Paule Latino-Martel. Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. *Lancet Oncol*, 10(2):173–180, Feb 2009.

[487] Shu-Juan Yang, Akira Yokoyama, Tetsuji Yokoyama, Yu-Chuan Huang, Si-Ying Wu, Ying Shao, Jin Niu, Jie Wang, Yu Liu, Xiao-Qiao Zhou, and Chun-Xia Yang. Relationship between genetic polymorphisms of aldh2 and adh1b and esophageal cancer risk: a meta-analysis. *World J Gastroenterol*, 16(33):4210–4220, Sep 2010.

[488] J. S. Tolstrup, M. Gronbaek, and B. G. Nordestgaard. Alcohol intake, myocardial infarction, biochemical risk factors, and alcohol dehydrogenase genotypes. 2:507–514–, 2009.

[489] L. L. Husemoen, T. Jorgensen, K. Borch-Johnsen, T. Hansen, O. Pedersen, and A. Linneberg. The association of alcohol and alcohol metabolizing gene variants with diabetes and coronary heart disease risk factors in a white population. 5(8):–, 2010.

[490] Dagmar Drogan, Abigail J. Sheldrick, Madlen Schtze, Sven Knppel, Frank Andersohn, Romina di Giuseppe, Bianca Herrmann, Stefan N. Willich, Edeltraut Garbe, Manuela M. Bergmann, Heiner Boeing, and Cornelia Weikert. Alcohol consumption, genetic variants in alcohol deydrogenases, and risk of cardiovascular diseases: a prospective study and meta-analysis. *PLoS One*, 7(2):e32176, 2012.

[491] Yi Peng, Hong Shi, Xue-bin Qi, Chun-jie Xiao, Hua Zhong, Run-lin Z. Ma, and Bing Su. The adh1b arg47his polymorphism in east asian populations and expansion of rice domestication in history. *BMC Evol Biol*, 10:15, 2010.

[492] Global status report on alcohol and health. *World Health Organisation*, 2011.

[493] WHO. "heart disease deaths by country", world health organization.

[494] Hui Li, Namita Mukherjee, Usha Soundararajan, Zsanett Tarnok, Csaba Barta, Shagufta Khaliq, Aisha Mohyuddin, Sylvester L B. Kajuna, S Qasim Mehdi, Judith R. Kidd, and Kenneth K. Kidd. Geographically separate increases in the frequency of the derived adh1b*47his allele in eastern and western asia. *Am J Hum Genet*, 81(4):842–846, Oct 2007.

[495] Hui Li, Sheng Gu, Yi Han, Zhi Xu, Andrew J. Pakstis, Li Jin, Judith R. Kidd, and Kenneth K. Kidd. Diversification of the adh1b gene during expansion of modern humans. *Ann Hum Genet*, 75(4):497–507, Jul 2011.

[496] H. W. Goedde, D. P. Agarwal, S. Harada, D. Meier-Tackmann, D. Ruofu, U. Bienzle, A. Kroeger, and L. Hussein. Population genetic studies on aldehyde dehydrogenase isozyme deficiency and alcohol sensitivity. *Am J Hum Genet*, 35(4):769–772, Jul 1983.

[497] F. Tanaka, Y. Shiratori, O. Yokosuka, F. Imazeki, Y. Tsukada, and M. Omata. Polymorphism of alcohol-metabolizing genes affects drinking behavior and alcoholic liver disease in japanese men. *Alcohol Clin Exp Res*, 21(4):596–601, Jun 1997.

[498] K. Okamoto, Y. Murawaki, I. Yuasa And, and H. Kawasaki. Effect of aldh2 and cyp2e1 gene polymorphisms on drinking behavior and alcoholic liver disease in japanese male workers. *Alcohol Clin Exp Res*, 25(6 Suppl):19S–23S, Jun 2001.

[499] F. Sun, I. Tsuritani, R. Honda, Z. Y. Ma, and Y. Yamada. Association of genetic polymorphisms of alcohol-metabolizing enzymes with excessive alcohol consumption in japanese men. *Hum Genet*, 105(4):295–300, Oct 1999.

[500] F. F. Zhang, L. Hou, M. B. Terry, J. Lissowska, A. Morabia, J. Chen, M. Yeager, W. Zatonski, S. Chanock, and W. H. Chow. Genetic polymorphisms in alcohol metabolism, alcohol intake and the risk of stomach cancer in warsaw, poland. pages –, 2007.

[501] S. J. Yin and G. S. Peng. Acetaldehyde, polymorphisms and the cardiovascular system. 285:52–63; discusion 63–8, 198–9–, 2007.

