Blood pressure management in type 2 diabetes: integrating clinical trials and genetic data

Milad Nazarzadeh Larzjan

Linacre College, University of Oxford

Michaelmas Term, 2022

Supervisor: Prof Kazem Rahimi

Co-supervisors: Dr Dexter Canoy, Dr Abbas Dehghan, Dr Derrick Bennett

A thesis submitted for the degree of Doctor of Philosophy

Contents

Abstract

Background: Blood pressure lowering is an established strategy for preventing microvascular and macrovascular complications of diabetes, but its role in the prevention of diabetes itself is unclear. On the other hand, controversy exists as to whether the threshold of blood pressure for initiation of antihypertensive therapy should differ between people with and without type 2 diabetes. I aimed to integrate individual participant data from major randomised controlled trials and genetic data to fill these knowledge gaps.

Objectives: This thesis sought to examine three main objectives: to investigate the effect of pharmacological blood pressure-lowering on the risk of new-onset type 2 diabetes; to investigate the separate effects of blood pressure-lowering drug classes on the risk of new-onset type 2 diabetes; to investigate the effect of pharmacological blood pressure-lowering treatment for the prevention of major cardiovascular disease in persons with and without type 2 diabetes.

Methods: For the first and second objectives, I conducted a one-stage individual participant-level data meta-analysis of major randomised controlled trials using data from the Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTTC). Analyses were complemented with Mendelian randomisation studies using naturally randomised genetic variants associated with systolic blood pressure and genetic variants in the gene that encodes the therapeutic targets of each drug class. For the third objective, I used one-stage individual participant-level data meta-analysis using the BPLTTC dataset. I expressed the treatment effect per 5 mmHg reduction in systolic blood pressure on the risk of developing a major cardiovascular event as the

primary outcome, defined as the first occurrence of fatal or non-fatal stroke or cerebrovascular disease, fatal or non-fatal ischaemic heart disease, or heart failure causing death or requiring hospitalisation. Cox proportional hazard models, stratified by trial, were used to estimate hazard ratios (HRs) separately by type 2 diabetes status at baseline, with further stratification by baseline categories of systolic blood pressure (in 10 mmHg increments from <120 mmHg to ≥170 mmHg).

Results: For the first and second objectives, blood pressure-lowering treatment was found to reduce the risk of diabetes by 11% (hazard ratio per 5 mmHg lower systolic blood pressure 0.89 [95% confidence interval [CI] 0.84 to 0.95]). Similarly, in the Mendelian randomisation study, each 5 mmHg genetically influenced lower systolic blood pressure was associated with an 11% lower risk of diabetes (odds ratio 0.89 [95% CI 0.86 to 0.93]). Evidence from genetic data and trials was also consistent in that angiotensin-converting enzyme inhibitors and angiotensin receptor blockers reduced the risk of diabetes, and beta-blockers increased this risk. There was no effect for calcium channel blockers and findings for thiazide diuretics were inconsistent. For the third objective, over 4.2 years median follow-up (IQR 3.0 to 5.0), a 5 mmHg reduction in systolic blood pressure decreased the risk of major cardiovascular events in both groups, but with a weaker relative treatment effect in participants with type 2 diabetes (HR 0.94 [95% CI 0.91 to 0.98]) compared with those without type 2 diabetes (0.89 [0.87 to 0.92]; p for interaction=0.001). However, absolute risk reductions did not differ substantially between people with and without type 2 diabetes (absolute risk reduction -1.54 [95% CI -2.04 to -1.04] in people with diabetes and -1.61 [-1.86 to -1.36] in people without diabetes, p for interaction =1). We found no reliable evidence for heterogeneity of treatment effects by baseline systolic blood pressure in either group.

Conclusions: Blood pressure lowering is an effective strategy for the prevention of new-onset type 2 diabetes. Established pharmacological interventions, however, have qualitatively and quantitively different effects on diabetes, likely due to their differing off-target effects, with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers having the most favourable outcomes. Additionally, although the relative beneficial effects of blood pressure reduction on major cardiovascular events were weaker in participants with type 2 diabetes than in those without, absolute effects were similar. The difference in relative risk reduction was not related to the baseline blood pressure or allocation to different drug classes. Therefore, the adoption of differential blood pressure thresholds, intensities of blood pressure lowering, or drug classes used in people with and without type 2 diabetes is not warranted.

Acknowledgements

Above all, I would like to express my deepest gratitude to Professor Kazem Rahimi, my main supervisor, for all of his support, inspiration, and excellent supervision during this project. I am extremely appreciative that you accepted me as a student and continued to have faith in me over the years, as I have benefited tremendously from your wealth of knowledge.

I would also like to thank my co-supervisors, Dr Dexter Canoy, Dr Abbas Dehghan and Dr Derrick Bennett, who supported me and offered deep insight into the study.

The acknowledgements section would not be complete without expressing my heartfelt gratitude to Ms Naseem Akhtar, DeepMedicine group administrator, whose unwavering support has been invaluable throughout these demanding academic years.

Parts of the thesis have previously been published, as indicated in each chapter. In all of the publications, I was the first author, and all materials were produced by myself during the DPhil programme. This thesis is supported by a PhD scholarship from the British Heart Foundation (grant number FS/19/36/34346).

Dedication

To my wife, Zeinab, I aspire to make my future research a meaningful tribute to the sacrifices you have made.

Publications

Publications contributing to DPhil thesis

Nazarzadeh M, Bidel Z, Canoy D, Copland E, Wamil M, Majert J, Smith Byrne K, Sundström J, Teo K, Davis BR, Chalmers J, Pepine CJ, Dehghan A, Bennett DA, Smith GD, Rahimi K; Blood Pressure Lowering Treatment Trialists' Collaboration. Blood pressure lowering and risk of new-onset type 2 diabetes: an individual participant data meta-analysis. Lancet. 2021 Nov 13;398(10313):1803-1810.

Nazarzadeh M, Bidel Z, Canoy D, Copland E, Bennett DA, Dehghan A, Davey Smith G, Holman RR, Woodward M, Gupta A, Adler AI, Wamil M, Sattar N, Cushman WC, McManus RJ, Teo K, Davis BR, Chalmers J, Pepine CJ, Rahimi K; Blood Pressure Lowering Treatment Trialists' Collaboration. Blood pressure-lowering treatment for prevention of major cardiovascular diseases in people with and without type 2 diabetes: an individual participant-level data meta-analysis. Lancet Diabetes Endocrinol. 2022 Jul 22:S2213-8587(22)00172-3.

Other relevant papers published during my DPhil

Rahimi K, Bidel Z, **Nazarzadeh M**, Copland E, Canoy D, Wamil M, et al. Age-stratified and blood-pressure-stratified effects of blood-pressure-lowering pharmacotherapy for the prevention of cardiovascular disease and death: an individual participant-level data meta-analysis. Lancet. 2021 Sep 18;398(10305):1053-1064.

Rahimi K, Bidel Z, **Nazarzadeh M**, Copland E, Canoy D, Ramakrishnan R, et al. Pharmacological blood pressure lowering for primary and secondary prevention of cardiovascular disease across different levels of blood pressure: an individual participant-level data meta-analysis. Lancet. 2021 May 1;397(10285):1625-1636.

Canoy D, **Nazarzadeh M**, Copland E, Bidel Z, Rao S, Li Y, Rahimi K. How Much Lowering of Blood Pressure Is Required to Prevent Cardiovascular Disease in Patients With and Without Previous Cardiovascular Disease? Curr Cardiol Rep. 2022 Jul;24(7):851-860.

Nazarzadeh M, Canoy D, Bidel Z, Copland E, Rahimi K, Teo K, Davis BR, Chalmers J, Pepine CJ, Woodward M. The Blood Pressure Lowering Treatment Trialists' Collaboration: methodological clarifications of recent reports. J Hypertens. 2022 May 1;40(5):847-852.

Canoy D, Copland E, **Nazarzadeh M**, Ramakrishnan R, Pinho-Gomes AC, Salam A, Dwyer JP, Farzadfar F, Sundström J, Woodward M, Davis BR, Rahimi K; Blood Pressure Lowering Treatment Trialists' Collaboration; Blood Pressure Lowering Treatment Trialists Collaboration. Antihypertensive drug effects on long-term blood pressure: an individual-level data meta-analysis of randomised clinical trials. Heart. 2022 Jul 27;108(16):1281-1289.

Canoy D, Harvey NC, Prieto-Alhambra D, Cooper C, Meyer HE, Åsvold BO, **Nazarzadeh M**, Rahimi K. Elevated blood pressure, antihypertensive medications and bone health in the population: revisiting old hypotheses and exploring future research directions. Osteoporos Int. 2022 Feb;33(2):315-326.

Tran J, Norton R, Canoy D, Ayala Solares JR, Conrad N, **Nazarzadeh M**, Raimondi F, Salimi-Khorshidi G, Rodgers A, Rahimi K. Multi-morbidity and blood pressure trajectories in hypertensive patients: A multiple landmark cohort study. PLoS Med. 2021 Jun 17;18(6):e1003674.

Pinho-Gomes AC, Azevedo L, Copland E, Canoy D, **Nazarzadeh M**, Ramakrishnan R, Berge E, Sundström J, Kotecha D, Woodward M, Teo K, Davis BR, Chalmers J, Pepine CJ, Rahimi K; Blood Pressure Lowering Treatment Trialists' Collaboration. Blood pressure-lowering treatment for the prevention of cardiovascular events in patients with atrial fibrillation: An individual participant data meta-analysis. PLoS Med. 2021 Jun 1;18(6):e1003599.

Copland E, Canoy D, **Nazarzadeh M**, Bidel Z, Ramakrishnan R, Woodward M, Chalmers J, Teo KK, Pepine CJ, Davis BR, Kjeldsen S, Sundström J, Rahimi K; Blood Pressure Lowering Treatment Trialists' Collaboration. Antihypertensive treatment and risk of cancer: an individual participant data meta-analysis. Lancet Oncol. 2021 Apr;22(4):558-570.

Nazarzadeh M, Pinho-Gomes AC, Bidel Z, Canoy D, Dehghan A, Smith Byrne K, Bennett DA, Smith GD, Rahimi K. Genetic susceptibility, elevated blood pressure, and risk of atrial fibrillation: a Mendelian randomization study. Genome Med. 2021 Mar 4;13(1):38.

Rahimi K, **Nazarzadeh M**, Pinho-Gomes AC, Woodward M, Salimi-Khorshidi G, Ohkuma T, Fitzpatrick R, Tarassenko L, Denis M, Cleland J; SUPPORT-HF2 Study Group. Home monitoring with technology-supported management in chronic heart failure: a randomised trial. Heart. 2020 Oct;106(20):1573-1578.

Nazarzadeh M, Pinho-Gomes AC, Bidel Z, Dehghan A, Canoy D, Hassaine A, Ayala Solares JR, Salimi-Khorshidi G, Smith GD, Otto CM, Rahimi K. Plasma lipids and risk of aortic valve stenosis: a Mendelian randomization study. Eur Heart J. 2020 Oct 21;41(40):3913-3920.

Nazarzadeh M, Rahimi K. Intensive Blood Pressure Lowering and Risk of Diabetes: Friend or Foe? Hypertension. 2020 Feb;75(2):293-294.

Nazarzadeh M, Pinho-Gomes AC, Smith Byrne K, Canoy D, Raimondi F, Ayala Solares JR, Otto CM, Rahimi K. Systolic Blood Pressure and Risk of Valvular Heart Disease: A Mendelian Randomization Study. JAMA Cardiol. 2019 Aug 1;4(8):788-795.

Rahimi K, Canoy D, **Nazarzadeh M**, Salimi-Khorshidi G, Woodward M, Teo K, Davis BR, Chalmers J, Pepine CJ; Blood Pressure Lowering Treatment Trialists' Collaboration. Investigating the stratified efficacy and safety of pharmacological blood pressure-lowering: an overall protocol for individual patient-level data meta-analyses of over 300 000 randomised participants in the new phase of the Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTTC). BMJ Open. 2019 May 22;9(5):e028698.

List of Tables

List of Figures

List of abbreviations

CI: Confidence interval

RCT: Randomised controlled trials

CCG: Clinical Commissioning Groups

IDF: International Diabetes Federation

NICE: The National Institute for Health and Care Excellence

BPLTTC: The Blood Pressure Lowering Treatment Trialists' Collaboration

IPD: Individual participant data

HR: Hazard ratio

GWAS: Genome-wide association studies

SD: Standard deviation

ACEIs: Angiotensin-converting enzyme inhibitors

ARBs: Angiotensin receptor blockers

RR: Relative risk

ICBP: International Consortium for Blood Pressure

MR-PRESSO: Mendelian Randomization Pleiotropy RESidual Sum and Outlier

RAPS: Robust Adjusted Profile Score

DIAGRAM: DIAbetes Genetics Replication And Meta-analysis Consortium

IVW: Inverse-variance weighted

SNPs: Single-nucleotide polymorphisms

Glossary of terms

Collider bias: Collider bias occurs when a shared third variable is influenced by both an exposure and an outcome, and this variable is subsequently controlled for either through study design or analytical methods.

Deep learning: A branch of machine learning that involves the use of neural networks consisting of three or more layers. Neural networks endeavour to mimic the structure and operation of the human brain, albeit falling short of its capacity, thereby enabling it to acquire knowledge from vast quantities of data. Although a neural network comprising only one layer can provide approximate predictions, incorporating additional hidden layers can enhance optimisation and precision for accuracy.

Ecological bias: Also known as the "ecological fallacy," it occurs when inferences about the character of individuals are drawn from the findings of the group to which those individuals belong. For example, the conclusion can be made that there exists a positive correlation between the incidence of breast cancer and the consumption of fat in countries with higher levels of fat intake. However, there is no indication that breast cancer is more likely to occur in women who consume a diet that is rich in fat.

Electronic health record: A longitudinal electronic documentation of a patient's medical background that is upheld by the healthcare provider. The patient's medical record encompasses essential administrative and clinical information that is relevant to their treatment by a specific healthcare provider. This may comprise demographic

details, progress notes, medical concerns, prescribed medications, vital signs, past medical history, immunisation records, laboratory findings, and radiology reports.

Epidemiology: Epidemiology is a discipline within the field of medicine that focuses on the study of the occurrence, distribution, and potential management of diseases and other health-related factors.

Genetic pleiotropy: A genetic phenomenon in which a DNA variant affects multiple traits. In Mendelian randomisation, a genetic variant is said to exhibit horizontal pleiotropy when it has an influence on the outcome variable in addition to the effect that it has on the exposure variable. Vertical pleiotropy is the effect of a variant on other traits through the exposure of interest. The presence of horizontal pleiotropy poses a challenge to Mendelian randomisation studies as it violates the instrumental variable assumptions. This is due to the fact that the genetic variant's effect on the outcome is not solely through the risk factor, thereby compromising the validity of the study. In practical terms, vertical pleiotropy, which demonstrates how one factor affects a subsequent outcome, is the core of Mendelian randomisation and is generally not problematic for study assumptions.

Heterogeneity of treatment effect: The concept of heterogeneity of treatment effects pertains to the non-random and explicable differences in the magnitude and direction of treatment effects that are observed among individuals within a given population. The main aim of the analysis is to estimate the effects of treatment within clinically relevant subgroups and predict the likelihood of treatment efficacy for an

individual. The most commonly employed approach for examining the heterogeneity of treatment effects is subgroup analysis.

Head-to-head trial: Trials that have been planned and conducted in order to make it possible to make formal comparisons between two active treatments. These trials are demanded by some health authorities to evaluate the positioning of newly developed therapies and assist clinicians in selecting the treatment alternatives that are most suitable for individual patients. In head-to-head trials, as opposed to placebo-controlled trials that compare therapy to placebo, two treatments are tested against each other.

Meta-analysis: Meta-analysis is an epidemiological study design used to systematically evaluate prior research evidence, combine it, and derive conclusions based on the available data. The results of a meta-analysis may provide a more precise estimate of the effect than any of the individual studies that contributed to the analysis. When commencing their quest for the best evidence to guide decision-making, clinicians are typically advised to consult the top layer of the evidence pyramid to ascertain whether a systematic review and meta-analysis have been executed.

Statistical power: The concept of statistical power pertains to the likelihood of accurately rejecting a null hypothesis in instances where it is false. In simpler terms, it denotes the capacity of statistical analysis to identify a genuine effect or association. In contrast, power calculation is a methodology employed to ascertain

the needed sample size for attaining a predetermined level of statistical power in a given study design and research question.

Chapter 1. Introduction

1.1. Epidemiology of elevated blood pressure

Elevated blood pressure is one of the most important and independent risk factors for cardiovascular disease and mortality,[1] which contributes to many cardiometabolic disruptions in the human body.[2] It is estimated that 1.38 billion people all across the globe have been diagnosed with hypertension as of the year 2010, making up 31.1% of the world's adult population.[3] Since 2000, national studies have revealed that the prevalence of hypertension is rising in low- and middle-income countries, while it is either remaining the same as in previous years or dropping in high-income nations.[4]

In 2015, a pooled analysis of 1,479 population-based studies with 19.1 million participants estimated that the worldwide mean age-standardised systolic blood pressure was 127.0 mmHg for men and 122.3 mmHg for women, while the corresponding global mean age-standardised diastolic blood pressure was 78.7 mmHg and 76.7 mmHg, respectively.[5] South Asia, Sub-Saharan Africa, and Central and Eastern Europe had higher mean systolic and diastolic blood pressures in both sexes than in high-income Western countries and high-income Asian countries.[5] Risk factors of elevated blood pressure, such as high body mass index, unhealthy diet, alcohol use, and sedentary lifestyle, differ by region, and this is largely due to social and environmental parameters including the availability of blood pressure lowering drugs and access to primary and secondary healthcare. These reasons may account for the observed geographical disparities.[6]

It has been estimated that in 2010, the worldwide age-standardised prevalence of hypertension, defined as systolic blood pressure 140 mmHg, diastolic blood pressure 90 mmHg, and/or current use of antihypertensive medication, was 31.1% (95% confidence interval [CI] 30.0 to 32.2%). This estimate was based on data from 135 population-based studies that included 968,419 individuals from 90 different countries.[3] Men had a slightly greater prevalence of hypertension (31.9%) compared to women (30.1%) while taking into account age.[3] Prevalence of hypertension in men was lowest in South Asia (26.4%), and highest in Eastern Europe and Central Asia (39.0%). The prevalence of hypertension amongst women ranged from 25.3% in high-income nations to 36.3% in Sub-Saharan Africa. Differences in the prevalence of risk factors for hypertension presumably play a role in explaining these regional variations in hypertension prevalence, but this is just a hypothesis.[3,6] From 2003 to 2009, 153,996 participants aged 35 to 70 were recruited from 628 rural and urban areas in 17 countries with varying economic and geographic profiles for the Prospective Urban Rural Epidemiology project. The inclusion of 142,042 individuals with baseline blood pressure data allows for a unique opportunity to examine the prevalence of hypertension in rural and urban populations across global areas. Hypertension was found to be more common in men (41%) than in women (37%), with a 95% CI of 40.8% (40.5 to 41.0%) for the whole study population.[7] In addition, the research found that the prevalence of hypertension was greater in rural than in urban regions of high- and middle-income nations, whereas the reverse was true in low-income countries.[7]

Hypertension in adults was re-defined in 2017 by the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines

as having a systolic blood pressure that is equal to or higher than 130 mmHg and/or diastolic blood pressure that is equal to or higher than 80 mmHg.[8] This shift was motivated by data from randomised controlled trials (RCTs), such as the SPRINT trial, which showed that intensive blood pressure lowering (target systolic blood pressure 120 mmHg) reduces cardiovascular disease and all-cause mortality more than standard blood pressure lowering strategy (target systolic blood pressure 140 mmHg).[9] The prevalence of hypertension in the general United States population rose from 32.0% (using the old criteria) to 45.4% when the new definition was implemented.[6] The rise was much more dramatic among the general Chinese population, going from 23.2% to 46.4%.[10] These results imply that the gap in hypertension prevalence between low-middle income nations and high-income countries would be significantly larger than previously reported if the revised criteria were adopted globally.

Opportunistic screening or the NHS Health Check in primary care are the most common methods of detecting hypertension in the United Kingdom.[11] It was found in a cross-sectional study of patients admitted to adult wards at four acute hospitals in Oxford, United Kingdom, between 2014 and 2018 that the proportion of patients meeting the diagnostic criteria for hypertension was 21.4% when the European guidelines were applied, and 47% when the American guidelines were applied. Patients who met diagnostic criteria for hypertension increased with age, but the number of patients who were undiagnosed with hypertension decreased.[12] According to the Health Survey for England 2017, the prevalence of hypertension is estimated to range from 16 to 33.8% at the Clinical Commissioning Groups (CCG) level.[13] The prevalence of hypertension in general practice varies from 18.8 to 31%, ignoring the

top and bottom 10% of practices.[13] CCGs in the East and South East of England had the greatest estimated hypertension prevalence, followed by the North of England, the coast, and regions with an older population. In 2017, it is estimated that 11.8 million individuals aged 16 and above in England had hypertension. This equates to around 26.2% of the adult population.[13]

1.2. Epidemiology of type 2 diabetes

Type 2 diabetes and its complications are major causes of death and disability across the globe. For instance, diabetes mellitus (in all its manifestations) was ranked as the ninth leading cause of shortened life expectancy in the world by the Global Burden of Disease Study 2013.[14] In 2010, diabetes mellitus was estimated to have caused 3.96 million deaths among individuals aged 20 to 79 (6.8% of worldwide mortality).[15] Since 1990, the number of people with diabetes mellitus who are disabled has risen dramatically, especially among those between the ages of 15 and 69.[16] According to the 2015 Global Burden of Diseases, Injuries, and Risk Factors Study, a high fasting glucose level was the tenth most prevalent global risk factor for disability-adjusted life years in 1990, the fourth most prevalent in 2005, and the third most prevalent in 2015.[17]

More than 113.9 million Chinese adults (11.6%) were diagnosed with diabetes mellitus in 2010; another 493.4 million Chinese adults (50.1%) were estimated to have prediabetes mellitus (defined based on the WHO criteria).[18] In 2011, a national survey in India indicated that 62 million people had diabetes and 77 million people had prediabetes.[19] In 2007, the cost of treating people hospitalised with diabetes mellitus but no complications ranged from 11% to 75% of per-capita income in India,

China, Thailand, and Malaysia. However, those admitted to hospitals who had complications had costs that were up to three times higher than those of patients who did not.[20] Adult diabetes mellitus reported prevalence in the Middle East range from 9.5% in Oman to 25.4% in Saudi Arabia, making the region another epicentre of the worldwide pandemic.[21,22] In Iran, the prevalence among individuals over the age of 40 was 24% and climbed by 0.4% every year after the age of 20.[23] As of 2015, the International Diabetes Federation (IDF) estimated a regional prevalence of 2.1 to 6.7% in sub-Saharan Africa, despite a lack of up-to-date regional statistics on the region.[20]

Half of all persons aged 65 and above in the United States had prediabetes mellitus in 2008, and in 2015, the nation was ranked third for having the largest number of patients with diabetes mellitus. North America and the Caribbean spent more on diabetes mellitus treatment than any other area, with a per capita cost that was 85 times higher than in Southeast Asia. Among the Pacific island countries, American Samoa has the highest rate of diabetes mellitus at over 30%, while other islands in Polynesia and Micronesia have a prevalence of 25% or higher.[20]

Diabetes is one of the most prevalent chronic diseases in the United Kingdom, and its incidence is rising.[24] An analysis of primary care cohort data from the General Practice Research Database involving 1.3 million patients in England and Wales revealed that the rate of diagnosis of new cases of diabetes increased by 26% during the study period, from 17.5 per 10,000 person-years in 1994 to 22.1 per 10,000 person-years in 1998.[25] The National Institute for Health and Care Excellence (NICE) predicts that more than 5 million individuals in the United Kingdom will be

diagnosed with diabetes by 2025.[24] National Diabetes Audit in England and Wales and the Scottish Diabetes Survey reported that one in 250 persons (0.4% of the population) in the United Kingdom have type 1 diabetes, one in 22 have type 2 diabetes (4.5%), and one in 1670 have another type of diabetes.[26]

1.3. The historical trend of blood pressure-lowering treatments over time

The history of blood pressure and its management is a story of scientific achievements, breakthroughs in medicine, and public health challenges. In this chapter, I will briefly discuss how our knowledge and treatment of high blood pressure have improved through decades, from scepticism and ambiguity to the present era of evidence-based medicine and technological innovations.

Blood pressure measurement is the initial step in diagnosing and treatment of hypertension. The first efforts for measuring direct blood pressure date back to the 18th century, when Stephen Hales placed a glass tube into a horse's artery and measured the height of blood rising in the tube.[27] This invasive and direct approach, however, was not appropriate for application in humans. Several non-invasive techniques were developed in the nineteenth century to indirectly monitor blood pressure by applying pressure to the arm or wrist with a cuff and listening to the sounds of blood flow using a stethoscope. Scipione Riva-Rocci devised the most extensively used technique in 1896, using a mercury manometer to measure systolic blood pressure (the greatest pressure when the heart contracts).[28] Nikolai Korotkoff developed diastolic blood pressure measurement (the lowest pressure when the heart relaxes) in 1905 by detecting the sounds of blood flow in the final stage.[29] The conventional sphygmomanometer is founded on the ideas of Riva-Rocci and

Korotkoff and comprises an inflated cuff, a manometer, and a stethoscope.[30] Automatic upper arm blood pressure monitoring devices have been developed by digital health companies over the last decade and are straightforward to use, which provides the opportunity for home-based monitoring and accurate measurement without any specialised training or experience.[31] Nowadays, innovation has pushed the measurement boundary to cuffless blood pressure sensors with new smart devices and software such as smartphones and smartwatches blood pressure monitoring technologies. However, these innovative cuffless technologies have not yet been completely validated for medical decision-making purposes, and further multidisciplinary research is required to verify these devices versus traditional cuff-based techniques.[32]

Hypertension was not immediately acknowledged as a significant health issue. The insurance industry was involved in the initial attempt to identify high blood pressure as a risk factor for human health and in 1906, a few companies started monitoring systolic blood pressure.[33] Until the middle of the nineteenth century, many medical professionals believed that elevated blood pressure is a natural response to ageing or stress and that moderating it could be detrimental. Several epidemiological studies, however, have altered this perception by disclosing the link between elevated blood pressure and an increased risk of cardiovascular disease. The Framingham Heart Study, which began in 1948 and followed more than 5,000 residents of Framingham, Massachusetts, for decades, was one of the most influential investigations.[34] In 1961, this study identified elevated blood pressure as one of the main risk factors for cardiovascular disease. It also demonstrated that hypertension can cause damage to different organs, such as eyes, kidneys, heart

and brain.[35] Another groundbreaking investigation, the Multiple Risk Factor Intervention Trial (MRFIT), recruited over 12,000 men with high blood pressure, high cholesterol, and smoking habits in 1972. This study showed that these three risk factors were independently associated with cause-specific cardiovascular mortality and that reducing them may cut cardiovascular risk.[36] Despite mounting data linking blood pressure to cardiovascular disease and death, there were sceptics in the medical community and media concerning the need to control high blood pressure.

Although there is no clear evidence of the early blood pressure-lowering treatments, it seems that food restrictions, bleeding, and purging were utilised in ancient Greece. These approaches were often futile and, in some cases, potentially dangerous. Potassium thiocyanate, a medication that has been around since the time of Claude Bernard in the eighteenth century, was first used to treat hypertension in the year 1940.[33] There is no evidence to suggest that this medication has any type of particular impact on the blood vessels, that it can lower the blood pressure of animals in a direct manner, or that it can influence the blood vessels of people who have hypertension.[37] Treatment of hypertension in the nineteenth century was primarily centred on a regimen of rest between activities, plenty of sleep at night, and avoidance of mental and physical stress.[38] The development of hypertension medications over the twentieth century had a significant influence on the trajectory of pharmacological blood pressure therapy.[39] The Veterans Administration Cooperative Studies, which were overseen by Edward Freis, were a significant milestone in clinical research. These studies provided initial evidence for the positive effects of antihypertensive agents in reducing blood pressure. The study conducted was a placebo-controlled trial which assessed the efficacy of active drug treatment in

patients with diastolic blood pressures ranging from 115 to 129 mm Hg. The results indicated a significant reduction in the incidence of stroke, aortic dissection, and malignant hypertension within a span of 2 years.[40] In the 1980s, it became widely recognised that systolic pressure provided a more accurate indication of cardiovascular events in individuals aged 50 years and older, compared to diastolic pressure. Consequently, numerous extensive, placebo-controlled trials were undertaken to explore the effects of antihypertensive drug treatment on isolated systolic hypertension in older individuals. Notable examples of such trials include the Systolic Hypertension in the Elderly Programme and Systolic Hypertension in Europe.[33,41,42] Concomitant with the progression and augmented accessibility of contemporary antihypertensive agents during the 1970s and 1980s, RCTs were conducted to assess the plausibility that distinct categories of antihypertensive agents have different potential to reduce cardiovascular and renal diseases, regardless of their efficacy in decreasing blood pressure.[39] In the last three decades, randomised controlled trials have provided overwhelming evidence regarding the beneficial effects of various classes of antihypertensives for the purpose of blood pressure lowering and also for primary and secondary prevention of different types of cardiovascular and cardiometabolic diseases.

Notwithstanding the scientific progress made in recent decades and its successful application in clinical settings; scientists, clinicians, and policy-makers must avoid becoming arrogant with prior achievements. There exists a multitude of substantial research questions that have yet to be addressed, and conventional research methodologies have reached their limits in addressing them. For example, individual trials have had limited data to investigate stratified effects, and the meta-

analyses of published aggregate study results have not been able to quantify benefits or harms in important patient subgroups.[43]

1.4. Aims of the thesis

This thesis aims to investigate key research questions concerning blood pressure management in the context of diabetes by integrating individual participant data meta-analyses of trials and genetic data analysis and to subsequently answer a series of research questions that cannot be investigated using individual study data or a single source of evidence.

The present thesis aims to achieve the following objectives:

(1) To investigate the effect of pharmacological blood pressure-lowering on the risk of new-onset type 2 diabetes using data from randomised clinical trials.

(2) To investigate the separate effects of blood pressure-lowering drug classes on the risk of new-onset type 2 diabetes using randomised clinical trial data.

(3) To assess the potential causal association between blood pressure reduction and risk of type 2 diabetes using genetic data analysis.

(4) To assess the separate effect of blood pressure-lowering drug classes on the risk of type 2 diabetes using genetic data analysis.

(5) To investigate the effect of pharmacological blood pressure-lowering treatment for the prevention of major cardiovascular disease in persons with and without type 2 diabetes, using randomised clinical trials.

1.5. Rationale for this thesis and the methods used

This thesis aims to investigate the effect of blood pressure reduction on the risk of developing diabetes. Additionally, it seeks to evaluate the potential differential effect of different antihypertensive drug classes on the risk of diabetes. Finally, the thesis aims to determine the effect of blood pressure reduction on the risk of major cardiovascular disease in individuals with and without diabetes at the onset of treatment. The selection of these topics was based on the clinical and public health impact of both diseases, as well as the existing uncertainties surrounding the prevention and management of diabetes through blood pressure reduction after several decades of research in the field of medicine.

Despite being one of the most affordable and widely accessible classes of medications in the world, the efficacy of blood pressure-lowering medications in preventing diabetes is still debatable. According to the most recent clinical guidelines around the world, there is insufficient well-documented evidence to support the recommendation of pharmacological and non-pharmacological blood pressure lowering for the prevention of type 2 diabetes (discussed in detail in chapters 3-6). Moreover, there is a paucity of randomised evidence to substantiate the evidence-based selection of antihypertensive drug classes for the prevention of diabetes. Despite the clinical guidelines advocating for blood pressure monitoring in individuals with diabetes, there exists a significant gap in knowledge regarding the optimal timing for recommending blood pressure reduction, the threshold at which treatment should be initiated, and the appropriate intensity and classification of medication to be utilised by clinicians. One potential reason for these long-lasting research questions is that providing convincing evidence for them using one study design or one set of data is difficult. As a result, considering the intricacy of both research

questions and the heterogeneous nature of elevated blood pressure and diabetes, I utilised separate study methods based on different data sets to present the strongest possible proof for these major research challenges. Given the number of methods utilised in this thesis, I dedicated one chapter (Chapter 2) to explaining broad principles about methods, and then subsequently in each chapter presented more concentrated information about each method used. In Chapter 3, I used individual participant data from randomised clinical trials to investigate the effect of blood pressure reduction on the risk of new-onset type 2 diabetes. Using trial data, I studied the effect of each antihypertensive medication class against placebo on the risk of new-onset type 2 diabetes in Chapter 4. In Chapters 5 and 6, I addressed the same research problems discussed in Chapters 2 and 3, but this time using a Mendelian randomisation study design and human genetic data. Finally, using individual participant data from randomised clinical trials, Chapter 7 investigates the effect of blood pressure reduction on the prevention of major cardiovascular diseases in people with and without diabetes. Chapter 8 is devoted to the interpretation of data, limitations of design, and future research suggestions.

Chapter 2. Summary of methods and data used in this thesis

2.1. Individual participant data meta-analysis of randomised clinical trials

A systematic review and meta-analysis of RCTs are considered the gold standard of evidence and should be the first stop for clinicians looking for the best available evidence to support decision-making for intervention or advice to their patients. Systematic reviews and meta-analyses are rapidly populating medical search indexes like PubMed and the Cochrane Library in an effort to address crucial clinical and public health concerns and provide the most credible information to influence practice and research. The purpose of a meta-analysis is to methodically synthesise or combine the data of separate, independent studies in order to statistically compute an overall effect size. An improved effect size estimate and greater applicability of individual study findings are two benefits of doing a meta-analysis. This means it has the potential to help settle disagreements across investigations and provide definitive conclusions when single studies have failed to do so.

We could classify meta-analysis research in a variety of ways. In the medical literature, there are two primary forms of meta-analysis based on the level of data used for analysis: 1) aggregate data meta-analysis, and 2) individual participant data meta-analysis. Aggregate data meta-analysis is the most common type of meta-analysis design, in which researchers extract summary-level data from published (or

sometimes unpublished) studies and combine them using statistical methods. However, this approach has some limitations and strengths. In terms of strengths, it is possible to conduct very quickly, it does not require extensive resources or computational hardware, it is possible to conduct with at least two scientists, and if properly conducted, it can provide the best available evidence for a research hypothesis. However, this method has significant drawbacks. It is reliant on estimations from previously published research, evaluating the quality of statistical methodologies used for the estimate is impossible, harmonisation for checking and unifying outcome and variable definitions is impossible, and detailed analysis of interaction is difficult. After realising the value of data sharing and collaborative research, a more powerful form of meta-analysis study has become increasingly popular in recent decades; individual participant data (IPD) meta-analysis.

In IPD meta-analysis, rather than obtaining summary (aggregate) data from study publications or investigators, the original research data for each study are obtained directly from the researchers who conducted each study. In IPD meta-analysis, strategy, design, and statistical analysis are quite different from aggregate data meta-analysis. A significant amount of time is required in IPD data to build collaboration with the PI of each study, persuade them to share the study individual-level data, comply with data sharing regulations specific to each country that studies were conducted in, provide sufficient computational resources for data storage, employ dedicated scientists for the harmonisation process, and finally run a complex statistical method for effect size estimation. Despite these challenges, IPD meta-analysis provides a strong framework to assess research questions that are not feasible to answer using aggregate data or data from single studies. In particular, in

the field of precision medicine, which is becoming very popular nowadays, having access to individual-level data from different studies and pooling it using IPD meta-analysis is crucial. For example, if one aims to investigate the effect of a treatment on a specific subgroup of patients and compare this effect with that of other patient groups, IPD meta-analysis is the best available design.

In terms of the blood pressure lowering treatment effect, the Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTTC) is one of the first and largest collaborations for gathering IPD data in order to conduct an IPD meta-analysis. I used IPD from the BPLTTC collaboration for my thesis. The BPLTTC was conceived and initiated in 1995 as a collaboration between the principal investigators of all the major ongoing clinical trials of blood pressure-lowering agents. The first cycle of the study included fifteen trials with more than 74,000 individuals. In the second cycle, fourteen more trials were included, which brought the total sample size to about 162,000 participants. The BPLTTC was extended in 2014, and more trialists were invited to take part. The third and most current cycle of the collaboration is coordinated by the University of Oxford and its core activities have recently been funded by the British Heart Foundation. There are currently more than 350,000 participants from 51 studies in the collaboration's third and ongoing phase.[44] With the use of this resource, it will be possible to address long-standing research questions about blood pressure-lowering therapy, in particular for certain patient subgroups. The current cycle is managed by an Oxford University-based research team. Prof. Kazem Rahimi presides over a Steering Committee comprising chosen Principal Investigators from some of the most important studies in the BPLTTC. The Steering Committee is responsible for overseeing the BPLTTC as a whole and providing

scientific leadership for all phases of proposal preparation, statistical analysis, and reporting. All scientific pursuits are supported by a worldwide network of BPLTTC collaborators.

There are two different analytical approaches in IPD meta-analysis: two-stage IPD meta-analysis and one-stage meta-analysis. The one-stage technique examines the IPD from all studies at the same time, such as in a single hierarchical regression model with fixed or random effects. The two-stage technique first re-estimates the effect size using IPD data for each study separately and then combines them in a standard meta-analysis model. In this thesis, I used a one-stage meta-analysis approach because it is more efficient for the assessment of interaction than a two-stage approach.[45]

A one-stage IPD meta-analysis relies on a single statistical model to synthesise IPD from several studies. This method estimates a pooled effect size in a single step while accounting for participant clustering within trials and heterogeneity across studies.[46] To prevent misleading findings, a one-stage IPD meta-analysis methodology must account for participant clustering within trials.[47] To address this important issue, I used the stratified Cox proportional hazard model to estimate the effect size in all of the one-stage IPD meta-analyses conducted in this thesis.[48,49] Standardisation for blood pressure reduction at the trial level is another important issue that should be addressed, particularly in the area of blood pressure lowering when the goal is to evaluate the risk of various outcomes associated with blood pressure lowering treatment per se. In the following, an overview of the rationale as

well as specifics of the methodologies employed for standardisation throughout this thesis is provided.