[502] T. L. Wall, H. R. Thomasson, M. A. Schuckit, and C. L. Ehlers. Subjective feelings of alcohol intoxication in asians with genetic variations of aldh2 alleles. 16(5):991–5–, 1992.

[503] T. L. Wall, S. H. Shea, K. K. Chan, and L. G. Carr. A genetic association with the development of alcohol and other substance use behavior in asian americans. 110(1):173–8–, 2001.

[504] H. R. Thomasson, H. J. Edenberg, D. W. Crabb, X. L. Mai, R. E. Jerome, T. K. Li, S. P. Wang, Y. T. Lin, R. B. Lu, and S. J. Yin. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in chinese men. 48(4):677–81–, 1991.

[505] H. R. Thomasson, D. W. Crabb, H. J. Edenberg, T. K. Li, H. G. Hwu, C. C. Chen, E. K. Yeh, and S. J. Yin. Low frequency of the adh2*2 allele among atayal natives of taiwan with alcohol use disorders. 18(3):640–3–, 1994.

[506] F. Tanaka, Y. Shiratori, O. Yokosuka, F. Imazeki, Y. Tsukada, and M. Omata. High incidence of adh2*1/aldh2*1 genes among japanese alcohol dependents and patients with alcoholic liver disease. 23(2):234–9–, 1996.

[507] Cheol Min Shin, Nayoung Kim, Sung-Il Cho, Joo Sung Kim, Hyun Chae Jung, and In Sung Song. Association between alcohol intake and risk for gastric cancer with regard to aldh2 genotype in the korean population. *Int J Epidemiol*, 40(4):1047–1055, Aug 2011.

[508] J. Wang, H. Wang, Y. Chen, P. Hao, and Y. Zhang. Alcohol ingestion and colorectal neoplasia: a meta-analysis based on a mendelian randomization approach. *Colorectal Dis*, 13(5):e71–e78, May 2011.

[509] Y. Wang, Y. Zhang, J. Zhang, X. Tang, Y. Qian, P. Gao, and D. Zhu. Association of a functional single-nucleotide polymorphism in the aldh2 gene with essential hypertension depends on drinking behavior in a chinese han population. *J Hum Hypertens*, May 2012.

[510] Chung-Tay Yao, Chun-An Cheng, Hsu-Kun Wang, Shao-Wen Chiu, Yi-Chyan Chen, Ming-Fang Wang, Shih-Jiun Yin, and Giia-Sheun Peng. The role of aldh2 and adh1b polymorphism in alcohol consumption and stroke in han chinese. *Hum Genomics*, 5(6):569–576, Oct 2011.

[511] S. Boccia, M. Hashibe, P. Galli, E. De Feo, T. Asakage, T. Hashimoto, A. Hiraki, T. Katoh, T. Nomura, A. Yokoyama, C. M. van Duijn, G. Ricciardi, and P. Boffetta. Aldehyde dehydrogenase 2 and head and neck cancer: a meta-analysis implementing a mendelian randomization approach. 18(1):248–54–, 2009.

[512] W. F. Bosron, L. Lumeng, and T. K. Li. Genetic polymorphism of enzymes of alcohol metabolism and susceptibility to alcoholic liver disease. 10(2):147–58–, 1988.

[513] G. K. Chambers, S. J. Marshall, G. M. Robinson, S. Maguire, J. Newton-Howes, and N. L. Chong. The genetics of alcoholism in polynesians: alcohol and aldehyde dehydrogenase genotypes in young men. 26(7):949–55–, 2002.

[514] Y. C. Chao, S. R. Liou, Y. Y. Chung, H. S. Tang, C. T. Hsu, T. K. Li, and S. J. Yin. Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in chinese patients. 19(2):360–6–, 1994.

[515] L. Chen, G. Davey Smith, R. M. Harbord, and S. J. Lewis. Alcohol intake and blood pressure: a systematic review implementing a mendelian randomization approach. 5(3):e52–, 2008.

[516] T. A. Cook, S. E. Luczak, S. H. Shea, C. L. Ehlers, L. G. Carr, and T. L. Wall. Associations of aldh2 and adh1b genotypes with response to alcohol in asian americans. 66(2):196–204–, 2005.

[517] P. A. Dickson, M. R. James, A. C. Heath, G. W. Montgomery, N. G. Martin, J. B. Whitfield, and A. J. Birley. Effects of variation at the aldh2 locus on alcohol metabolism, sensitivity, consumption, and dependence in europeans. 30(7):1093–100–, 2006.

[518] N. Druesne-Pecollo, B. Tehard, Y. Mallet, M. Gerber, T. Norat, S. Hercberg, and P. Latino-Martel. Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. 10(2):173–80–, 2009.