Several questions were investigated in this thesis and different models were used for answering each of them. The initial question was about the treatment effect across the whole population per 5 mmHg systolic blood pressure reduction. For this, I ran the main model without any interaction term. This was just a preliminary step towards the investigation of heterogeneity of treatment effects by type 2 diabetes status and blood pressure categories. To assess the effects of blood pressure-lowering treatment on primary and secondary outcomes by type 2 diabetes status at baseline, I ran a model that included type 2 diabetes status at baseline as an interaction. Finally, to estimate the treatment effect on primary and secondary outcomes stratified by baseline systolic blood pressure and type 2 diabetes status at baseline, I used the third model (i.e., with interaction terms for type 2 diabetes and systolic blood pressure categories). I standardised the analyses for differences in systolic blood pressure between trial arms (per trial) because I was interested in the proportional effects of lowering systolic blood pressure (scaled to a 5 mmHg difference) and because the trials varied in their relative intensity of blood pressure lowering achieved (by design). Conventional meta-analyses weight trials by their standard error (i.e., statistical power). Our standardisation approach refines that weighting of trials by additionally weighting them by their achieved intensity of blood pressure reduction. This enables comparison of like-with-like when primary hypotheses are concerned with the blood pressure-mediated effects. In practical terms, this means that everything else being equal, trials with very little blood pressure reduction between treatment arms are given a proportionately lower weight

than when no standardisation is applied (and vice versa). In statistical terms, we eliminate one source of heterogeneity (different intensities of blood pressure reduction) by ‘adjusting’ for it, without violating the intention to treat analysis principle. Consider the case in which I did not perform standardisation. This would mean that trials with very little blood pressure reduction would dilute treatment effects towards the null, and trials with an extreme blood pressure reduction would bias them towards the extreme. This is of concern given that we did not restrict the inclusion of trials to those that had a minimum blood pressure reduction. It could also lead to false conclusions in the investigation of the heterogeneity of effects. For instance, imagine that participants belonging to a particular subgroup (e.g., type 2 diabetes or systolic blood pressure <130 mmHg) happened to be predominantly recruited into trials with little blood pressure reduction. On the other hand, participants belonging to another subgroup (e.g., no type 2 diabetes, or systolic blood pressure >140mmHg) happened to be coming from trials that had a disproportionately large blood pressure reduction between trial arms. Without standardisation, one could find an apparent heterogeneous treatment effect among those subgroups, when in fact no such heterogeneity exists. The reverse could also be true in that we may fail to identify heterogeneous treatment effects because they are masked by different intensities of blood pressure reduction across subgroups.

It should be noted that these analyses were all pre-specified as our primary analyses. However, as a sensitivity analysis, the findings without standardisation were done for comparison to the main results because the scenario that blood pressure reduction would substantially differ between subgroups and hence bias findings did not materialise.

Different methods have been used to standardise treatment effect estimates for the intensity of systolic blood pressure reduction or cholesterol lowering in aggregate data and two-stage IPD meta-analyses.[50,51] For a better grasp of the concept, for example, in a previous aggregate data meta-analysis,[51] investigators standardised the analyses by multiplying the log of the summary statistic of each trial by 10/d, where d was the average systolic blood pressure reduction in that trial. If the log hazard ratio [HR] was −0.2 and the systolic blood pressure reduction was 4 mmHg, the standardised logHR would be −0.2 × (10/4) = −0.5 per 10 mmHg reduction in blood pressure. This approach is useful when individual-level information is not available or when the IPD meta-analysis has a simpler 'two-stage' design (as has been the case with the Cholesterol Lowering Treatment Trialists' Collaboration).[50] One of the strengths of the current cycle of the BPLTTC is that for the first time, we have pooled the data from all trials into a single harmonised dataset (like having a large clinical trial). This enables analyses of treatment interactions by multiple patient characteristics (blood pressure, type 2 diabetes status, drug etc.). This, however, requires alternative models that better utilise this strength without losing information. More specifically, the most appropriate method here is to include the standardisation as part of the main model, by using regression models adjusted for the intensity of systolic blood pressure lowering. We opted for hierarchical Cox models and included additional interaction terms when required (as described above).

For clarity, we illustrate below the three steps for estimating the effects of systolic blood pressure lowering treatment on primary outcomes by type 2 diabetes status at baseline. The other analyses follow the same approach.

Step 1. Running the model

First, I ran the Cox model (Model #1), adjusted for systolic blood pressure reduction at the trial level. The value for systolic blood pressure reduction for each trial was derived from our previous study.[52]

The following model has been fitted:

(*Model #1*): treatment + delta + (treatment × delta) + (treatment × T2D) + (treatment × delta × T2D)

Where "treatment" is a binary variable for treatment (0 comparator, 1 intervention), "delta" is systolic blood pressure reduction at the trial level and "T2D" is prior diabetes status (0 non-diabetes at baseline, 1 diabetes at baseline).

Stage 2. Obtaining a summary of the model and the variance-covariance matrix

At this stage, the following regression coefficients are extracted from the model summary and variance-covariance matrix:

b treatment arm = regression coefficient for treatment arm

b delta= regression coefficient for systolic blood pressure reduction at the trial level

b treatment arm: delta = regression coefficient for interaction between treatment arm and systolic blood pressure reduction at the trial level

b comparator arm: T2D = regression coefficient for interaction between comparator arm and diabetes status at baseline

b treatment arm:T2D = regression coefficient for interaction between treatment arm and diabetes status at baseline

b comparator arm:delta:T2D = regression coefficient for interaction between comparator arm, systolic blood pressure reduction at the trial level and diabetes status at baseline

b treatment arm:delta:T2D = regression coefficient for interaction between treatment arm, systolic blood pressure reduction at the trial level and diabetes status at baseline

Stage 3. Rescaling to 5 mmHg as the mean blood pressure reduction in BPLTTC

Up to this point, the model has been adjusted for systolic blood pressure reduction at the trial level. This means that the model accounts for variations in blood pressure reduction across trials. Now we rescale the HR to a 5 mmHg reduction, for people with and without type 2 diabetes at baseline. From the Cox model, we multiplied appropriate terms in the prediction model by 5 to estimate the hazard ratio per 5 mmHg reduction for each category of diabetes at baseline:

(*Model #2*): Standardised logHR per 5 mmHg systolic blood pressure reduction in participants with diabetes at baseline = b treatment arm + (b treatment arm:delta×5) + b treatment arm:T2D + (b treatment arm:delta:T2D×5) - b comparator arm:T2D – (b comparator arm:delta:T2D×5)

(*Model #3*): Standardised log hazard ratio per 5 mmHg systolic blood pressure reduction in participants without diabetes at baseline = b treatment arm + (b treatment arm:delta×5)

We used this approach in previous BPLTTC studies.[43,49,51,53-56]

2.2. Individual participant data network meta-analysis of randomised clinical trials

Typically, when conducting meta-analyses of clinical trials or other types of intervention studies, we estimate the true effect size of a single intervention. In many areas of research, however, there is more than one conclusive type of treatment. For instance, in the field of blood pressure lowering, there are many different drug classes that lower blood pressure via different biological pathways. Therefore, it is important to estimate each drug class's separate effect on the outcome, particularly when the outcome of interest is a complex cardiometabolic condition such as type 2 diabetes. This generates new problems. For a conventional meta-analysis to examine the comparative efficacy of multiple treatments, adequate head-to-head comparisons between two treatments are required. Regretfully, this is frequently not the case. In many research fields, only a small number of trials have directly compared the effects of two treatments, as opposed to a usually weaker control group. Consequently, using pair-wise comparison, we cannot have a precise estimation of each treatment effect in comparison to an interested comparator.

By incorporating both direct and indirect evidence from a network of trials, network meta-analysis is a method for evaluating three or more treatments simultaneously in a single study. Network meta-analysis, also known as mixed

treatments comparison or multiple treatments comparison meta-analysis, broadens the scope of a traditional pair-wise meta-analysis by comparing interventions directly within trials and indirectly between trials using a common comparator such as a placebo.[57] When appropriate, the two sources of information may be combined as a weighted average when both direct and indirect evidence is available. This type of data structure may be expanded to n-comparisons to enable simultaneous inference about all potential treatments and to provide support for choosing the most effective alternative among several.[57]

These are two distinct techniques for network meta-analysis, frequentist and Bayesian, both of which might provide valuable findings under certain conditions. Bayesian meta-analysis differs from frequentist meta-analysis in that both data and model parameters are treated as random variables. In this thesis, I used a Bayesian network meta-analysis approach to estimate the effect of each class of blood pressure-lowering drug on the risk of type 2 diabetes. Then, in a separate analysis to assess the effect of each class of drug on the risk of major cardiovascular diseases in people with and without type 2 diabetes, I conducted a stratified IPD network meta-analysis to estimate the stratified effect in each subgroup. I used a so-called Bayesian hierarchical model to conduct a Bayesian meta-analysis. The uncertainty in the estimation (tau^2) can be directly modelled using Bayesian approaches. They may also perform better than traditional methods of calculating pooled effects, especially when the number of studies being pooled together is low.[58] Methods sections in subsequent chapters provide further information on the specific statistical analyses performed.

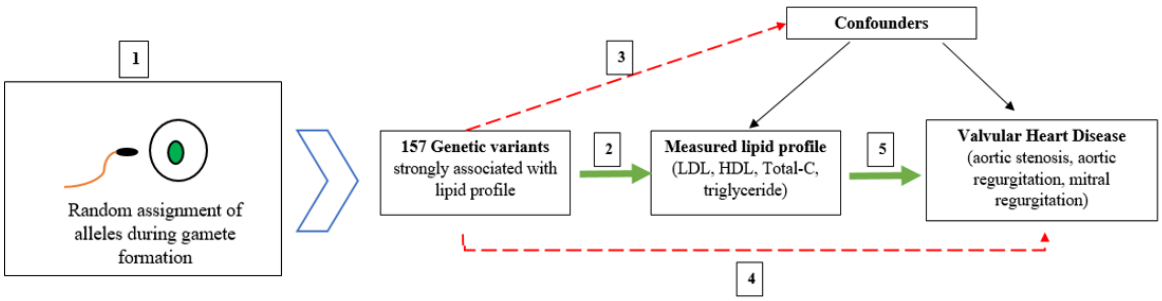
2.3. Mendelian randomisation

The use of naturally randomised genetic variants to answer causal questions about how modifiable exposures affect various outcomes is referred to as Mendelian randomisation.[59] Mendel's rules of heredity and instrumental variable estimate techniques provide the foundation for the concepts of Mendelian randomisation. This framework makes it possible to draw conclusions about the existence of causal effects despite the presence of unmeasured confounding factors.[60] Exposures may be any factor that is strongly linked to genetic variants in people; for example, an exposure might include clinically measured traits such as blood pressure or less directly observable traits like gene expression or specific protein expression of a specific gene in a specific tissue.[59]

The Mendelian randomisation statistical approach is mainly based on instrumental variable analysis. Comparing the propensity score technique with the instrumental variable approach, the latter is used to control observable and measured confounders while the former is used to control unobservable and unmeasured causes of confounding.[61] In the case of Mendelian randomisation, genetic variants or a genetic risk score developed based on multiple genetic variants associated with exposure of interest serve as an instrumental variable. Mendelian randomisation is based on three important assumptions: the genetic variant(s) should be associated with the exposure; the genetic variant(s) should not associate with confounder; the genetic variant(s) should influence the outcome only through the exposure.[62] **Figure 2-1** shows the framework and assumptions of Mendelian randomisation in the context of the effect of lipid profiles on the risk of valvular heart diseases from my recent publication.[63]

Figure 2-1. Diagram of Mendelian randomisation framework.

1. A zygote consists of gametes formed from the union of the sperm cell and the ovum. During the formation of the zygote, one allele from the father (1/2) and one from the mother (1/2) randomly inherit by the baby. A well-known example is the probability of baby gender (1/2 male and 1/2 female); 2.genetic variants should have a strong association with lipid profile measures; 3.genetic variants should not have a significant association with confounders; 4.genetic variants should not have any significant association with outcome of interest; 5. If the mentioned assumptions exist, we can investigate the causality of association.



Using this framework, Mendelian randomisation minimises the potential for bias caused by unobserved factors that might be influencing both the exposure and the outcome. However, in order to make causal inferences and effect size estimating, one must first make a number of key extra assumptions, which vary from those used by other techniques for conducting causal effect estimation. Estimates of the causal effect obtained by Mendelian randomisation may be examined within the context of a triangulation of evidence framework. This framework requires the findings to be interpreted in conjunction with the findings obtained from complementary methods that depend on distinct assumptions. When employing this method, it is essential that the sources of bias in the various research paradigms are not interconnected to one another. In this way, the size and direction of the bias in one study will not be able to predict the size and direction of the bias in the other studies.[59] For example, to make causal inferences for the effect of blood pressure lowering on the risk of type 2 diabetes, in this thesis, I used both Mendelian

randomisation and IPD meta-analysis of RCTs, two high-quality sources of randomised evidence with independent assumptions and independent data sources.

There are two main types of Mendelian randomisation designs, one-sample and two-sample. In single-sample or one-sample Mendelian randomisation to get a causal estimate of the effect of the risk factor on the outcome, the investigator only utilises a single dataset for the instrumental variable analysis.[60] This sample should be a large enough dataset to provide sufficient statistical power. One such resource for conducting one-sample Mendelian randomisation is the UK Biobank, a massive biological database and research resource including in-depth genetic and health information from half a million UK individuals.[64] This type of Mendelian randomisation study needs extensive individual-level data from large-scale biobanks, or it is necessary to pool individual-level data from different biobanks to have enough statistical power; therefore, it is not very popular.

A more popular type of Mendelian randomisation is a two-sample design. To conduct this type of study, it is not necessary to have access to individual-level data from large-scale biobanks, which can be expensive and require substantial computing resources. Instead, investigators only rely on summary-level data from published genome-wide association studies (GWAS). Given the publicly available summary data from GWAS studies, this type of Mendelian randomisation is getting very popular. We need summary data from two independent GWAS studies to conduct this study, one to extract variant-exposure association and the other to extract variant-outcome association. This design provides much stronger statistical power for instrumental variable analysis and causal inference.[60] In this thesis, I used

both approaches. Given the fact that a two-sample design has better statistical power, it was my main design, and using the UK Biobank, I conducted one-sample Mendelian randomisation to investigate the research questions from different angles. In the method section of each Chapter, I explained the statistical approach and provided details about both methods.

Chapter 3. Effect of blood pressure-lowering on the risk of type 2 diabetes: An individual participant data meta-analysis of randomised control trials

3.1. Introduction

Diabetes is a major cardiovascular risk factor that affects about 9% of the adult population worldwide, with a rising prevalence in many regions of the world.[65] Patients with diabetes often suffer from elevated blood pressure and are disproportionately at high risk of developing cardiovascular disease.[66,67] On the other side, type 2 diabetes type is associated with a higher risk of major cardiovascular events such as stroke, ischemic heart disease, and heart failure.[68] Although blood pressure lowering is an established strategy for preventing micro-and macrovascular events in people with type 2 diabetes,[69] its benefit for the prevention of diabetes itself has been less clear. Thus, whether elevated blood pressure is a modifiable risk factor for diabetes remains to be established.

Given the shared pathways between elevated blood pressure and type 2 diabetes, it has been hypothesised that elevated blood pressure increases the risk of type 2 diabetes as it does with major cardiovascular disease. For instance, elevated blood pressure may decrease insulin sensitivity by altering the transport of glucose or insulin to cells, which is linked to reduced glucose tolerance.[70] Furthermore, it has been shown that elevated blood pressure may lead to chronic inflammation as well as endothelial dysfunction; both of these conditions are linked to the onset of diabetes.[71,72] Thus, there is biological evidence to support the hypothesis that elevated blood pressure may contribute to the development of new-onset type 2 diabetes.

Evidence from observational cohort studies suggests that higher systolic blood pressure is associated with an increased risk of type 2 diabetes. One of the largest meta-analyses of observational evidence, including thirty prospective observational studies with 285,664 participants and 17,388 incident diabetes events, showed that each 20 mmHg higher systolic blood pressure is associated with a 77% higher risk of new-onset type 2 diabetes.[73] However, the causality of that association remains uncertain, as observational evidence is prone to confounding and reverse causation. Evidence from RCTs[74-76] and Mendelian randomisation studies [77] have been uncertain, with previous studies lacking statistical power and failing to consider potentially opposing effects of different blood pressure-lowering drug classes on the risk of type 2 diabetes. Details of the literature review have been reported in **Table 3-1**. In this part of the thesis, I aimed to assess the effect of pharmacological blood pressure lowering on the risk of new-onset type 2 diabetes by taking advantage of individual participant data of RCTs.

Table 3-1. Selected published reports on the effect of blood pressure lowering per se and specific drug class effect on the risk of new-onset type 2 diabetes.

Study	Publication date	Design	Study name	Size	Time of follow-up	Exposure in cohort studies or trial arms in RCTs	Finding
Studies investigating the effect of blood pressure lowering per se on the risk of type 2 diabetes mellitus							
1) *Meta-analysis of observational studies*							
Emdin CA[73]	2015	-Observational cohort - Meta-analysis of observational cohort studies	Clinical Practice Research Datalink (CPRD)	Observational cohort: 4.1 million Meta-analysis: 30 studies with 285,664 participants and 17,388 incident diabetes events	A median follow-up of 6.8 years	Systolic BP per 20 mmHg increase	**Observational cohort**: 20 mmHg higher systolic BP was associated with a 58% higher risk of new-onset diabetes (hazard ratio 1.58; 95% CI 1.56 to 1.59) **Meta-analysis**: The pooled relative risk of diabetes for a 20 mmHg higher usual systolic BP across studies was 1.77 (1.53 to 2.05).
2) Individual randomised controlled trial							
Roumie CL[75]	2020	Randomized clinical trial	The Systolic Blood Pressure Intervention Trial (SPRINT)	8,380	3 years	More intense vs less intense treatment	The adjusted hazard ratio for the incidence of diabetes was 1.19 (95% CI, 0.95–1.49)
3) Mendelian randomisation							
Sun D[77]	2019	Mendelian randomisation	UK Biobank	318,664	NA	Genetically determined hypertension	Genetically determined hypertension has no relationship with diabetes (odds ratio 0.96 [CI 95% 0.88 to 1.04])
Aikens RC[78]	2017	Mendelian randomisation	NA	Summary statistics from GWAS meta-analysis	NA	Genetically determined higher systolic BP	A 2% increase in the risk of diabetes per 1 mmHg genetically determined higher systolic BP (odds ratio 1.02, 95% CI 1.01 to 1.03)
Zhu Z[79]	2018	Mendelian randomisation	NA	Summary statistics from GWAS meta-analysis	NA	Genetically determined higher systolic BP	- Analysis based on GWAS meta-analysis of two community-based studies (GERA and UKB) showed no association (odds ratio 1.07, 95% CI 0.89 to 1.29) - Analysis based on published independent case-control studies revealed significant findings (odds ratio 1.46, 95% CI 0.13 to 1.89)
Studies investigating the effect of antihypertensives on the risk of type 2 diabetes							
1) Observational studies							
Gress TW[80]	2000	Prospective cohort study	The Atherosclerosis Risk in Communities (ARIC)	12,550	6 years	Antihypertensive medications use in people without diabetes at baseline	- Thiazide diuretics were not significantly associated with a greater risk of the subsequent development of diabetes (hazard ratio, 0.91; 95% CI, 0.73 to 1.13) - ACEIs were not associated with a greater risk of the subsequent development of diabetes (hazard ratio, 0.98; 95% CI, 0.72–1.34) - Calcium channel blockers were not associated with a significantly greater risk of the subsequent development of diabetes hazard ratio 1.17, 95% CI 0.83 to 1.66) - Beta-blocker increased the risk of new-onset diabetes (hazard ratio 1.28, 95% CI 1.04 to 1.57)
1) Individual randomised controlled trial							
Fletcher AE[81]	1991	Randomized clinical trial	The European Working Party on High Blood Pressure in the Elderly (EWPHE)	840	NA	Diuretics vs placebo	No effect on new-onset diabetes (risk ratio 1.47, 95% CI 0.84 to 1.57)
Savage PJ[76]	1998	Multicenter, randomized, double-blind, placebo-controlled clinical trial	The Systolic Hypertension in the Elderly Program (SHEP)	4,736	3 years	Thiazide diuretic or beta blockers vs placebo	New cases of diabetes were reported by 8.6% of the participants in the active treatment group and 7.5% of the participants in the placebo group (risk ratio 1.14, 95% CI 0.90 to 1.45)
Cooper-DeHoff R[82]	2006	Randomized clinical trial	International Verapamil SR-Trandolapril Study (INVEST)	16,176	2.8 years	Calcium channel blockers versus beta-blockers	The risk of new-onset diabetes was lower in patients who took calcium channel blockers than beta-blocker (hazard ratio 0.85, 95% CI 0.76 to 0.95).
2) Meta-analyses of trials							
Elliott WJ[83]	2007	Network meta-analysis of clinical trials	NA	22 trials with 143,153 participants	NA	Antihypertensive agents	Considering the diuretics as the comparator group, the odds ratios were: 0.57 (95% CI 0.46 to 0.72) for ARB, 0.67 (0.56 to 0.80) for ACEI, 0.75 (0.62 to 0.90) for Calcium channel blockers, 0.77 (0.63 to 0.94) for placebo and 0.90 (0.75 to 1.09) for beta-blockers.

BP: blood pressure, RCT: randomized controlled trial, CI: confidence intervals, GWAS: genome-wide association study, ACEI: Angiotensin-converting-enzyme inhibitors, ARB: angiotensin II receptor blocker, NA: not applicable

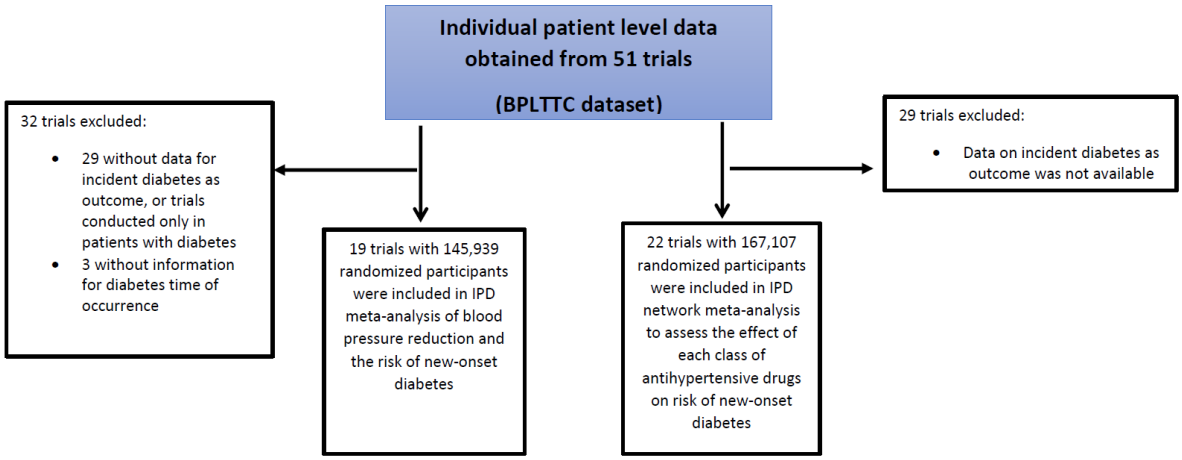
3.2. Objective

- To investigate the effect of pharmacological blood pressure-lowering on the risk of new-onset type 2 diabetes using data from randomised clinical trials.

3.3. Methods

I used the resource provided by the BPLTTC, a collaboration of principal investigators and trialists of major RCTs of pharmacological blood pressure-lowering treatment (Please see Chapter 2 for more details about the BPLTTC). For this study, I included all primary and secondary prevention trials that used a specific class(es) of antihypertensive drugs versus placebo or other classes of blood pressure-lowering medications and had at least 1,000 persons-years of follow-up in each randomly allocated arm. **Figure 3-1** shows the details of trial selection from the main BPLTTC dataset.

Figure 3-1. Trial selection flowchart for investigating the effect of blood pressure-lowering per se on the risk of type 2 diabetes.



All participants with a known diagnosis of diabetes at baseline or trials conducted in patients with prevalent diabetes were excluded. New-onset type 2 diabetes was defined based on the diagnostic criteria reported by each trial (**Table 3-2**). The risk of bias for each trial was assessed by the revised Cochrane risk-of-bias tool and has been reported in a previous study.[54]

Table 3-2. Diagnostic criteria for the definition of type 2 diabetes in each trial.

Trial name	New-onset type 2 diabetes definition
ACTIVE I	Adverse event report
ALLHAT	Fasting blood glucose of ≥ 7.0 mmol/L (126 mg/dL)
ANBP	Adverse event report
ANBP2	Adverse event report
ASCOT-BPLA	Fasting blood glucose of ≥ 7.0 mmol/L (126 mg/dL)
CAPPP	Two determinations of fasting blood glucose of ≥ 6.7 mmol/L (120.6 mg/dL) according to the 1985 World Health Organization criteria.
CASEJ	Self-reported diabetes or anti-diabetic agents
COLM	Initial diagnosis by participating physicians and final ascertainment by the endpoint committee
COPE	Adverse event report
HIJ-CREATE	Fasting blood glucose ≥ 7.0 mmol/L (126 mg/dL) or commencement of anti-diabetic agents and/or glycohemoglobin A1c ≥ 6.5%
HOPE	Adverse event report
INSIGHT	World Health Organization criteria
MOSES	Adverse event report
NORDIL	Adverse event report
ONTARGET	Fasting blood glucose of ≥ 7.0 mmol/L (126 mg/dL)
PEACE	Adverse event report
PROGRESS	Adverse event report
STOP2	Two determinations of fasting blood glucose of ≥ 6.7 mmol/L (120.6 mg/dL)
SYSTEUR	International Classification of Diseases, ninth revision
TRANSCEND	Fasting blood glucose of ≥ 7.0 mmol/L (126 mg/dL)
VALUE	Fasting blood glucose of > 7.8 mmol/L (140.4 mg/dL)
PROFESS	Adverse event report

ACTIVE I: Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events; ALLHAT: Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial; ANBP: Australian National Blood Pressure Study; ANBP2: Second Australian National Blood Pressure Study; ASCOT-BPLA: Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm; CAPPP: Captopril Prevention Project; CASE-J: Candesartan Antihypertensive Survival Evaluation in Japan Trial; COLM: Combination of OLMesartan study; COPE: Combination Therapy of Hypertension to Prevent Cardiovascular Events; HIJ-CREATE: Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease; HOPE: Heart Outcomes Prevention Evaluation; INSIGHT: International Nifedipine GITS study: Intervention as a Goal in Hypertension Treatment; MOSES: Morbidity and Mortality After Stroke, Eprosartan Compared With Nitrendipine for Secondary Prevention; NORDIL: Nordic Diltiazem Study; ONTARGET: Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial; PEACE: Prevention of Events with Angiotensin-Converting Enzyme Inhibition; PRoFESS: Prevention Regimen for Effectively Avoiding Second Strokes; PROGRESS: Perindopril Protection Against Recurrent Stroke Study; STOP Hypertension-2: Swedish Trial in Old Patients with Hypertension-2; Syst-Eur: Systolic Hypertension in Europe; TRANSCEND: Telmisartan Randomized Assessment Study in ACE Intolerant Subjects with Cardiovascular Disease; VALUE:Valsartan Antihypertensive Long-term Use Evaluation

Participants were grouped into the intervention and comparator arms. For placebo-controlled trials, the placebo arm was considered as the comparator and the active arm as the intervention. For head-to-head trials that compared two or more drug classes, the arm with the greater systolic blood pressure reduction was considered as the intervention and the other(s) as the comparator. The summary characteristics of the included trials are shown in **Table 3-3**.

Table 3-3. General characteristics of trials included in the individual patient data meta-analysis as well as Bayesian network meta-analysis.

Trial	Design	Inclusion criteria	Exclusion criteria	Follow-up duration (years)	Trial arms		Incident diabetes cases†	Total participants	Diabetes event date¶
					Intervention*	Comparator			
ACTIVE I	Placebo-controlled	Atrial fibrillation, ≥1 risk factor (age ≥75 years, on antihypertensive treatment, history of stroke, TIA or non-CNS embolism, LVEF <45%, PVD, or age 55-74 years with either CAD or diabetes)	Use of anticoagulant, peptic ulcer disease in past 6 months, history of intracerebral haemorrhage, thrombocytopenia, or mitral stenosis	4.1	ARB	Placebo	409	7,231	Available
ALLHAT	Head-to-head	Age ≥55 years stage 1 or 2 hypertension plus ≥1 risk factor (MI or stroke >6 months previously, left ventricular hypertrophy, T2D, smoking, HDL <0.91 mmol/l), other atherosclerotic CVD	Symptomatic or hospitalization for heart failure, LVEF <35%	4.8	Diuretic	ACEI, CCB or alpha-blocker	3,184	27,135	Available
ANBP	Placebo-controlled	Age 30-69 years with mild hypertension (DBP 95-110 mmHg and SBP <200 mmHg)	Antihypertensive treatment in the past 3 months, recent angina or MI, stroke, hormone therapy, asthma, diabetes, gout, severe disease, tricyclic antidepressant use	3.6	Diuretic	Placebo	27	3,427	Available
ANBP2	Head-to-head	Age 65-84 years, SBP ≥160 mmHg or DBP ≥90 mmHg (if SBP≥140 mmHg), no recent CVD	Serious illness, plasma creatinine >221 μmol/l, malignant hypertension, dementia	4.1	Diuretic	ACEI	341	5,642	Available
ASCOT-BPLA	Head-to-head	Age 40-79 years, untreated (SBP ≥160 or DBP ≥100 mmHg) or treated hypertension (SBP ≥140 or DBP ≥90 mmHg), ≥3 CVD risk factors (documented LVH, abnormal ECG, T2D, PAD, previous stroke or TIA, male sex, age ≥55 years, microalbuminuria or proteinuria, smoking, TC: HDL ≥6, family history of premature coronary heart disease	Previous MI, current treatment for angina, recent CeVD, fasting triglycerides >4.5 mmol/l, heart failure, arrhythmia, haematological or biochemical abnormality at screening	5.3	CCB-based (+ACEI)	BB-based (+ diuretic)	1,358	14,112	Available
CAPPP	Head-to-head	Age 25-66 years, DBP ≥100 mmHg on two occasions	Secondary hypertension, serum creatinine >150 μmol/, a condition requiring BB treatment	5.8	BB and diuretic	ACEI	717	10,413	Available
CASEJ	Head-to-head	Age 20-85 years, ≥1 high-risk factor: SBP ≥180 or DBP ≥110 mmHg, T2D, history of angina pectoris, MI, stroke, TIA >6 months before screening, LVH, proteinuria or serum creatinine ≥1.3 mg/100 ml, peripheral artery obstruction	BP ≥200/120 mmHg, T1D, heart failure, LVEF <40%, atrial fibrillation, cancer	3.1	CCB	ARB	97	2,685	Available
COLM	Head-to-head	Age 65-84 years, hypertension (treated: BP ≥140/90 mmHg; untreated: BP ≥160/100 mmHg), CVD history or CVD risk factors (diabetes, dyslipidemia)	Secondary/malignant hypertension, recent major CVD, revascularization, angina pectoris hospitalization or severe heart failure, atrial fibrillation, hepatic or renal dysfunction	3.0	ARB and CCB	ARB and diuretic	26	3,779	Available
COPE	Head-to-head	Age 40-85 years, BP ≥140/90 mmHg	SBP ≥200 or DBP ≥120 mmHg, secondary hypertension, diabetes, recent CVD or revascularization, heart failure, atrial fibrillation/flutter, hepatic or renal dysfunction, congenital or rheumatic heart disease, cancer	3.6	CCB and ARB	CCB and diuretic or CCB and BB	89	2,827	Available
HIJ-CREATE	Head-to-head	Age 20-80 years, CAD hospitalization and hypertension (BP ≥140/90 mmHg or antihypertensive treatment use)	Secondary hypertension, recent AMI or CeVD, severe aortic valve stenosis, cardiomyopathy, serum creatinine >2 mg/dl, serum potassium >5 mmol/l, hepatic dysfunction, malignancy	4.0	ARB	non-ARB (including ACEI)	25	1269	Available
HOPE	Placebo-controlled	Age ≥55 years, CAD, stroke, PVD or diabetes, plus ≥1 risk factor (hypertension, dyslipidemia, smoking, or documented microalbuminuria)	Heart failure, LVEF <40%, using ACEI or Vitamin E, uncontrolled hypertension, nephropathy, or recent MI or stroke	4.5	ACEI	Placebo	257	5,720	Unavailable
INSIGHT	Head-to-head	Age 55-80 years, hypertensive (SBP ≥150 or DBP ≥95 mmHg, or SBP ≥160 mmHg), ≥1 other risk factor (TC ≥6.43 mmol/l, smoking, family history of premature MI, CAD, other CVD	None specified	2.8	Diuretic	CCB	312	5,015	Unavailable
MOSES	Head-to-head	Hypertension requiring treatment, documented TIA, ischemic stroke or cerebral haemorrhage	Internal carotid artery occlusion or stenosis >70%, heart failure, age >85 years, on anticoagulant for cardiac arrhythmia, high-grade aortic or mitral valve stenosis, unstable angina	3.3	CCB	ARB	34	854	Available
NORDIL	Head-to-head	Age 50-74 years, untreated hypertension (DBP ≥100 mmHg on two occasions); if previously treated, DBP ≥100 mmHg on two consecutive visits at one week apart during the run-in period and no treatment was given	Age <50 or ≥70 years, bradycardia, secondary hypertension, atrial fibrillation, recent CeVD or MI, heart failure	4.2	BB and diuretic	CCB	465	10,154	Available
ONTARGET	Head-to-head	CAD, PAD, CeVD or diabetes with end-organ damage	Heart failure, pericarditis, congenital heart disease, unexplained syncope, planned revascularization <3 months of consent, uncontrolled hypertension, heart transplant, subarachnoid haemorrhage, renal artery disease, proteinuria, hepatic dysfunction, volume, or sodium depletion, primary hyper-aldosteronism, hereditary fructose intolerance, other serious conditions	4.8	ARB or ACEI	ACEI, ARB	1,088	16,008	Available

Trial	Design	Inclusion criteria	Exclusion criteria	Follow-up duration (years)	Trial arms		Incident diabetes cases†	Total participants	Diabetes event date¶
					Intervention*	Comparator			
PEACE	Placebo-controlled	Age ≥50 years, documented CAD	Unstable angina, severe valvular heart disease, recent revascularization, planned elective revascularization, limited 5-year survival, serum creatinine >177 μmol/l, serum potassium >5.5 mmol/l	4.7	ACEI	Placebo	734	6,910	Available
PROGRESS	Placebo-controlled	Stroke or TIA in the past 5 years	Indication or contraindication for ACEI	3.9	ACEI and/or diuretic	Placebo	168	5,344	Available
STOP2	Head-to-head	Aged 70-84 years, SBP ≥180 mmHg and/or DBP ≥105 mmHg	Not specified	4.5	BB and/or diuretic	ACEI and CCB	288	5,895	Available
SYSTEUR	Placebo-controlled	Age ≥60 years, sitting SBP 160-219 mmHg, sitting DBP <95 mmHg, and standing SBP ≥140 mmHg	Secondary hypertension, retinal haemorrhage/papilledema, heart failure, dissecting aortic aneurysm, serum creatinine ≥180 μmol/l, recent severe nosebleeds, stroke or MI, dementia, disorders prohibiting standing position, severe CVD/non-CVD	2.6	CCB	Placebo	185	4,246	Available
TRANSCEND	Placebo-controlled	Intolerant to ACEI and with established CAD, PVD, CeVD or diabetes with end-organ damage	Heart failure, valvular/cardiac outflow tract obstruction, pericarditis, congenital heart disease, unexplained syncope, recent revascularization, SBP >160 mmHg, heart transplantation, subarachnoid haemorrhage, significant renal stenosis, renal or hepatic dysfunction	4.9	ARB	Placebo	454	3,808	Available
VALUE	Head-to-head	Age ≥50 years, hypertension, CVD, CVD risk factors (male sex, age >50 years, diabetes, current smoking, high cholesterol, LVH, proteinuria, serum creatinine 150 to 265 μmol/l)	Renal artery stenosis, recent CAD or CeVD, severe hepatic disease or chronic renal failure, heart failure, on monotherapy with BB for CAD and hypertension	4.2	CCB-based	ARB-based	1,535	10,422	Unavailable
PRoFESS	Placebo-controlled	Age ≥55 years with ischemic stroke <90 days before randomization (later modified to include age 50 to 54 years or had stroke 90 to 120 days before randomization if with ≥2 additional risk factors: diabetes, hypertension, smoker, obesity previous CVD, end-organ damage or hyperlipidemia) and remained stable	Hemorrhagic stroke, severe disability after the qualifying stroke, contraindication to treatments	2.5	ARB	Placebo	248	14211	Available

* Treatment arm in head-to-head trials compared two or more drug classes defined based on the following predefined structure: the arm with the greater systolic blood pressure reduction was considered the intervention and the other(s) as the comparator.

† All patients with known diabetes diagnosis at baseline have been excluded.

¶ All trials without information for the time of diabetes occurrence were excluded from the individual patient data meta-analysis to assess the effect of blood pressure reduction and risk of diabetes.

BP: blood pressure; GFR: Glomerular filtration rate; DBP: diastolic blood pressure; CKD: chronic kidney disease; T2D: type 2 diabetes; CAD: coronary artery disease; CeVD: cerebrovascular disease; CVD: cardiovascular disease; HbA1c: glycated hemoglobin; SBP: systolic blood pressure; TIA: transient ischemic attack; PAD: peripheral artery disease; MI: myocardial infarction; CNS: the central nervous system; LVEF: left ventricular ejection fraction; HDL: high-density lipoprotein; ECG: electrocardiogram; TC: total cholesterol; LVH: left ventricular hypertrophy; T1D: type 1 diabetes; CCB: calcium channel-blocker; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; BB: beta blocker

ACTIVE I: Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events; ALLHAT: Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial; ANBP: Australian National Blood Pressure Study; ANBP2: Second Australian National Blood Pressure Study; ASCOT-BPLA: Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm; CAPPP: Captopril Prevention Project; CASE-J: Candesartan Antihypertensive Survival Evaluation in Japan Trial; COLM: Combination of OLMesartan study; COPE: Combination Therapy of Hypertension to Prevent Cardiovascular Events; HIJ-CREATE: Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease; HOPE: Heart Outcomes Prevention Evaluation; INSIGHT: International Nifedipine GITS study: Intervention as a Goal in Hypertension Treatment; MOSES: Morbidity and Mortality After Stroke, Eprosartan Compared With Nitrendipine for Secondary Prevention; NORDIL: Nordic Diltiazem Study; ONTARGET: Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial; PEACE: Prevention of Events with Angiotensin-Converting Enzyme Inhibition; PRoFESS: Prevention Regimen for Effectively Avoiding Second Strokes; PROGRESS: Perindopril Protection Against Recurrent Stroke Study; STOP Hypertension-2: Swedish Trial in Old Patients with Hypertension-2; Syst-Eur: Systolic Hypertension in Europe; TRANSCEND: Telmisartan Randomized Assessment Study in ACE Intolerant Subjects with Cardiovascular Disease; VALUE: Valsartan Antihypertensive Long-term Use Evaluation

A one-stage IPD meta-analysis framework was used for statistical analysis.[54] We used stratified Cox proportional hazard models, with fixed treatment effects and participants as the unit of analysis.[84] We standardised the effect sizes for a 5 mmHg reduction in systolic blood pressure between randomised groups as a convenient round value close to the average systolic blood pressure reduction across all trials.[54,85] Standardisation of effect size is necessary when the aim is to assess the effects of blood pressure reduction per se through pooling of the data from different trials with differing levels of achieved blood pressure reduction.[54] Patients entered the analysis at the date of randomisation and were followed until the earliest occurrence of type 2 diabetes, death, study exit, or end of the trial. Kaplan-Meier survival curves were used to compare the probability of survival during the follow-up time. A subgroup analysis was conducted to assess the heterogeneity of effect by body mass index categories, as the possible mediator on the causal pathway between blood pressure and type 2 diabetes. The likelihood ratio test was used to test the heterogeneity of treatment effect across subgroups of body mass index categories at baseline.[49]

I also performed a series of sensitivity analyses to check the robustness of the results**.** I conducted a stratified analysis based on the various diabetes ascertainment techniques given by each study in order to evaluate the consistency of the results across ascertainment methods. In addition, I reported a one-stage Cox proportional hazards model with random effects terms and different levels of possible confounding variables. A two-stage meta-analysis was performed to further check the findings. Finally, I used Egger's regression test to check whether failure to obtain data from all trials may lead to acquisition bias.