[519] H. J. Edenberg. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. 30(1):5–13–, 2007.

[520] M. Y. Eng, S. E. Luczak, and T. L. Wall. Aldh2, adh1b, and adh1c genotypes in asians: a literature review. 30(1):22–7–, 2007.

[521] H. W. Goedde, D. P. Agarwal, G. Fritze, D. Meier-Tackmann, S. Singh, G. Beckmann, K. Bhatia, L. Z. Chen, B. Fang, R. Lisker, and et al. Distribution of adh2 and aldh2 genotypes in different populations. 88(3):344–6–, 1992.

[522] L. L. Husemoen, M. Fenger, N. Friedrich, J. S. Tolstrup, S. Beenfeldt Fredriksen, and A. Linneberg. The association of adh and aldh gene variants with alcohol drinking habits and cardiovascular disease risk factors. 32(11):1984–91–, 2008.

[523] D. E. Irons, M. McGue, W. G. Iacono, and W. S. Oetting. Mendelian randomization: a novel test of the gateway hypothesis and models of gene-environment interplay. 19(4):1181–95–, 2007.

[524] T. Konishi, M. Calvillo, A. S. Leng, J. Feng, T. Lee, H. Lee, J. L. Smith, S. H. Sial, N. Berman, S. French, V. Eysselein, K. M. Lin, and Y. J. Wan. The adh3*2 and cyp2e1 c2 alleles increase the risk of alcoholism in mexican american men. 74(2):183–9–, 2003.

[525] S. J. Lewis and G. D. Smith. Alcohol, aldh2, and esophageal cancer: a meta-analysis which illustrates the potentials and limitations of a mendelian randomization approach. 14(8):1967–71–, 2005.

[526] C. Chen, G. Davey Smith, R. Harbord, and S. J. Lewis. Genotype influencing alcohol consumption is positively associated with blood pressure and the risk of hypertension: a systematic review using a mendelian randomization approach. pages –, Ahead of publication.

[527] Suthat Liangpunsakul, Rong Qi, David W. Crabb, and Frank Witzmann. Relationship between alcohol drinking and aspartate aminotransferase:alanine aminotransferase (ast:alt) ratio, mean corpuscular volume (mcv), gamma-glutamyl transpeptidase (ggt), and apolipoprotein a1 and b in the u.s. population. *J Stud Alcohol Drugs*, 71(2):249–252, Mar 2010.

[528] P. Jousilahti, D. Rastenyte, and J. Tuomilehto. Serum gamma-glutamyl transferase, self-reported alcohol drinking, and the risk of stroke. *Stroke*, 31(8):1851–1855, Aug 2000.

[529] National Institute for Health and Clinical Excellence. Hypertension. clinical management of primary hypertension in adults. nice clinical guidance 127 available at: http://www.nice.org.uk/nicemedia/live/13561/56008/56008.pdf. *NICE*, 2011.

[530] Cholesterol Treatment Trialists' (C. T. T) Collaborators , B. Mihaylova, J. Emberson, L. Blackwell, A. Keech, J. Simes, E. H. Barnes, M. Voysey, A. Gray, R. Collins, and C. Baigent. The effects of lowering ldl cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet*, 380(9841):581–590, Aug 2012.

[531] Hester M. Den Ruijter, Sanne A E. Peters, Todd J. Anderson, Annie R. Britton, Jacqueline M. Dekker, Marinus J. Eijkemans, Gunnar Engstrm, Gregory W. Evans, Jacqueline de Graaf, Diederick E. Grobbee, Bo Hedblad, Albert Hofman, Suzanne Holewijn, Ai Ikeda, Maryam Kavousi, Kazuo Kitagawa, Akihiko Kitamura, Hendrik Koffijberg, Eva M. Lonn, Matthias W. Lorenz, Ellisiv B. Mathiesen, Giel Nijpels, Shuhei Okazaki, Daniel H. O'Leary, Joseph F. Polak, Jackie F. Price, Christine Robertson, Christopher M. Rembold, Maria Rosvall, Tatjana Rundek, Jukka T. Salonen, Matthias Sitzer, Coen D A. Stehouwer, Jacqueline C. Witteman, Karel G. Moons, and Michiel L. Bots. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA*, 308(8):796–803, Aug 2012.

[532] Mauro Amato, Piero Montorsi, Alessio Ravani, Elisa Oldani, Stefano Galli, Paolo M. Ravagnani, Elena Tremoli, and Damiano Baldassarre. Carotid intima-media thickness by b-mode ultrasound as surrogate of coronary atherosclerosis: correlation with quantitative coronary angiography and coronary intravascular ultrasound findings. *Eur Heart J*, 28(17):2094–2101, Sep 2007.

[533] Julian P T. Higgins and Simon G. Thompson. Quantifying heterogeneity in a meta-analysis. *Stat Med*, 21(11):1539–1558, Jun 2002.

[534] Martin D. Tobin, Nuala A. Sheehan, Katrina J. Scurrah, and Paul R. Burton. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med*, 24(19):2911–2935, Oct 2005.

[535] M. G. Marmot, G. D. Smith, S. Stansfeld, C. Patel, F. North, J. Head, I. White, E. Brunner, and A. Feeney. Health inequalities among british civil servants: the whitehall ii study. *Lancet*, 337(8754):1387–1393, Jun 1991.

[536] Folkert W. Asselbergs, Yiran Guo, Erik P A. van Iperen, Suthesh Sivapalaratnam, Vinicius Tragante, Matthew B. Lanktree, Leslie A. Lange, Berta Almoguera, Yolande E. Appelman, John Barnard, Jens Baumert, Amber L. Beitelshees, Tushar R. Bhangale, Yii-Der Ida Chen, Tom R. Gaunt, Yan Gong, Jemma C. Hopewell, Toby Johnson, Marcus E. Kleber, Taimour Y. Langaee,

Mingyao Li, Yun R. Li, Kiang Liu, Caitrin W. McDonough, Matthijs F L. Meijs, Rita P S. Middelberg, Kiran Musunuru, Christopher P. Nelson, Jeffery R. O'Connell, Sandosh Padmanabhan, James S. Pankow, Nathan Pankratz, Suzanne Rafelt, Ramakrishnan Rajagopalan, Simon P R. Romaine, Nicholas J. Schork, Jonathan Shaffer, Haiqing Shen, Erin N. Smith, Sam E. Tischfield, Peter J. van der Most, Jana V. van Vliet-Ostaptchouk, Niek Verweij, Kelly A. Volcik, Li Zhang, Kent R. Bailey, Kristian M. Bailey, Florianne Bauer, Jolanda M A. Boer, Peter S. Braund, Amber Burt, Paul R. Burton, Sarah G. Buxbaum, Wei Chen, Rhonda M. Cooper-Dehoff, L Adrienne Cupples, Jonas S. deJong, Christian Delles, David Duggan, Myriam Fornage, Clement E. Furlong, Nicole Glazer, John G. Gums, Claire Hastie, Michael V. Holmes, Thomas Illig, Susan A. Kirkland, Mika Kivimaki, Ronald Klein, Barbara E. Klein, Charles Kooperberg, Kandice Kottke-Marchant, Meena Kumari, Andrea Z. LaCroix, Laya Mallela, Gurunathan Murugesan, Jose Ordovas, Willem H. Ouwehand, Wendy S. Post, Richa Saxena, Hubert Scharnagl, Pamela J. Schreiner, Tina Shah, Denis C. Shields, Daichi Shimbo, Sathanur R. Srinivasan, Ronald P. Stolk, Daniel I. Swerdlow, Herman A Taylor, Jr, Eric J. Topol, Elina Toskala, Joost L. van Pelt, Jessica van Setten, Salim Yusuf, John C. Whittaker, A. H. Zwinderman, LifeLines Cohort Study , Sonia S. Anand, Anthony J. Balmforth, Gerald S. Berenson, Connie R. Bezzina, Bernhard O. Boehm, Eric Boerwinkle, Juan P. Casas, Mark J. Caulfield, Robert Clarke, John M. Connell, Karen J. Cruickshanks, Karina W. Davidson, Ian N M. Day, Paul I W. de Bakker, Pieter A. Doevendans, Anna F. Dominiczak, Alistair S. Hall, Catharina A. Hartman, Christian Hengstenberg, Hans L. Hillege, Marten H. Hofker, Steve E. Humphries, Gail P. Jarvik, Julie A. Johnson, Bernhard M. Kaess, Sekar Kathiresan, Wolfgang Koenig, Debbie A. Lawlor, Winfried Mrz, Olle Melander, Braxton D. Mitchell, Grant W. Montgomery, Patricia B. Munroe, Sarah S. Murray, Stephen J. Newhouse, N Charlotte Onland-Moret, Neil Poulter, Bruce Psaty, Susan Redline, Stephen S. Rich, Jerome I. Rotter, Heribert Schunkert, Peter Sever, Alan R. Shuldiner, Roy L. Silverstein, Alice Stanton, Barbara Thorand, Mieke D. Trip, Michael Y. Tsai, Pim van der Harst, Ellen van der Schoot, Yvonne T. van der Schouw, W M Monique Verschuren, Hugh Watkins, Arthur A M. Wilde, Bruce H R. Wolffenbuttel, John B. Whitfield, G Kees Hovingh, Christie M. Ballantyne, Cisca Wijmenga, Muredach P. Reilly, Nicholas G. Martin, James G. Wilson, Daniel J. Rader, Nilesh J. Samani, Alex P. Reiner, Robert A. Hegele, John J P. Kastelein, Aroon D. Hingorani, Philippa J. Talmud, Hakon Hakonarson, Clara C. Elbers, Brendan J. Keating, and Fotios Drenos. Large-scale gene-centric meta-analysis across 32 studies identifies multiple lipid loci. *Am J Hum Genet*, 91(5):823–838, Nov 2012.