3.4. Results

The characteristics of participants included in the IPD meta-analysis are shown in **Table 3-4**. Overall, 145,939 randomised participants from 19 trials were included in this analysis. For survival analysis, we excluded 631 participants with missing information for follow-up time.

Table 3-4. Baseline characteristics of participants included in individual participant data meta-analysis of randomised controlled trials to assess the effect of blood pressure lowering per se on the risk of new-onset type 2 diabetes mellitus.

Characteristic	Comparator (n=80887)	Treatment (n=65042)
Sex		
Women	31788 (39.3)	25641 (39.4)
Men	49099 (60.7)	39401 (60.6)
Age (years)	65.5 (9.7)	64.9 (9.9)
Systolic blood pressure (mmHg)	153 (22.1)	154 (21.8)
Diastolic blood pressure (mmHg)	89 (12.4)	89 (12.5)
Categories of systolic blood pressure (mmHg)		
<120	3827 (4.7)	2826 (4.3)
120-129	6724 (8.3)	5195 (8.0)
130 to 139	10250 (12.7)	8019 (12.3)
140 to 149	15408 (19.1)	11925 (18.3)
150 to 159	14224 (17.6)	11040 (17.0)
160 to 169	12688 (15.7)	11153 (17.2)
≥170	17734 (21.9)	14861 (22.9)
Body mass index (kg/m^2)		
<18.5	888 (1.2)	692 (1.1)
18.5 to 24.9	23303 (30.5)	19048 (31.3)
25 to 29.9	33480 (43.8)	26588 (43.6)
≥30	18849 (24.6)	14605 (24.0)
Comorbidity		
Peripheral vascular disease	888 (4.2)	882 (4.3)
Atrial fibrillation	4915 (6.1)	4616 (7.1)
Diabetes	0	0
Chronic kidney disease	5919 (20.0)	5581 (19.1)
Cerebrovascular disease	15794 (24.9)	14383 (26.0)
Ischaemic heart disease	22791 (28.2)	17012 (26.2)
Previous use of non-study medications		
Angiotensin-converting enzyme inhibitor	12479 (40.3)	9507 (38.6)
Angiotensin II receptor blocker	1695 (9.2)	1640 (9.4)
Calcium channel blocker	11877 (32.0)	9563 (31.1)
Diuretic	7800 (21.0)	6529 (21.3)
Beta-blocker	14590 (39.3)	11251 (36.6)
Alpha blocker	1110 (3.6)	917 (3.8)
Anti-platelet drug	14264 (67.7)	9611 (65.4)
Anticoagulant	2902 (9.9)	2564 (11.2)
Lipid-lowering treatment	14189 (41.4)	10310 (34.7)
Follow-up duration (years)	4.47 (1.89)	4.46 (1.98)

Among the 145,929 participants, 39.4% were female, 18.2% were current smokers, 53.3% had a history of blood pressure-lowering medication use and 43.9% had a history of cardiovascular diseases. The mean age of the participants was 65.2 years with a standard deviation (SD) of 9.8 years. At the baseline, the overall mean (SD) values for body mass index [kg/m2] and systolic blood pressure were 27.3 (4.7) and 153.3 (21.9), respectively. The distribution of baseline variables was roughly similar between the comparator and treatment groups (**Table 3-4)**.

Over 4.4 years of median follow-up (interquartile range 2.0), I identified 9,883 cases of new-onset type 2 diabetes. The incidence rate for developing a new-onset type 2 diabetes event per 1000 person-years was 16.44 (95% CI 16.01 to 16.87) in the comparator group and 15.94 (95% CI 15.47 to 16.42) in the intervention group. The HR and 95% CIs for diagnosis of new-onset type 2 diabetes during follow-up for a 5 mmHg reduction in systolic blood pressure were 0.89 (95% CI 0.84 to 0.95) (**Figure 3-2**). I performed a subgroup analysis by body mass index categories down to less than 25. As **Figure 3-3** shows, there is no heterogeneity of effect by body mass index; any difference is likely due to chance.

In the sensitivity analysis, which was followed by a stratified analysis based on the various diabetes diagnostic methods, the main results were verified without any significant changes in the subgroups (**Table 3-5**). The findings of a one-stage Cox proportional hazards model that included random effects terms and was adjusted for several possible confounding factors demonstrated consistency with the main results (**Table 3-6**). The finding of the two-stages meta-analysis was in line with

the main estimation (**Figure 3-4**). I also did not find any evidence of acquisition bias using funnel plot and Egger regression analysis (**Figure 3-5**).

Figure 3-2. Kaplan–Meier estimates of survival in the intervention and comparator groups.

The curve has been truncated at 7 years after randomisation and adjusted for systolic blood pressure reduction achieved at the trial level. All participants with a known diagnosis of diabetes were excluded at baseline. The incidence rate in each arm refers to the number of new-onset type 2 diabetes per 1000 person-years at risk.

No: numbers; py: person-years at risk; CI: confidence intervals

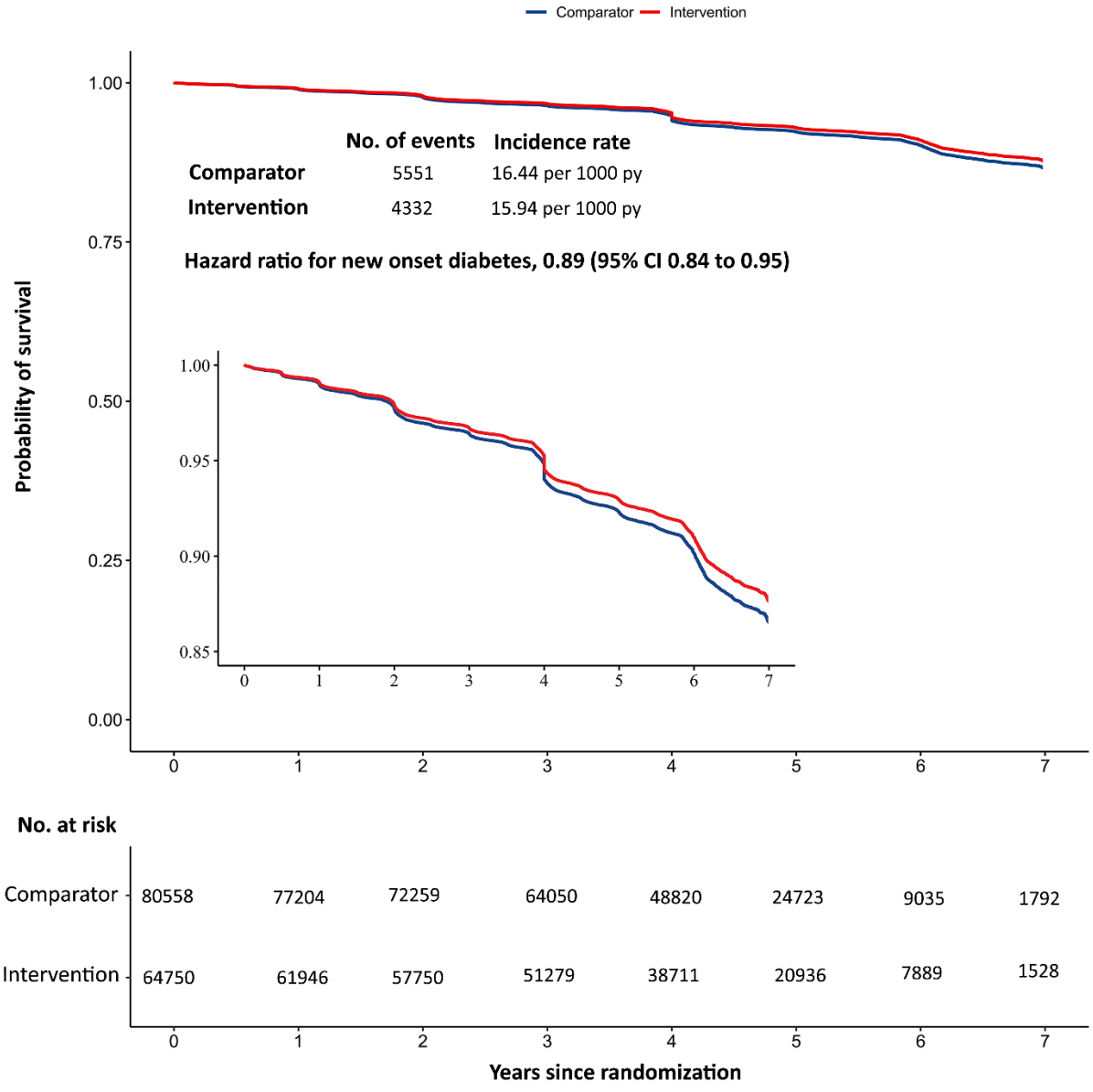


Figure 3-3. Blood pressure lowering treatment and risk of new-onset type 2 diabetes, by body mass index categories at baseline

The forest plot shows the hazard ratios and 95% CI per 5 mm Hg reduction in systolic blood pressure.

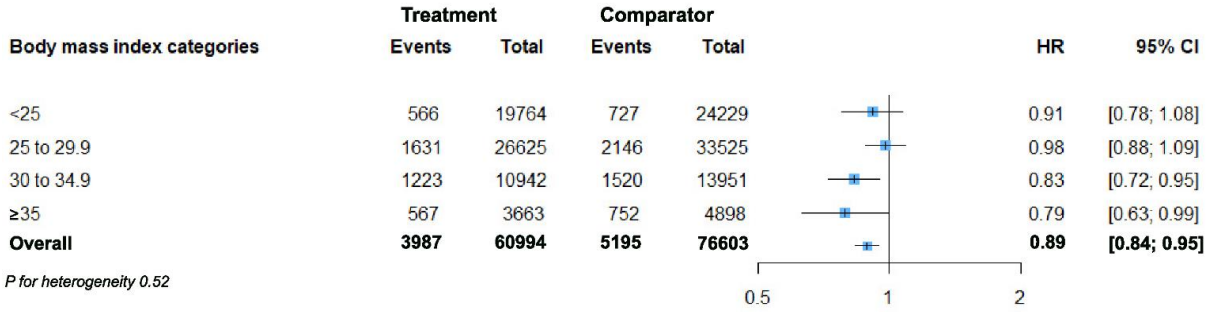


Table 3-5. Sensitivity analysis of the effect of systolic blood pressure lowering per se on the risk of new-onset type 2 diabetes, stratified by different diabetes ascertainment methods reported by each trial.

Trial name	Trial Design	Diabetes ascertainment type as the outcome	Diabetes ascertainment type at baseline	Treatment	Comparator	Treatment		Comparator		BP difference	Incidence rate	HR (95% CIs)
						Events	Total	Events	Total			
ACTIVE I	Placebo	AE	History/glucose lowering treatment	ARBs	Placebo	196	3614	213	3617	2.91	13.6	
ALLHAT	Drug-drug	FPG	History/FPG	Diuretics	ACEIs, CCBs and ARBs	1374	9719	1810	17393	1.45	24.3	
ANBP	Placebo	AE	History/diagnosis	Diuretics	Placebo	14	1717	13	1704	9.74	2.2	
ANBP2	Drug-drug	AE	History/diagnosis	Diuretics	ACEIs	184	2817	127	2795	1.85	13.6	
ASCOT-BPLA	Drug-drug	FPG	FPG/GTT/ glucose lowering treatment/history	CCBs	BBs	565	7032	792	6982	3.52	18.3	
CAPPP	Drug-drug	FPG	FPG/GTT	BBs/Diuretics	ACEIs	380	5205	337	5154	1.18	12.0	
CASEJ	Drug-drug	ICD-self report	FPG/HbA1c/GTT/ glucose lowering treatment	CCBs	ARBs	59	1293	38	1302	2.5	11.9	
COLM	Drug-drug	AE	History/FPG/GTT	ARBs and Diuretics	ARBs and CCBs	15	1840	11	1844	0.01	2.3	
COPE	Drug-drug	AE	History/diagnosis	CCBs and ARBs	CCBs and Diuretics or CCBs and BBs	20	956	69	1871	0.36	8.7	
HIJCREATE	Drug-drug	FPG	FPG/ glucose lowering treatment	ARBs	No- ARBs	7	645	18	624	0.09	4.9	
MOSES	Drug-drug	AE	History/diagnosis	CCBs	ARBs	11	416	19	433	3.24	10.7	
NORDIL	Drug-drug	AE	History/diagnosis	BBs/Diuretics	CCBs	249	5026	216	4980	3.26	10.9	
ONTARGET	Drug-drug	FPG	History/diagnosis	ACEIs/ARBs	ARBs and ACEIs	323	5280	761	10717	2.52	14.8	
PEACE	Placebo	AE	History/diagnosis	ACEIs	Placebo	334	3417	399	3457	5.04	22.4	
PROGRESS	Placebo	AE	History/diagnosis	ACEIs/Diuretics	Placebo	80	2657	86	2685	8.35	8.0	
STOP2	Drug-drug	FPG	History/diagnosis	BBs/Diuretics	ACEIs or CCBs	97	1954	190	3923	2.57	10.8	
SYSTEUR	Placebo	ICD-self report	History/diagnosis/FPG	CCBs	Placebo	107	2165	78	2069	9.46	16.8	
TRANSCEND	Placebo	FPG	History/diagnosis/FPG	ARBs	Placebo	205	1889	238	1905	5	25.0	
PRoFESS	Placebo	AE	History/diagnosis	ARBs	Placebo	112	7108	136	7103	4.41	6.9	
Diagnosis subgroups												
Overall estimation (n trials=19)											15.7	0.89 (0.84 to 0.95)
Subgroup 1: Both outcome and baseline diabetes ascertained using at least one laboratory test (n trials=5) *											19.8	0.64 (0.56 to 0.73)
Subgroup 2: Outcome ascertained using at least one laboratory test (n trials=7) †											18.3	0.63 (0.55 to 0.72)
Subgroup 3: Outcome reported as AE (n trials =10) ‡											11.3	0.92 (0.84 to 1.00)
Subgroup 4: Outcome ascertained using at least one laboratory test or ICD codes-self report (n trials =9) §											18.1	0.87 (0.79 to 0.95)

Drug-drug: drug-drug comparison trials; Placebo: Placebo-controlled trial; ICD: International Classification of Diseases code; AE: adverse event, GTT: Glucose Tolerance Test; FPG: fasting plasma glucose test; History: history of type 2 diabetes; diagnosis: diagnosis of type 2 diabetes by clinical staff; BP difference: systolic blood pressure difference; Incidence rate: overall incidence rate per 1000 person-years of follow-up, CCBs: calcium channel-blockers; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin II receptor blockers; BBs: beta-blockers; HR and 95% CI: hazard ratio and 95% confidence intervals standardized for 5 mmHg reduction in systolic blood pressure

* Included trials: ALLHAT, ASCOT-BPLA, CAPPP, HIJCREATE, TRANSCEND

† Included trials: ALLHAT, ASCOT-BPLA, CAPPP, HIJCREATE, TRANSCEND, ONTARGET, STOP2

‡ Included trials: ACTIVE I, ANBP, ANBP2, COLM, COPE, MOSES, NORDIL, PEACE, PROGRESS, PRoFESS

§ Included trials: ALLHAT, ASCOT-BPLA, CAPPP, HIJCREATE, TRANSCEND, ONTARGET, STOP2, SYSTEUR, CASEJ

Table 3-6. One-stage Cox proportional hazards model included random effects terms and adjusted for multiple potential confounders.

Model number	Sensitivity analysis	HR (95% CI)
#1	**Main model (fixed effect)**	0.89 (0.84 to 0.95)
	Main model with different adjustment levels for baseline variables	
#2	Adjusted for age and sex	0.89 (0.84 to 0.95)
#3	Adjusted for variables in model #2 + SBP at baseline	0.89 (0.84 to 0.95)
#4	Adjusted for variables in model #3 + BMI	0.90 (0.84 to 0.96)
#5	Adjusted for variables in model #4 + Comorbidities	0.88 (0.82 to 0.95)
#6	Adjusted for variables in model #5 + previous use of non-study antihypertensive medications	0.88 (0.81 to 0.94)
#7	Adjusted for variables in model #6 + previous use of non-study medications (anti-platelet drug, anticoagulants, lipid-lowering treatment)	0.86 (0.75 to 1.00)
	Post-hoc sensitivity analysis model (random effect)	
#8	Age as random effect term	0.91 (0.86 to 0.97)
#9	Sex as random effect term	0.91 (0.86 to 0.97)
#10	SBP categories at baseline as random effect term	0.91 (0.86 to 0.97)
#11	BMI categories as random effect term	0.92 (0.86 to 0.98)
#12	Comorbidities as random effect term	0.88 (0.83 to 0.94)
#13	Previous use of non-study antihypertensive medications as random effect term	0.93 (0.86 to 0.99)
#14	Previous use of non-study medications (anti-platelet drug, anticoagulants, lipid-lowering treatment) as random effect term	0.88 (0.80 to 0.97)

SBP: systolic blood pressure, HR: hazard ratio, CI: confidence intervals, BMI: body mass index

Figure 3-4. Forest plot showing the effect of systolic blood pressure lowering per se on the risk of new-onset type 2 diabetes, overall and separately for each trial.

The estimated heterogeneity indexes were I^2 = 86% and tau^2 = 0.11. The hazard ratio (HR) is standardised for blood pressure reduction between included trials. BP difference: systolic blood pressure difference, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin II receptor blockers, BB: beta-blockers, CI: confidence intervals, ICD: international classification of diseases diagnosis codes

			Treatment		Comparator						
Trials	**Treatment**	**Comparator**	**Events**	**Total**	**Events**	**Total**	**BP diff**	**HR**		**95% CI**	**Weight**
ACTIVE I	ARB	Placebo	196	3614	213	3617	2.91	0.86	[0.62;	1.20]	3.4%
ALLHAT	Diuretic	ACEI, CCB and ARB	1374	9719	1810	17393	1.45	1.99	[1.56;	2.54]	6.4%
ANBP	Diuretic	Placebo	14	1717	13	1704	9.74	1.00	[0.68;	1.48]	2.5%
ANBP2	Diuretic	ACEI	184	2817	127	2795	1.85	2.78	[1.51;	5.11]	1.0%
ASCOT-BPLA	CCB	BB	565	7032	792	6982	3.52	0.58	[0.50;	0.68]	15.9%
CAPPP	BB/Diuretic	ACEI	380	5205	337	5154	1.18	1.64	[0.88;	3.06]	1.0%
CASEJ	CCB	ARB	59	1293	38	1302	2.50	2.43	[1.07;	5.49]	0.6%
COLM	ARB and Diuretic	ARB and CCB	15	1840	11	1844	0.01				0.0%
COPE	CCB and ARB	CCB and Diuretic or CCB and BB	20	956	69	1871	0.36				0.0%
HIJCREATE	ARB	No- ARB	7	645	18	624	0.09				0.0%
MOSES	CCB	ARB	11	416	19	433	3.24	0.47	[0.15;	1.48]	0.3%
NORDIL	BB/Diuretic	CCB	249	5026	216	4980	3.26	1.21	[0.92;	1.60]	4.8%
ONTARGET	ACEI/ARB	ARB and ACEI	323	5280	761	10717	2.52	0.75	[0.58;	0.97]	5.6%
PEACE	ACEI	Placebo	334	3417	399	3457	5.04	0.83	[0.72;	0.96]	18.0%
PROGRESS	ACE/Diuretic	Placebo	80	2657	86	2685	8.35	0.96	[0.80;	1.16]	11.3%
STOP2	BB/Diuretic	ACEI or CCB	97	1954	190	3923	2.57	1.04	[0.64;	1.67]	1.7%
SYSTEUR	CCB	Placebo	107	2165	78	2069	9.46	1.15	[0.99;	1.34]	15.7%
TRANSCEND	ARB	Placebo	205	1889	238	1905	5.00	0.86	[0.71;	1.03]	10.7%
PRoFESS	ARB	Placebo	112	7108	136	7103	4.41	0.80	[0.45;	1.40]	1.2%
Overall								**0.89**	**[0.84;**	**0.95]**	

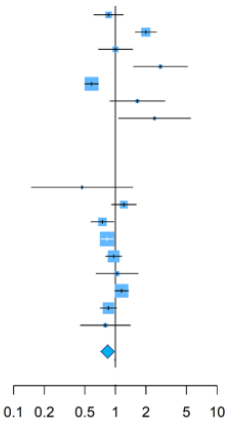
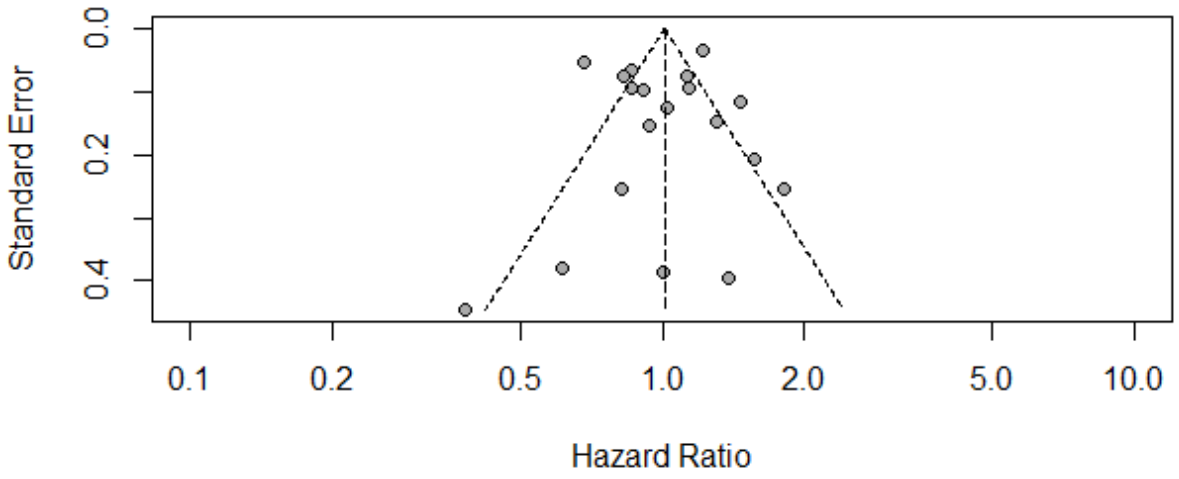


Figure 3-5. Funnel plot for assessment of publication (acquisition) bias on the effect of blood pressure reduction and risk of new-onset type 2 diabetes.

Egger's regression test: T statistics = - 0.2, df = 17, bias coefficient -0.22, standard error 1.09, p-value = 0.83.



3.5. Discussion

Using individual participant data analyses of RCTs including 145,929 individuals and 9,883 cases of new-onset type 2 diabetes, I found evidence for the preventive effect of blood pressure lowering on the risk of new-onset type 2 diabetes. On average, a 5 mmHg reduction in systolic blood pressure reduced the risk of type 2 diabetes by 11%.

Previous observational evidence has shown conflicting associations between elevated blood pressure and the risk of new-onset type 2 diabetes. For instance, in a prospective cohort analysis of 7,735 participants with 12.8 years follow-up, no association was found between elevated systolic blood pressure and type 2 diabetes;[86] and the Whitehall II Study, a prospective occupational cohort study that included 10,308 participants at baseline, showed no increased risk of type 2 diabetes per unit increase of systolic blood pressure.[87] A large observational cohort study, including 4.1 million UK adults and about 186,000 diabetes cases, revealed a 58% higher risk of new-onset diabetes per 20 mmHg increase in systolic blood pressure.[73] Although this study has been the most comprehensive analysis to date, due to its non-randomised design, it could not reliably rule out residual confounding effects and reverse causation.

Previous reports from individual randomised controlled trials have not been able to fill this knowledge gap, in part because analyses were focused on drug classes as opposed to blood pressure reduction per se.[75,83] Results from a secondary data analysis of the SPRINT trial, which included 8,380 people with no

history of diabetes at baseline, showed an increased risk of type 2 diabetes of 19% among those who received an intensive strategy for blood pressure lowering compared to those in the standard arm, but the difference was not statistically significant (adjusted HR, 1.19 [95% CI, 0.95 to 1.49]).[75] However, this secondary data analysis of the SPRINT trial has several limitations. First, it is unclear whether the lack of an effect is due to the blood pressure reduction per se or to the interaction between different classes of antihypertensive medications used in the intensive blood pressure lowering arm (we answered this important question in the next chapters). Second, the length of time that participants were followed up in the SPRINT trial was insufficient to identify enough cases of type 2 diabetes for the study (median follow-up time 3 years). This could be an important justification for the null finding in this analysis. We were able to have sufficient statistical power for analysis by pooling the data from the individual participants of the randomised clinical trial, with a significantly higher total number of events (number of cases 9,883).[48] These uncertainties have prevented international guideline committees from making firm recommendations on the relative merits of pharmaceutical and non-pharmaceutical approaches to lowering blood pressure in the primary prevention of type 2 diabetes.[88,89]

This gap in the evidence is filled by the findings of our study, which evaluates the effects of a standardised fixed degree of blood pressure decrease using IPD derived from RCTs. We have shown that hypertension, or high blood pressure, is in fact a modifiable risk factor for new-onset type 2 diabetes in people who have not previously been diagnosed with diabetes, with a relative effect size comparable to that seen for prevention of major cardiovascular events.[54] The evidence that lower

blood pressure is associated with a lower risk of diabetes provides clinicians and those who make decisions about health policy with an opportunity to modify disease risk. This can be done, for example, either through the use of appropriate antihypertensive medications or by promoting lifestyle behaviours that are known to reduce blood pressure, such as maintaining a healthy weight through physical activity[90] and a balanced diet.[91] On the other hand, these findings have significant implications for clinical practice given the disappointing results of pharmacological interventions through glucose-modifying pathways and the well-known increase in the risk of type 2 diabetes with lipid-lowering medications as one of the main strategies for cardiovascular disease risk prevention.[51,54,92] Randomised controlled studies have shown that lipid-lowering medication, especially statin therapy, is associated with a 10% increased risk of new-onset type 2 diabetes.[93,94] This effect, which has also been proven in genetic studies, is regarded to be one of the most significant adverse effects of pharmacological lipid-lowering therapy.[95,96] In this context, our findings that blood pressure reduction reduces the risk of new-onset type 2 diabetes will highlight the importance of a blood pressure reduction strategy in high-risk populations.

This part of the project has some limitations. Although we were unable to get data from several eligible RCTs, our results did not show any evidence of acquisition bias. Another limitation that can result in case ascertainment bias is the fact that diabetes was not the primary outcome in any of the included trials. However, randomised trials are robust to bias from case ascertainment and the main risk resulting from incomplete case identification is the dilution of the true treatment effects.[97] To evaluate this issue further, we retrieved data on the method of diabetes

identification at baseline, diabetes detection during follow-up, and the incidence rate. We found that the overall incidence of diabetes was lower in trials that relied mostly on adverse event reports as opposed to those that used more extensive laboratory testing. Nonetheless, relative risk reductions were comparable across trial groups with different methods of case ascertainment; this finding supports the validity of the overall estimation and the study's results.

Using randomised evidence from major pharmacological blood pressure lowering trials, this study has shown consistent evidence to suggest that the preventive effect of blood pressure reduction on type 2 diabetes risk is causal, and therefore reducing blood pressure is likely to prevent new-onset type 2 diabetes. The results of this study have been published in the Lancet, and parts of it were used for the writing of this chapter.[48]

Chapter 4. Effect of each class of blood pressure-lowering drugs on the risk of type 2 diabetes: Individual participant data network meta-analysis

4.1. Introduction

Using IPD data from RCTs, I show in Chapter 3 that reducing systolic blood pressure is a modifiable risk factor for new-onset type 2 diabetes, with a 5 mmHg reduction in systolic blood pressure associated with an 11% decrease in risk of new-onset type 2 diabetes. However, a crucial question remained unanswered, which I addressed in this chapter.

There is evidence that each class of blood pressure-lowering drugs affects the risk of newly-onset type 2 diabetes differently. This potentially opposing effects of blood pressure-lowering drug classes on the risk of type 2 diabetes has further complicated previous research and contributed to the existing uncertainties around the link between blood pressure and type 2 diabetes. For instance, several individual RCTs have shown that renin-angiotensin system inhibitors decrease the risk of new-onset type 2 diabetes,[98-100] while diuretics do the opposite.[83,101] Even less evidence exists for the effects of other classes of blood pressure-lowering drugs on the risk of diabetes. Several observational studies attempted to investigate the association between blood pressure-lowering drugs and the risk of diabetes; however, the results of these studies have been limited, mainly as a result of inadequate adjustments, small sample sizes, and the natural limitations of using observational evidence to evaluate drug effects.[102-105] Furthermore, evidence from individual RCTs and/or their secondary data analysis has been limited. In the ALLHAT trial, the risk of developing incident type 2 diabetes was shown to be 1.4 times greater in the

chlorthalidone (thiazide-like diuretics) arm compared to the lisinopril (Angiotensin-converting enzyme inhibitors [ACEIs]) arm.[106] The ASCOT trial reported a lower risk of incident diabetes in the amlodipine (calcium channel blockers) arm compared to atenolol (beta blockers).[107] However, the evidence from RCTs is diverse and it is difficult to determine the distinct effect of each drug class, mostly because each study utilised a different comparison group and drug class as an intervention.

To achieve a better understanding of the distinct effects of each drug class, we need to choose a more uniform comparison group and combine the data in a more efficient manner. To deal with this situation, in this chapter I used the IPD network meta-analysis model to compare the effect of different antihypertensive drugs with placebo on the risk of diabetes.

4.2. Objective

- To investigate the separate effects of blood pressure-lowering drug classes on the risk of new-onset type 2 diabetes using randomised clinical trial data.

4.3. Methods

In this part of the project, similar to Chapter 3, I used the BPLTTC dataset to conduct an IPD network meta-analysis. 22 trials with 167,107 randomised participants were included in this IPD network meta-analysis to assess the effect of each class of antihypertensive drugs on the risk of new-onset diabetes (**Figure 3-1**). In an approach similar to that described in Chapter 3, I did not include individuals who had a known diagnosis of diabetes at basline or trials that were specifically

conducted on patients with diabetes. The inclusion and exclusion criteria, as well as the definition of the outcome, were the same as in the one-stage IPD meta-analysis described in Chapter 3, with the exception that in this chapter I included trials for which the information about the time of incidence for diabetes was not available (**Table 3-3**).

I calculated the odds ratio (OR) for each potential comparison using patient-level data for each trial and the logistic regression model, excluding all known cases of diabetes from the baseline. Because data on the time of occurrence of type 2 diabetes was not available for some trials (**Table 3-3**), I estimated the OR using the logistic regression model, which allowed me to utilise all available data from trials to have better statistical power.

I used a Bayesian fixed-effect network meta-analysis model to evaluate the effect of major blood pressure-lowering drugs versus placebo on diabetes risk.[108,109] When there is limited direct evidence from individual trials for comparison, the network meta-analysis approach can incorporate all direct (randomised comparison within trials) and indirect (non-randomised evidence across trials) evidence and enables drug effect comparisons using a common comparator. For instance, it is challenging to obtain a reliable estimation of the separate effect of each class of drug versus a placebo due to the low number of RCTs that compare one particular class of blood pressure-lowering drugs with a placebo.

To perform the network meta-analysis model, I used the Markov chain Monte-Carlo simulation method with four chains and 100,000 iterations after an initial burn-

in of 10,000.[109] I utilised the network graph to have a better understanding of the geometry of the network before I started the data analysis. The network geometry outlines which treatments have been subjected to direct comparisons in RCTs and which can only be informed via an indirect route. The Nodesplit analysis was done to check the consistency of the network model. I used ‘gemtc’ package in R software to run this analysis. The development of network meta-analysis models and node-splitting models to uncover inconsistency are all facilitated by this package. Estimation of models is done using JAGS (through the rjags package).

4.4. Results

Twenty-two trials that evaluated antihypertensive drug treatments and collected diagnostic information for incident diabetes were included in this analysis (**Table 3-3**). Of the 22 included trials, eight were placebo-controlled, and 14 were head-to-head trials. The calculated effect sizes for each trial and structure of the dataset used for Bayesian network meta-analysis are shown in **Table 4-1**.

Table 4-1. Structure of data used for Bayesian network meta-analysis.

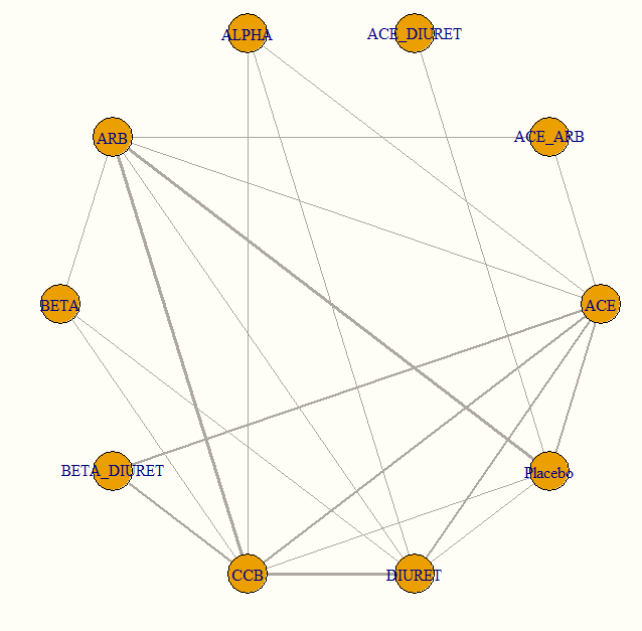
Trial	Treatment	Log odds ratio	Standard error
ACTIVE I	ARB	-0.087	0.101
ACTIVE I	Placebo	Ref	Ref
ALLHAT	ACEI	Ref	0.042
ALLHAT	Alpha-blocker	-0.236	0.063
ALLHAT	CCB	0.180	0.058
ALLHAT	Diuretic	0.340	0.051
ANBP	Diuretic	0.065	0.386
ANBP	Placebo	Ref	Ref
ANBP2	Diuretic	0.290	0.112
ANBP2	ACEI	Ref	Ref
ASCOT-BPLA	BB	Ref	Ref
ASCOT-BPLA	CCB	-0.379	0.057
CAPPP	BB and diuretic	0.119	0.077
CAPPP	ACEI	Ref	Ref
CASEJ	ARB	Ref	Ref
CASEJ	CCB	0.456	0.211
COLM	Diuretic	0.306	0.398
COLM	CCB	Ref	Ref
COPE	ARB	Ref	0.226
COPE	BB	0.657	0.281
COPE	Diuretic	0.503	0.288
HOPE	ACEI	-0.421	0.130
HOPE	Placebo	Ref	Ref
INSIGHT	Diuretic	0.272	0.117
INSIGHT	CCB	Ref	Ref
MOSES	ARB	Ref	Ref
MOSES	CCB	-0.455	0.359
NORDIL	BB and diuretic	0.141	0.095
NORDIL	CCB	Ref	Ref
ONTARGET	ACE	Ref	0.054
ONTARGET	ARB or ACEI	-0.104	0.078
ONTARGET	ARB	0.119	0.075
PEACE	ACEI	-0.186	0.078
PEACE	Placebo	Ref	Ref
PROGRESS	ACEI and/or diuretic	-0.062	0.156
PROGRESS	Placebo	Ref	Ref
STOP2	ACE	Ref	0.103
STOP2	BB and/or diuretic	-0.005	0.146
STOP2	CCB	-0.052	0.148
SYSTEUR	CCB	0.281	0.152
SYSTEUR	Placebo	Ref	Ref
TRANSCEND	ARB	-0.169	0.100
TRANSCEND	Placebo	Ref	Ref
VALUE	ARB	Ref	Ref
VALUE	CCB	0.256	0.055
PRoFESS	ARB	-0.198	0.128
PRoFESS	Placebo	Ref	Ref

Ref: reference category for calculation of odds ratio
Analysis comparing the effects of drug classes was not standardised for the intensity of blood pressure reduction. This was to account for potential variations in blood pressure-lowering efficacy, tolerability, or non-blood pressure-mediated effects of the different drug classes.
CCB: calcium channel-blocker; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; BB: beta blocker

The network graph and treatment nodes are presented in **Figure 4-1**. This figure illustrates the overall structure of comparisons in the network, enabling us to understand which treatments were compared with each other in the BPLTTC dataset. The edges in the network have varying thicknesses, indicative of the amount of information (sample size) available for any specific comparisons in our database.

Figure 4-1. The network plot exhibits the treatment arms included in the network meta-analysis.