[537] Hadassa M. Jochemsen, Raoul P. Kloppenborg, Lisette C P G M. de Groot, Ellen Kampman, Willem P T M. Mali, Yolanda van der Graaf, Mirjam I. Geerlings, and S. M. A. R. T Study Group . Homocysteine, progression of ventricular enlargement, and cognitive decline. the second manifestations of arterial disease-magnetic resonance study. *Alzheimers Dement*, Aug 2012.

[538] Stephen R. Cole, Robert W. Platt, Enrique F. Schisterman, Haitao Chu, Daniel Westreich, David Richardson, and Charles Poole. Illustrating bias due to conditioning on a collider. *Int J Epidemiol*, 39(2):417–420, Apr 2010.

[539] M Maria Glymour, Eric J Tchetgen Tchetgen, and James M. Robins. Credible mendelian randomization studies: approaches for evaluating the instrumental variable assumptions. *Am J Epidemiol*, 175(4):332–339, Feb 2012.

[540] Brendan J. Keating, Sam Tischfield, Sarah S. Murray, Tushar Bhangale, Thomas S. Price, Joseph T. Glessner, Luana Galver, Jeffrey C. Barrett, Struan F A. Grant, Deborah N. Farlow, Hareesh R. Chandrupatla, Mark Hansen, Saad Ajmal, George J. Papanicolaou, Yiran Guo, Mingyao

Li, Stephanie Derohannessian, Paul I W. de Bakker, Swneke D. Bailey, Alexandre Montpetit, Andrew C. Edmondson, Kent Taylor, Xiaowu Gai, Susanna S. Wang, Myriam Fornage, Tamim Shaikh, Leif Groop, Michael Boehnke, Alistair S. Hall, Andrew T. Hattersley, Edward Frackelton, Nick Patterson, Charleston W K. Chiang, Cecelia E. Kim, Richard R. Fabsitz, Willem Ouwehand, Alkes L. Price, Patricia Munroe, Mark Caulfield, Thomas Drake, Eric Boerwinkle, David Reich, A Stephen Whitehead, Thomas P. Cappola, Nilesh J. Samani, A Jake Lusis, Eric Schadt, James G. Wilson, Wolfgang Koenig, Mark I. McCarthy, Sekar Kathiresan, Stacey B. Gabriel, Hakon Hakonarson, Sonia S. Anand, Muredach Reilly, James C. Engert, Deborah A. Nickerson, Daniel J. Rader, Joel N. Hirschhorn, and Garret A. Fitzgerald. Concept, design and implementation of a cardiovascular gene-centric 50 k snp array for large-scale genomic association studies. *PLoS One*, 3(10):e3583, 2008.

[541] Duncan C. Thomas, Debbie A. Lawlor, and John R. Thompson. Re: Estimation of bias in nongenetic observational studies using "mendelian triangulation" by bautista et al. *Ann Epidemiol*, 17(7):511–513, Jul 2007.

[542] E. B. Rimm, E. L. Giovannucci, W. C. Willett, G. A. Colditz, A. Ascherio, B. Rosner, and M. J. Stampfer. Prospective study of alcohol consumption and risk of coronary disease in men. 338(8765):464–8–, 1991.

[543] Paul Poirier, Thomas D. Giles, George A. Bray, Yuling Hong, Judith S. Stern, F Xavier Pi-Sunyer, Robert H. Eckel, American Heart Association , Physical Activity , Obesity Committee of the Council on Nutrition, and Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 american heart association scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. *Circulation*, 113(6):898–918, Feb 2006.

[544] Fiona Turnbull and Blood Pressure Lowering Treatment Trialists' Collaboration . Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet*, 362(9395):1527–1535, Nov 2003.

[545] C. L. Han, C. S. Liao, C. W. Wu, C. L. Hwong, A. R. Lee, and S. J. Yin. Contribution to first-pass metabolism of ethanol and inhibition by ethanol for retinol oxidation in human alcohol dehydrogenase family–implications for etiology of fetal alcohol syndrome and alcohol-related diseases. *Eur J Biochem*, 254(1):25–31, May 1998.

[546] Paul E. Ronksley, Susan E. Brien, Barbara J. Turner, Kenneth J. Mukamal, and William A. Ghali. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ*, 342:d671, 2011.

[547] Norihiro Kato, Fumihiko Takeuchi, Yasuharu Tabara, Tanika N. Kelly, Min Jin Go, Xueling Sim, Wan Ting Tay, Chien-Hsiun Chen, Yi Zhang, Ken Yamamoto, Tomohiro Katsuya, Mitsuhiro Yokota, Young Jin Kim, Rick Twee Hee Ong, Toru Nabika, Dongfeng Gu, Li-Ching Chang, Yoshihiro Kokubo, Wei Huang, Keizo Ohnaka, Yukio Yamori, Eitaro Nakashima, Cashell E. Jaquish, Jong-Young Lee, Mark Seielstad, Masato Isono, James E. Hixson, Yuan-Tsong Chen, Tetsuro Miki, Xueya Zhou, Takao Sugiyama, Jae-Pil Jeon, Jian Jun Liu, Ryoichi Takayanagi, Sung Soo Kim, Tin Aung, Yun Ju Sung, Xuegong Zhang, Tien Yin Wong, Bok-Ghee Han, Shotai Kobayashi, Toshio Ogihara, Dingliang Zhu, Naoharu Iwai, Jer-Yuarn Wu, Yik Ying Teo, E Shyong Tai, Yoon Shin Cho, and Jiang He. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east asians. *Nat Genet*, 43(6):531–538, Jun 2011.

[548] dbsnp short genetic variations - reference snp(refsnp) cluster report: rs671. available at: $http://www.ncbi.nlm.nih.gov/projects/snp/snp_ref.cgi?searchtype = adhoc_search\&type = rs\&rs = rs671$. *NCBI*.

[549] Shuen-Iu Hung, Wen-Hung Chung, Shiou-Hwa Jee, Wen-Chieh Chen, Yun-Ting Chang, Woan-Ruoh Lee, Shu-Ling Hu, Meng-Tse Wu, Gwo-Shing Chen, Tak-Wah Wong, Pa-Fan Hsiao, Wei-Hsuan Chen, Han-Yu Shih, Wu-Hsiang Fang, Chun-Yu Wei, Yi-Hui Lou, Yau-Li Huang, Juei-Jueng Lin, and Yuan-Tsong Chen. Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet Genomics*, 16(4):297–306, Apr 2006.

[550] Carbamazepine, oxcarbazepine and eslicarbazepine: potential risk of serious skin reactions associated with the hla-a* 3101 allele. available at: http://www.mhra.gov.uk/safetyinformation/drugsafetyupdate/con214944. *MHRA*, 2012.

[551] N. J. Wald, A. K. Hackshaw, and C. D. Frost. When can a risk factor be used as a worthwhile screening test? *BMJ*, 319(7224):1562–1565, Dec 1999.

[552] John Arrowsmith. A decade of change. *Nat Rev Drug Discov*, 11:17, 2012.

[553] Ismail Kola and John Landis. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*, 3:711–716, 2004.

[554] Ron Leuty. Anthera lays off 45http://www.bizjournals.com/sanfrancisco/blog/biotech/2012/03/anthera-layoff-lupus.html. *San Fransisco Bussiness Times*, 2012.

[555] Catherine M. Phillips. Metabolically healthy obesity: Definitions, determinants and clinical implications. *Rev Endocr Metab Disord*, Aug 2013.

[556] Catherine M. Phillips and Ivan J. Perry. Does inflammation determine metabolic health status in obese and nonobese adults? *J Clin Endocrinol Metab*, Aug 2013.

[557] R. Silverwood, M. V. Holmes, C. Dale, D. A. Lawlor, J. Whittaker, G. Davey Smith, D. Leon, T.M. Palmer, B. J. Keating, J.P. Casas, and Dudbridge F. Testing for non-linear causal effects using a binary genotype in a mendelian randomisation study: application to alcohol and cardiovascular traits. *In Preparation*, 2013.

[558] P. M. Rautaharju, H. P. Calhoun, and B. R. Chaitman. Novacode serial ecg classification system for clinical trials and epidemiologic studies. *J Electrocardiol*, 24 Suppl:179–187, 1992.

[559] N. J. Samani, P. Burton, M. Mangino, S. G. Ball, A. J. Balmforth, J. Barrett, T. Bishop, and A. Hall. A genomewide linkage study of 1,933 families affected by premature coronary artery disease: The british heart foundation (bhf) family heart study. *Am J Hum Genet*, 77(6):1011–20, 2005.