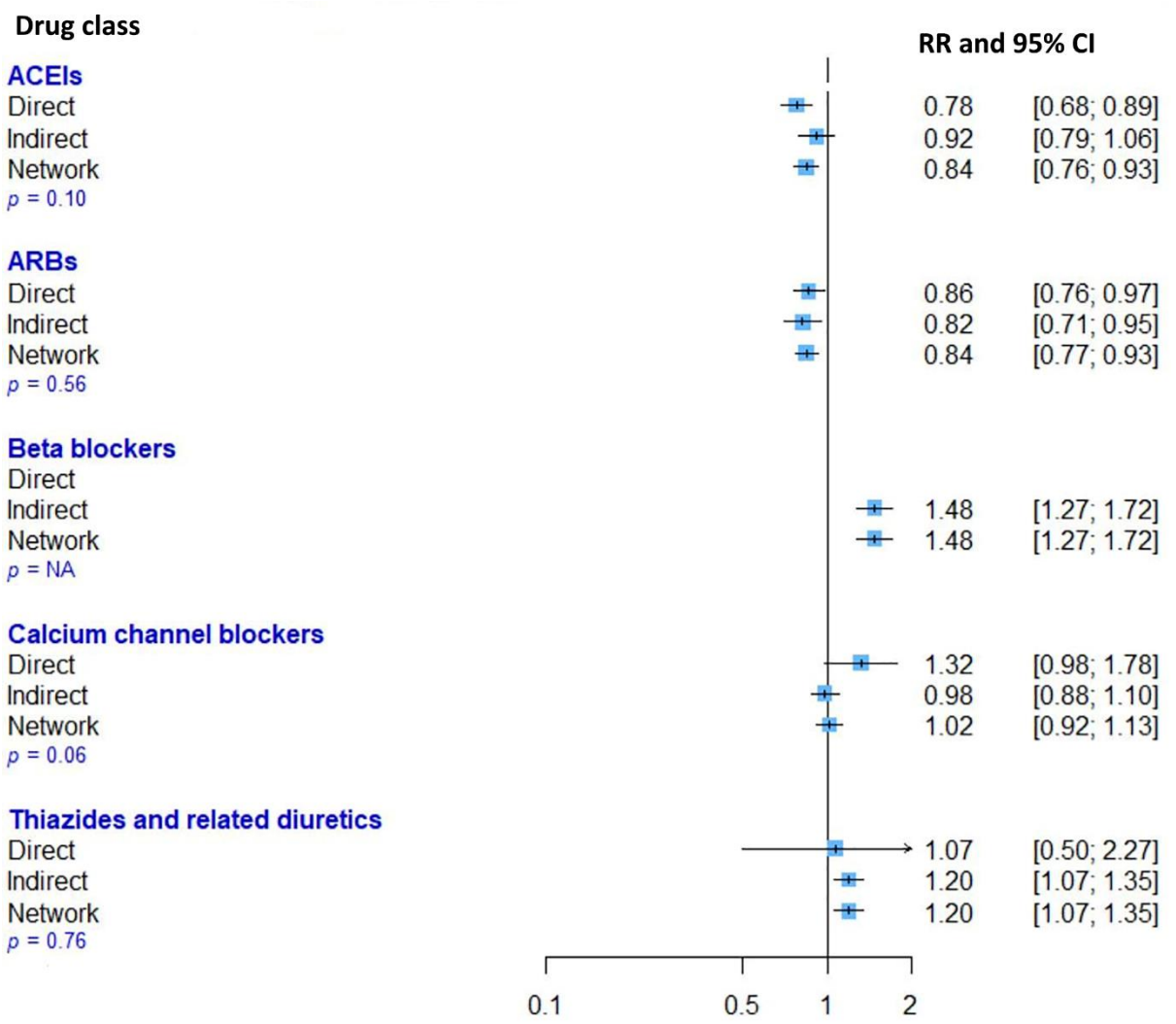
ALPHA: Alpha-blockers, ACE_DIURET: angiotensin-converting enzyme inhibitors AND Diuretics; ARB: angiotensin receptor blockers; BETA: beta-blocker; ACE_ARB: angiotensin-converting enzyme inhibitors AND angiotensin receptor blockers; ACE: angiotensin-converting enzyme inhibitors; BETA_DIURET: beta-blocker AND Diuretic; CCB: calcium channel blocker



The net splitting plot confirmed the consistency of the network model, except for calcium channel blockers where a marginal inconsistency was suggested (p =0.06) (**Figure 4-2**). In addition, all information on beta-blockers is derived from indirect evidence, therefore no direct comparison could be performed.

Figure 4-2. Node-splitting analysis to assess the inconsistency of the network model.

The plot shows the estimates when we used only direct comparison, only indirect comparison, and a combination of both (network estimates). Significant heterogeneity (p-value in the plot) between direct and indirect estimates is an indicator of inconsistency in the model.



I found that ACEIs and angiotensin II receptor blockers (ARBs) classes of drugs reduce the risk of diabetes compared with placebo (OR 0.84 [95% CI 0.76 to 0.93] for ACEIs and OR 0.84 [95% CI 0.77 to 0.93] for ARBs). The network estimates revealed no effect for calcium channel blockers (OR 1.02 [95% CI 0.92 to

1.13]) while beta-blockers and thiazide diuretics were found to increase the risk of diabetes compared with placebo (OR 1.48 [95% CI 1.27 to 1.72] for beta-blockers and OR 1.20 [95% CI 1.07 to 1.35] for thiazide diuretics).

4.5. Discussion

The result from Chapter 3 using a one-stage IPD meta-analysis of RCTs provides compelling evidence for the effect of blood pressure lowering per se on the risk of new-onset type 2 diabetes. I found that each 5 mmHg reduction in systolic blood pressure, on average, reduces the risk of diabetes by about 10 per cent. However, in the current chapter using an IPD network meta-analysis approach, I showed that the effects are not consistent across different classes of antihypertensives. While I found strong evidence that ACEIs and ARBs reduce the risk of diabetes, the use of beta-blockers and thiazide diuretics showed an increase in risk. Calcium channel blockers had no material influence on diabetes risk.

ARBs and ACEIs are least related to the incidence of new-onset diabetes, followed by calcium channel blockers, placebo, beta-blockers, and thiazide diuretics (reference group), according to the only network meta-analysis utilising aggregate data.[83] When the placebo was applied as a control group, they found that ACEIs had no statistically significant effect (OR 0.87, 95% CI 0.75 to 1.01) whereas ARBs had a substantial protective effect (OR 0.75, 95% CI 0.61 to 0.91).[83] One of the limitations of this aggregate data network meta-analysis was that a high proportion of evidence was estimated through indirect comparisons as well as low statistical power for the effect of ACEIs effect. As a consequence, the clinical implications of these findings

remain uncertain. Thus, it remains to be established whether the ACEIs and ARBs class of drugs had a net beneficial or adverse effect (in comparison to a placebo) on the incidence of new-onset diabetes. By using IPD-level data from RCTs, I addressed this question more directly. My study, with consistent results from direct and indirect comparisons, suggests that in comparison to the placebo, ACEIs or ARBs lead to a noticeable reduction in the risk of new-onset diabetes. This result was in keeping with the previous evidence that suggested the protective effect of ACEIs/ARBs.[98-100,110] By including large trials such as TRANSCEND and PROFESS in my analysis, I provided more robust and direct evidence on the similarities of the effects of ACEIs and ARBs on diabetes risk than previous aggregate data network meta-analyses.[83] Possible biological mechanism for this protective effect is the improvement of insulin resistance by suppression of inflammatory mediators, such as C-reactive protein and reactive oxygen species.[111]

My findings, which were consistent with prior aggregate data meta-analysis results,[83,112] indicated that the effect of calcium channel blockers is neutral, which did not support the notion that calcium channel blockers may prevent diabetes.[113] The strength of my research is that it was entirely based on IPD data from large-scale RCTs, which allowed me to exclude known cases of diabetes from the baseline and also provided a larger number of diabetes cases than earlier studies. The individual-level data included in my research was all of high quality and seemed to have enough statistical power to detect any clinically important effect of calcium channel blockers on the risk of the outcome. Experimental studies suggested that verapamil inhibits TXNIP expression in INS-1 cells and human islets, and oral administration of verapamil reduces TXNIP expression and beta-cell apoptosis, increases

endogenous insulin levels, and protects mice against diabetes.[113] According to animal research, Verapamil also improved beta-cell survival and enhanced glucose homeostasis and insulin sensitivity.[113] However, neither a beneficial effect nor an increase in risk was found in my research. This might be because the magnitude of the preventive effect is too small to have a clinical relevance on type 2 diabetes risk in a clinical setting. In addition, I did not evaluate the impact of calcium channel blockers on diabetes biomarkers such as insulin sensitivity or glucose tolerance; thus, more clinical and experimental research is necessary to investigate the effect of calcium channel blockers on diabetes biomarkers.

My study shows that beta-blockers versus placebo could increase the risk of type 2 diabetes by 48%. This was not in line with the result of a previous study that showed a null effect for this class of drug (OR 1.17 [95% CI 0.98 to 1.40]).[83] Furthermore, studies conducted in clinical settings showed that blood pressure reduction with beta-blockers resulted in a considerable reduction in insulin sensitivity in hypertensive individuals.[114] It is anticipated that this worsening of insulin sensitivity induced by beta-blockers would have a direct detrimental impact on glycaemic management in individuals who suffer from both hypertension and type 2 diabetes. Most evidence from observational studies and RCTs suggests that beta-blockers increase the risk of type 2 diabetes, particularly in patients with elevated blood pressure. For example, in the Atherosclerosis Risk in Communities cohort study, people taking beta-blockers had a 28% increased risk of type 2 diabetes after accounting for all potentially important confounders.[80] In the LIFE trials, 9,193 patients with hypertension and left ventricular hypertrophy were randomly assigned to receive blood pressure-lowering treatment based on losartan (ARBs) or atenolol

(beta-blocker) for at least four years. In patients without diabetes at the time of randomisation, the incidence of new-onset type 2 diabetes was 25% lower among those receiving losartan compared to those receiving atenolol-based treatment (relative risk [RR] 0.75 [95% CI 0.55 to 0.88]).[115] Overall, these findings support clinical guidelines classifying beta-blockers as a low-priority class for treating hypertension in general and especially in people with diabetes.[88,116]

Using individual trial data, it is challenging to estimate the net benefit of beta-blockers since most trials compared calcium channel blockers or ACEIs to standard treatment that included diuretics or beta-blockers in different combinations. However, in this Chapter, using the IPD network meta-analysis method, I provided evidence for the adverse effect of beta-blockers versus placebo on the risk of type 2 diabetes. As shown in my network analysis, there was limited direct evidence from trials to compare the effect of beta-blockers with the placebo. But, this is the strength of the network meta-analysis method, in which we could combine direct and indirect evidence to have a better view of the effect of a specific class of drug. To deal with the limitation of indirect comparison, I will use genetic data analysis to further investigate this effect in Chapter 6.

On the other hand, my analysis showed a 20% increase in the risk of new-onset type 2 diabetes with diuretics usage. Similar to beta-blockers, the evidence for the direct effect of diuretics on the risk of diabetes was limited. Out of several placebo-controlled trials of blood pressure-lowering pharmacological therapy, only two trials with a limited number of new-onset diabetes events assessed the effect of diuretics against the placebo and confirmed a neutral effect. In the SHEP trial, which

comprised 4,736 participants who were randomly assigned to receive a placebo or therapy with chlorthalidone (thiazide-like diuretics), 8.6% of the active treatment group and 7.5% of the placebo group reported new cases of diabetes (RR 1.14 [95% CI 0.90 to 1.45]).[76] Similarly, the EWPHE trial, which included 840 participants, investigated the effect of diuretics versus placebo and found no significant difference in the incidence of diabetes between the two groups (risk ratio 1.47 [95% CI 0.84 to 1.57]).[81] Similar to my findings, the previous aggregate data meta-analysis revealed a 30% increase in diabetes risk for diuretics compared to placebo (OR 1.30 [95% CI 1.07 to 1.58]).[83]

Although the exact biological mechanism by which diuretics affect diabetes is not yet understood, potassium-related metabolic justifications have been suggested. Hypokalemia has long been suspected to have a role in the deterioration of glucose tolerance in individuals receiving diuretics.[117] There is evidence of a substantial negative association between lower potassium levels and higher glucose levels, which may result in diabetes.[118] The mechanism underlying this glucose elevation may include insulin secretion, in which hyperkalemia increases insulin secretion and causes cellular potassium uptake.[119] This suggests that decreased plasma potassium levels may hinder insulin production and therefore raise plasma glucose levels. More experimental studies are required to get better mechanistic insights into diuretic-Induced diabetes.

This study has some limitations that should be discussed. All of the limitations discussed in Chapter 3 pertain here. Furthermore, since we did not account for the effect of dose in the analysis, the results of this research represent the average

dosage of medications used in the trials. As a result, it cannot establish whether the treatment dose plays a role, which warrants further investigations. Finally, in the estimation for beta-blockers, I only used indirect comparison, which, similar to observational studies, is prone to bias and confounding. However, in the next Chapters, I will use genetic data to re-evaluate the effect of each drug class on diabetes risk.

Up until this point, I found evidence to support the hypothesis that lower systolic blood pressure is associated with a reduced risk of developing type 2 diabetes. In this chapter, I demonstrated that this protective effect is not consistent among the different classes of antihypertensive drugs. Beta-blockers and thiazide diuretics were shown to increase the risk of developing diabetes, in contrast to ACEIs and ARBs, which were found to reduce the risk. The results of this study have been published in the Lancet, and parts of it were used for the writing of this chapter.[48]

Chapter 5. Effect of blood pressure-lowering on the risk of type 2 diabetes: A Mendelian randomisation study

5.1. Introduction

In Chapter 3, using IPD data of RCTs, I showed that each 5 mmHg reduction in systolic blood pressure reduced the risk of new-onset type 2 diabetes by 11%. Although blood pressure-lowering RCTs and corresponding meta-analyses are given the highest quality of evidence, the nature of data or study designs, including short-term follow-up and limited generalisability of findings in trials with a focus on high-risk patients precluded direct translation of previous evidence into the clinical practice. In this chapter, I will re-test the same hypothesis using genetic data in order to further evaluate the validity of my results from the IPD meta-analysis and also to check the effect of blood pressure lowering on data that is more representative of the general population.

Several previous Mendelian randomisation studies have tried to assess the causal link between blood pressure and the risk of type 2 diabetes, all with inconsistent findings. For example, in a bidirectional Mendelian randomisation using the UK Biobank, investigators reported that type 2 diabetes may causally affect hypertension as a binary outcome, while the effect of hypertension on diabetes is unlikely to be causal.[77] Another Mendelian randomisation using summary statistics from GWAS meta-analysis revealed a 2% increase in the risk of diabetes per 1 mmHg genetically determined higher systolic blood pressure.[78] In light of the inconclusive findings from previous studies and also the global prevalence of hypertension and diabetes, in Chapter 3 I tried to first establish the effect using IPD

meta-analysis of RCTs and then investigate the possible causal effect using Mendelian randomisation approaches in the current Chapter.

Recent advances in GWAS studies have made it possible to use genetic information for assessing causal relations and drug-target effects. Mendelian randomisation is a study design that takes the opportunity of naturally randomised genetic variants during conception to mimic the random allocation process in RCTs and thus establish causal inference.[120] This feature can be used as a complementary design to overcome the limitations of RCTs. The main novelty of this thesis lies in the innovative idea of integrating large-scale epidemiological and genetic biobank data with evidence from RCTs to answer important questions that no single data resource or method alone could address.[121]

5.2. Objective

- To assess the possible causal association between blood pressure lowering and risk of type 2 diabetes using Mendelian randomisation approaches

5.3. Methods

For this part of the thesis, the exposure was genetically-influenced systolic blood pressure used as an instrumental variable, which was estimated using genetic variants with minor allele frequency >0.01 that were independently (linkage disequilibrium $r^2 < 0.05$) associated with systolic blood pressure at a genome-wide significance level ($P < 5 \times 10^{-8}$). Overall, 246 genetic variants were selected from a GWAS meta-analysis, including over one million participants of European ancestry (**Appendix Table 1**).[122] I extracted the corresponding beta coefficients and standard

errors from the International Consortium for Blood Pressure GWAS (ICBP),[123] which did not include the UK Biobank, in order to avoid weak instrument bias resulting from the overlap between the GWAS selected for exposure and outcome[124] (UK Biobank contributing to both). ICBP is a GWAS meta-analysis, including about 200,000 participants from European countries, and its estimations were adjusted for sex, age, age-squared, body mass index, within-cohort stratification, and also for blood pressure-lowering medication use. The ICBP analyses were conducted using a linear regression model and combined across studies using inverse-variance weighted meta-analysis.[123]

The summary statistics for variants associated with diabetes were extracted from the GWAS, including 21,147 type 2 diabetes cases and 434,460 controls from the European subset of UK Biobank participants.[125] In this GWAS study, diabetes outcomes were defined using UK Biobank self-reports of the disease and ICD-10 diagnostic codes, and analysis was controlled for age and sex, population stratification, relatedness, and polygenic effect.[125] I used a two-sample Mendelian randomisation framework and a random-effect inverse variance weighted method to estimate the effect, along with several sensitivity analyses. In the following, I will discuss the details of statistical analyses.

The summary estimations of variants-exposure and variants-outcome were harmonised before conducting the statistical analysis.[124,126] The inverse-variance weighted method has been used as the main method and assumes that either all the instruments are valid or any horizontal pleiotropy is balanced.[127] I applied various

Mendelian randomisation methods with different assumptions as sensitivity analyses to check the robustness and reliability of our findings.

I employed the weighted median method,[128] which is consistent if at least 50% of the weight comes from valid instrumental variables.[129] The Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method was used to test and, if needed, to correct for any possible horizontal pleiotropic outliers in the analysis.[130] The MR-Egger regression method was used to assess the presence of pleiotropy.[131] Although the MR-Egger method is a worthwhile sensitivity analysis for detecting pleiotropy, it is susceptible to outlier genetic variants.[132] Therefore, I calculated Cook's distance measure to detect the outlier variants and then re-ran the MR-Egger analysis after removing the outlier variants.[132,133] Robust Adjusted Profile Score (RAPS) estimator is robust to systematic and idiosyncratic pleiotropy and is recommended for complex traits and diseases.[134] MRMix method provides unbiased estimation in the presence of a large number of invalid genetic instruments. A methodological study suggested that MRMix produces a more robust estimation compared to other conventional approaches.[135] Finally, I used Steiger filtering to remove genetic variants that are likely associated with diabetes through other causal pathways other than blood pressure.[136] Furthermore, I examined the heterogeneity of the estimates by using a scatter plot and applying Cochran's Q test.[137] I also assessed the probable directional pleiotropy using a funnel plot similar to that being used to assess for publication bias in meta-analysis.[137] A leave-one-out sensitivity analysis was conducted by removing a single variant from the analysis in turn. The fluctuation of the estimates in response to excluding each variant reflects the possibility of an outlier variant in the effect estimation. The

'MendelianRandomization' and 'TwoSampleMR' packages for R were used to implement the Mendelian randomisation analyses.[138,139]

The GWAS studies with blood pressure as the phenotype of interest routinely adjust for the effect of body mass index.[122,123] Using body mass index-adjusted GWAS summary estimates to undertake two-sample Mendelian randomisation might induce collider bias. Therefore, we explored whether the identified association is driven by body mass index using unadjusted blood pressure estimations and by including body mass index as a phenotype in multivariable Mendelian randomisation. The UK Biobank dataset was used to derive the unadjusted estimates.[140] We used multivariable Mendelian randomisation through the inverse-variance weighted method to calculate adjusted versus unadjusted effect estimations.[141,142]

Additionally, I tested the validity of the analysis by examining the effect between systolic blood pressure and coronary heart disease, myocardial infarction, and ischemic stroke as positive control outcomes. For this analysis, we utilised the same genetic variants for systolic blood pressure, but the variants-outcome association was extracted from independent GWAS studies.[143,144]

In a sensitivity analysis to further replicate the findings using different GWAS data, we extracted variants-outcome estimations from stage 1 of the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium.[145] The first stage of DIAGRAM consisted of 12,171 diabetes cases and 56,862 controls across 12 GWAS studies of individuals of European descent. In DIAGRAM, each genetic

variant with a minor allele frequency of >1% passing quality control was tested for association with diabetes under an additive model.