[560] A. G. Shaper, S. J. Pocock, M. Walker, N. M. Cohen, C. J. Wale, and A. G. Thomson. British regional heart study: cardiovascular risk factors in middle-aged men in 24 towns. *Br Med J (Clin Res Ed)*, 283(6285):179–86, 1981.

[561] D. A. Lawlor, C. Bedford, M. Taylor, and S. Ebrahim. Geographical variation in cardiovascular disease, risk factors, and their control in older women: British women's heart and health study. *J Epidemiol Community Health*, 57(2):134–40, 2003.

[562] B. G. Nordestgaard, B. Agerholm-Larsen, H. H. Wittrup, and A. Tybjaerg-Hansen. A prospective cardiovascular population study used in genetic epidemiology. the copenhagen city heart study. *Scand J Clin Lab Invest Suppl*, 226:65–71, 1996.

[563] A. Panayiotou, A. Nicolaides, M. Griffin, T. Tyllis, N. Georgiou, R. M. Martin, D. Bond, C. Tziakouri-Shiakalli, C. Fessas, and C. Deltas. Serum total homocysteine, folate, 5,10-methylenetetrahydrofolate reductase (mthfr) 677c–¿t genotype and subclinical atherosclerosis. *Expert Opin Ther Targets*, 13(1):1–11, 2009.

[564] F. G. Fowkes, E. Housley, E. H. Cawood, C. C. Macintyre, C. V. Ruckley, and R. J. Prescott. Edinburgh artery study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol*, 20(2):384–92, 1991.

[565] J. W. Beulens, E. M. Monninkhof, W. M. Verschuren, Y. T. van der Schouw, J. Smit, M. C. Ocke, E. H. Jansen, S. van Dieren, D. E. Grobbee, P. H. Peeters, and H. B. Bueno-de Mesquita. Cohort profile: the epic-nl study. *Int J Epidemiol*, 39(5):1170–8, 2010.

[566] M. D. Tobin, S. M. Raleigh, S. Newhouse, P. Braund, C. Bodycote, J. Ogleby, D. Cross, J. Gracey, S. Hayes, T. Smith, C. Ridge, M. Caulfield, N. A. Sheehan, P. B. Munroe, P. R. Burton, and N. J. Samani. Association of wnk1 gene polymorphisms and haplotypes with ambulatory blood pressure in the general population. *Circulation*, 112(22):3423–9, 2005.

[567] D. Baldassarre, K. Nyyssonen, R. Rauramaa, U. de Faire, A. Hamsten, A. J. Smit, E. Mannarino, S. E. Humphries, P. Giral, E. Grossi, F. Veglia, R. Paoletti, and E. Tremoli. Cross-sectional analysis of baseline data to identify the major determinants of carotid intima-media thickness in a european population: the improve study. *Eur Heart J*, 31(5):614–22, 2010. Baldassarre, Damiano Nyyssonen, Kristiina Rauramaa, Rainer de Faire, Ulf Hamsten, Anders Smit, Andries J Mannarino, Elmo Humphries, Steve E Giral, Philippe Grossi, Enzo Veglia, Fabrizio Paoletti, Rodolfo Tremoli, Elena IMPROVE study group RG2008/014/British Heart Foundation/United Kingdom Multicenter Study Research Support, Non-U.S. Gov't England European heart journal Eur Heart J. 2010 Mar;31(5):614-22. Epub 2009 Dec 1.

[568] G. J. Miller, K. A. Bauer, S. Barzegar, J. A. Cooper, and R. D. Rosenberg. Increased activation of the haemostatic system in men at high risk of fatal coronary heart disease. *Thromb Haemost*, 75(5):767–71, 1996.

[569] G. F. Diercks, W. M. Janssen, A. J. van Boven, A. A. Bak, P. E. de Jong, H. J. Crijns, and W. H. van Gilst. Rationale, design, and baseline characteristics of a trial of prevention of cardiovascular and renal disease with fosinopril and pravastatin in nonhypertensive, nonhypercholesterolemic subjects with microalbuminuria (the prevention of renal and vascular endstage disease intervention trial [prevend it]). *Am J Cardiol*, 86(6):635–8, 2000.

[570] P. R. O. C. A. R. D. I. S Consortium . A trio family study showing association of the lymphotoxin-alpha n26 (804a) allele with coronary artery disease. *Eur J Hum Genet*, 12(9):770–774, Sep 2004.

[571] J. Shepherd, G. J. Blauw, M. B. Murphy, E. L. Bollen, B. M. Buckley, S. M. Cobbe, I. Ford, A. Gaw, M. Hyland, J. W. Jukema, A. M. Kamper, P. W. Macfarlane, A. E. Meinders, J. Norrie, C. J. Packard, I. J. Perry, D. J. Stott, B. J. Sweeney, C. Twomey, and R. G. Westendorp. Pravastatin in elderly individuals at risk of vascular disease (prosper): a randomised controlled trial. *Lancet*, 360(9346):1623–30, 2002. Shepherd, James Blauw, Gerard J Murphy, Michael B Bollen, Edward L E M Buckley, Brendan M Cobbe, Stuart M Ford, Ian Gaw, Allan Hyland,

Michael Jukema, J Wouter Kamper, Adriaan M Macfarlane, Peter W Meinders, A Edo Norrie, John Packard, Chris J Perry, Ivan J Stott, David J Sweeney, Brian J Twomey, Cillian Westendorp, Rudi G J PROSPER study group. PROspective Study of Pravastatin in the Elderly at Risk Clinical Trial Randomized Controlled Trial Research Support, Non-U.S. Gov't England Lancet Lancet. 2002 Nov 23;360(9346):1623-30.

[572] A. Hofman, M. M. Breteler, C. M. van Duijn, G. P. Krestin, H. A. Pols, B. H. Stricker, H. Tiemeier, A. G. Uitterlinden, J. R. Vingerling, and J. C. Witteman. The rotterdam study: objectives and design update. *Eur J Epidemiol*, 22(11):819–29, 2007.

[573] T. W. Meade, H. C. Wilkes, Y. Stirling, P. J. Brennan, C. Kelleher, and W. Browne. Randomized controlled trial of low dose warfarin in the primary prevention of ischaemic heart disease in men at high risk: design and pilot study. *Eur Heart J*, 9(8):836–43, 1988.

[574] D. B. van Wieren-de Wijer, A. H. Maitland-van der Zee, A. de Boer, B. H. Stricker, A. A. Kroon, P. W. de Leeuw, O. Bozkurt, and O. H. Klungel. Recruitment of participants through community pharmacies for a pharmacogenetic study of antihypertensive drug treatment. *Pharm World Sci*, 31(2):158–64, 2009.

[575] S. Yusuf, F. Zhao, S. R. Mehta, S. Chrolavicius, G. Tognoni, K. K. Fox, and Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators . Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without st-segment elevation. *N Engl J Med*, 345(7):494–502, Aug 2001.

[576] J. Pitha, J. A. Hubek, R. Poledne, V. Stank, M. Aschermann, M. Gebauerov, P. Hjek, P. Niederle, M. Pnika, and J. Veselka. Genetic determination of the prognosis in survivors of acute coronary syndromes. study design and rationale for a multicenter study. *Cor et Vasa*, 49(4):134–137, 2007. Cited By (since 1996): 5 Export Date: 23 February 2012 Source: Scopus.

[577] J. S. Zebrack, J. L. Anderson, C. A. Maycock, B. D. Horne, T. L. Bair, and J. B. Muhlestein. Usefulness of high-sensitivity c-reactive protein in predicting long-term risk of death or acute myocardial infarction in patients with unstable or stable angina pectoris or acute myocardial infarction. *Am J Cardiol*, 89(2):145–9, 2002. Zebrack, James S Anderson, Jeffrey L Maycock, Chloe Allen Horne, Benjamin D Bair, Tami L Muhlestein, Joseph Brent Intermountain Heart Collaborative (IHC) Study Group 5T32HL07576/HL/NHLBI NIH HHS/United States Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. United States The American journal of cardiology Am J Cardiol. 2002 Jan 15;89(2):145-9.

[578] D. A. Morrow, B. M. Scirica, E. Karwatowska-Prokopczuk, S. A. Murphy, A. Budaj, S. Varshavsky, A. A. Wolff, A. Skene, C. H. McCabe, and E. Braunwald. Effects of ranolazine on recurrent cardiovascular events in patients with non-st-elevation acute coronary syndromes: the merlin-timi 36 randomized trial. *JAMA*, 297(16):1775–83, 2007. Morrow, David A Scirica, Benjamin M Karwatowska-Prokopczuk, Ewa Murphy, Sabina A Budaj, Andrzej Varshavsky, Sergei Wolff, Andrew A Skene, Allan McCabe, Carolyn H Braunwald, Eugene MERLIN-TIMI 36 Trial Investigators Multicenter Study Randomized Controlled Trial United States JAMA : the journal of the American Medical Association JAMA. 2007 Apr 25;297(16):1775-83.

[579] C. P. Cannon, E. Braunwald, C. H. McCabe, D. J. Rader, J. L. Rouleau, R. Belder, S. V. Joyal, K. A. Hill, M. A. Pfeffer, and A. M. Skene. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med*, 350(15):1495–504, 2004.