To further replicate the result of two-sample Mendelian randomisation through a different framework, I followed a one-sample Mendelian randomisation approach using individual participant data from the UK Biobank. I used the UK Biobank data, which is a large prospective cohort study that included 502,602 participants aged 40 to 69 years, recruited between 2006 and 2010 from 22 assessment centres across the United Kingdom. Details of the UK Biobank design have been published elsewhere.[146] UK Biobank genotype data were imputed with IMPUTE4 using the Haplotype Reference Consortium and the UK10K + 1000 Genomes panel to identify ~96 million variants for 487,381 participants.[64] I excluded 55,208 individuals who were not white British, had a variant call rate <98% and were outliers based on heterozygosity. Finally, I included 432,173 participants in the one-sample Mendelian randomisation study. I built a weighted polygenic risk score as an instrumental variable for systolic blood pressure using independent genetic variants (linkage disequilibrium $r^2 < 0.05$) with minor allele frequency > 0.01 and $P < 5\times10^{-8}$ at a genome-wide level. Overall, 246 genetic variants were selected, all with imputation quality > 0.9 that have been shown to be associated with systolic blood pressure in a GWAS meta-analysis, including over one million participants of European ancestry.[122] To build a genetic risk score, first, each variant was recoded additively (0, 1, and 2) according to the number of alleles that decrease the log beta of systolic blood pressure. Then, each variant was weighed according to the regression coefficient obtained from the GWAS meta-analysis to give more weight to variants with stronger effects.[147] A weighted genetic risk score was constructed using the

following formula: ($\beta_1 \times variant_1 (+) \beta_2 \times variant_2 (+ \cdots (\beta_n \times variant_n)$, where β_i was the regression coefficient associated with $variant_i$ and obtained from the GWAS study.[122]

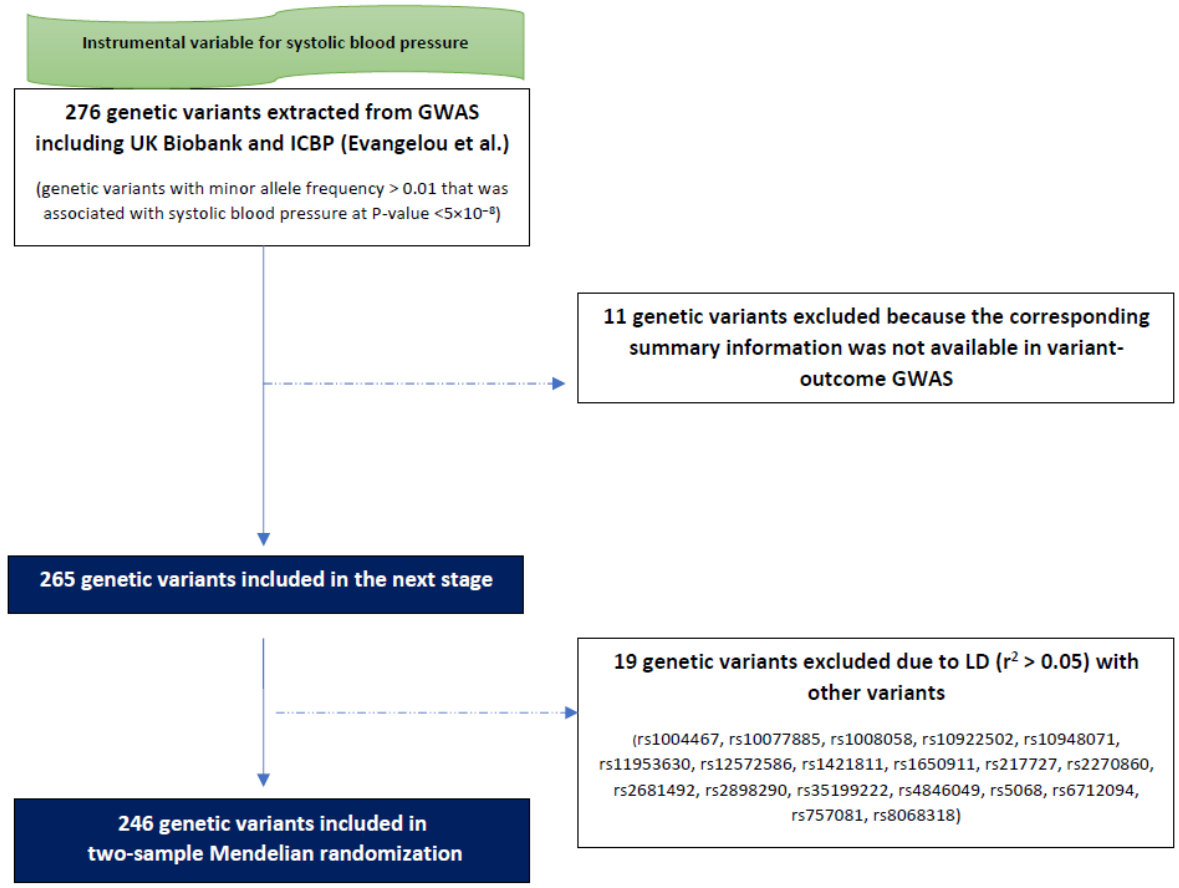
Additionally, I replicated the one-sample Mendelian randomisation using a new genetic risk score. In this sensitivity analysis, to build the genetic risk score, I selected 370 genetic variants that have been reported to be associated with systolic blood pressure (linkage disequilibrium $r^2 < 0.05$, minor allele frequency > 0.01 and $p < 5 \times 10^{-8}$ at a genome-wide level which passed quality control) in the final ICBP genome-wide association dataset included 77 cohorts (n = 299,024, no overlap with UK Biobank).[123] The instrumental variable analysis was performed using an adjusted, two-stage predictor substitution method that used the unweighted genetic risk score as an instrument variable.

5.4. Results

The selection of reliable instrumental variables for the exposure of interest is the most crucial element of any Mendelian randomisation study. My quality control process for variant selection returned 246 eligible variants for use as an instrumental variable in the two-sample Mendelian randomisation (**Figure 5-1**).

Figure 5-1. The genetic variant selection workflow for systolic blood pressure.

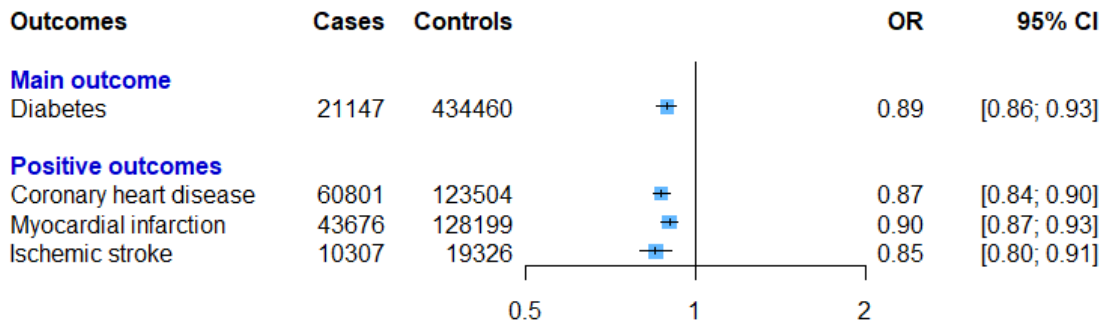
LD: linkage disequilibrium; GWAS: genome-wide association study



Using two-sample Mendelian randomisation, I found that each 5 mmHg genetically predicted lower systolic blood pressure was associated with an 11% lower risk of type 2 diabetes (OR: 0.89 [95% CI 0.86 to 0.93]) (**Figure 5-2**). The positive control analysis revealed strong associations between systolic blood pressure reduction and lower risk of coronary heart disease (OR: 0.87 [95% CI 0.84 to 0.90]), myocardial infarction (OR: 0.90 [95% CI 0.87 to 0.93]), and ischemic stroke (OR: 0.85 [95% CI 0.80 to 0.91]) further supporting the validity of our instrumental variable (**Figure 5-2**).

Figure 5-2. Mendelian randomisation estimates for the association between genetically predicted 5 mmHg systolic blood pressure reduction and diabetes as the main outcome, and coronary heart disease, myocardial infarction, and ischemic stroke as positive control outcomes.

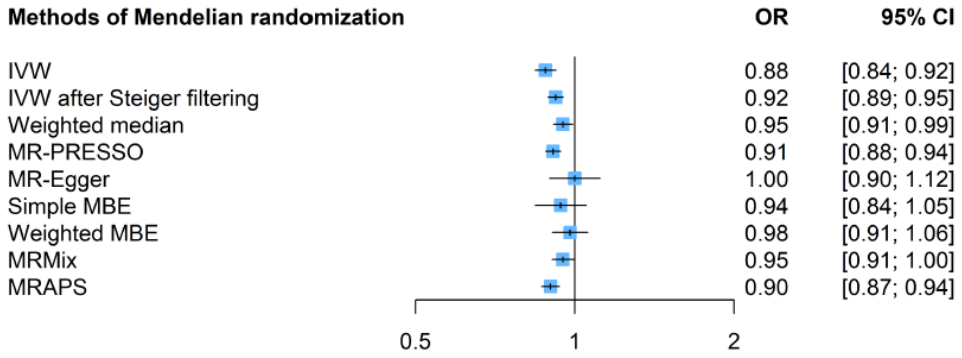
Solid squares represent point estimates and vertical lines represent 95% confidence intervals (CI). Cases and controls: number of cases and controls in genome-wide association studies. Odds ratio (OR): estimated using the inverse-variance weighted method



In sensitivity analysis, I observed that the estimate from the main model (IVW random effect model) and other methods are not consistent when utilising various analytical techniques for Mendelian randomisation (**Figure 5-3**). In particular, when the IVW method shows significant findings but the MR-Egger effect estimation is attenuated and MR-Egger intercept as an indicator of average pleiotropic effect is not zero, this is an alarming sign for possible outlier variants that could affect the estimation.

Figure 5-3. The association between systolic blood pressure and risk of diabetes estimated by random-effect inverse variance weighted and applied various sensitivity analysis methods of two-sample Mendelian randomisation.

Blue squares represent the point estimation and size of squares is the same. The horizontal solid lines represent 95% confidence intervals. The MBE method was implemented using both simple and weighted options with bandwidth ϕ = 1 under the no measurement error (NOME) assumption.OR: odds ratio



Therefore, I calculated Cook's distance measure to detect the outlier variants and then re-estimated the Mendelian randomisation results after removing the outlier variants. Cook's distance is a common measurement of a data point's influence. It is a way to find influential outliers in a set of predictor variables when performing a least-squares regression analysis. The following variants stood up as outliers when I used this method: rs10274928, rs11191548, rs12454712, rs1446468, rs17249754, rs17477177, rs2972146, rs34072724, rs4841569, rs5219, rs6712203, rs9368222 (**Figure 5-4**). By eliminating the outlier variants, the findings became more in line with one another, and MR-egger estimates verified that the likelihood of a pleiotropic effect is low (**Figure 5-5, Panel B**). We can be confident in the estimate from the main IVW method when it is consistent across many statistical approaches of

Mendelian randomisation, each of which makes a unique assumption about pleiotropic effects.

Figure 5-4. Bar plot of Cook's distance to detect genetic variants that strongly influence fitted values of the model.

Any variants over 4/n are considered an outlier, where n is the number of included genetic variants.

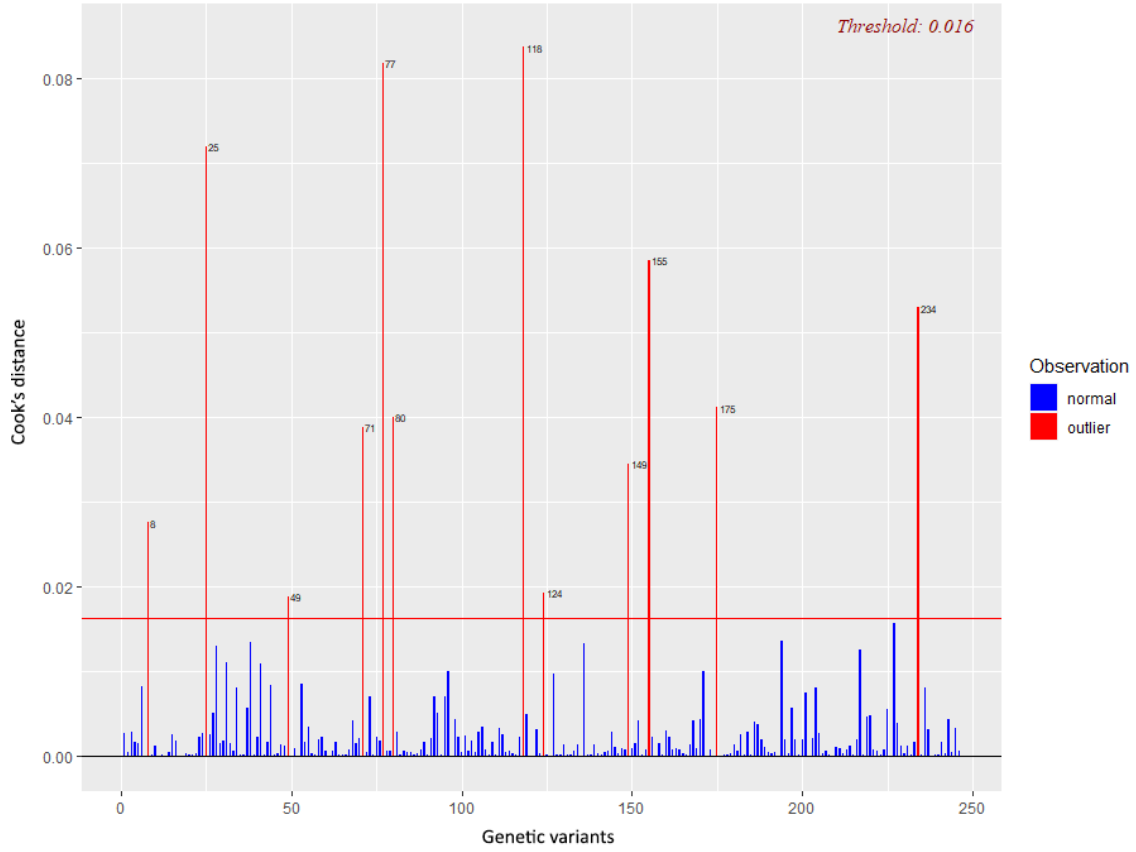
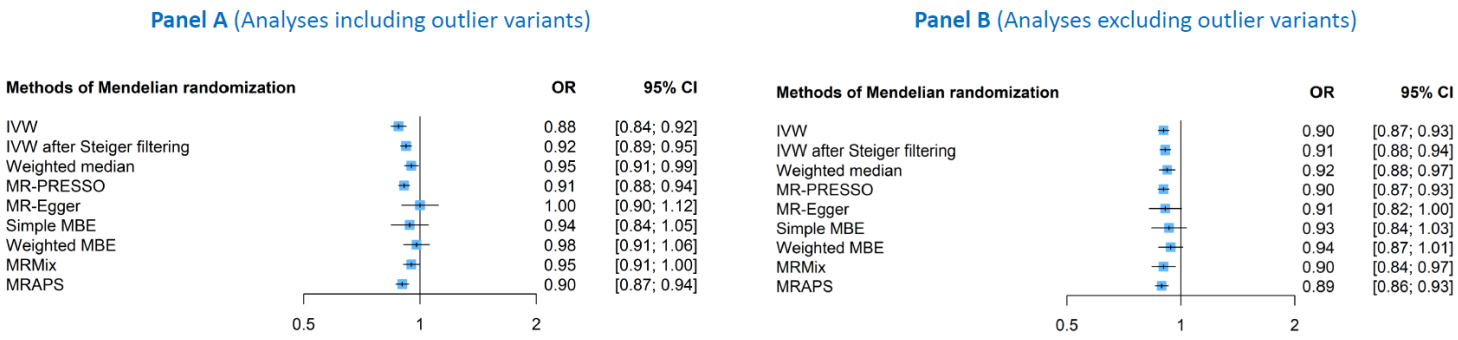


Figure 5-5. The association between systolic blood pressure and risk of diabetes estimated by random-effect inverse variance weighted and applied various sensitivity analysis methods of two-sample Mendelian randomisation, before (main analysis) and after excluding outlier variants (sensitivity analysis).

Blue squares represent the point estimation and the size of the squares is the same. The horizontal solid lines represent 95% confidence intervals. The MBE method was implemented using both simple and weighted options with bandwidth $\phi = 1$ under the no measurement error (NOME) assumption. The following outlier variants were excluded based on Cook's distance measure over 4/n, where n is the number of included genetic variants: rs10274928, rs11191548, rs12454712, rs1446468, rs17249754, rs17477177, rs2972146, rs34072724, rs4841569, rs5219, rs6712203, rs9368222



In order to visually examine heterogeneity, we can plot the genetic associations with the outcome versus the genetic associations with the exposure, along with their corresponding confidence intervals. The possible directional pleiotropy and heterogeneity of the estimates were assessed using the funnel and scatter plots. Scatter plots for the association of systolic blood pressure and risk of diabetes risk are given in **Figure 5-6, Panel A**. The plot demonstrates heterogeneity, with several outliers. The same outliers were found in the funnel plot (**Figure 5-7, Panel A).** In these diagrams, each dot represents a genetic variant; under the null

hypothesis, all of the dots should lie in a straight line. If there is a significant departure from this line, it may indicate pleiotropy that warrants further exploration. To further explore the observed heterogeneity, I excluded outlier variants based on Cook's distance, as described above. **Figures 5-6** and **5-7**, **Panel B** show that after the outliers were removed, heterogeneity was much reduced and estimates from several models were consistent (**Figure 5-5**).

Figure 5-6. Scatter plot of genetic variant-outcome associations versus variant-exposure associations for the association between systolic blood pressure and risk of type 2 diabetes.

Circles indicate marginal genetic associations with systolic blood pressure and risk of diabetes for each variant. Error bars indicate 95% CIs. SNP: single-nucleotide polymorphism

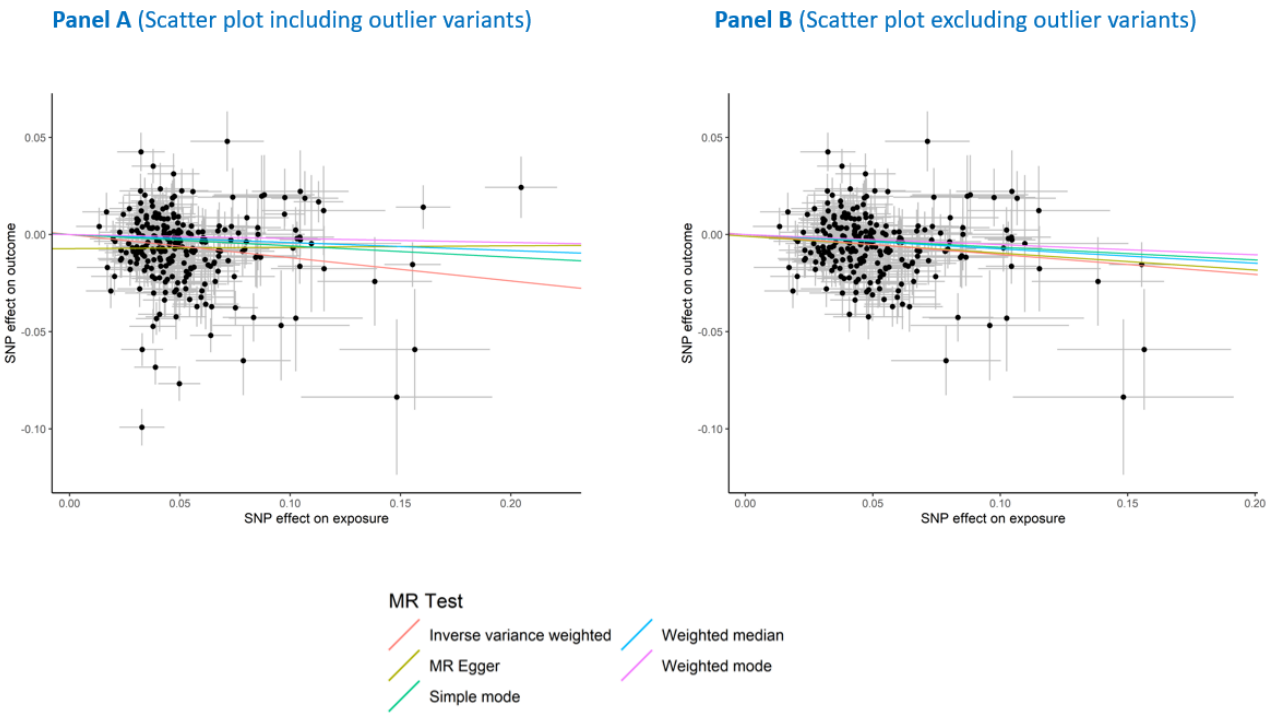
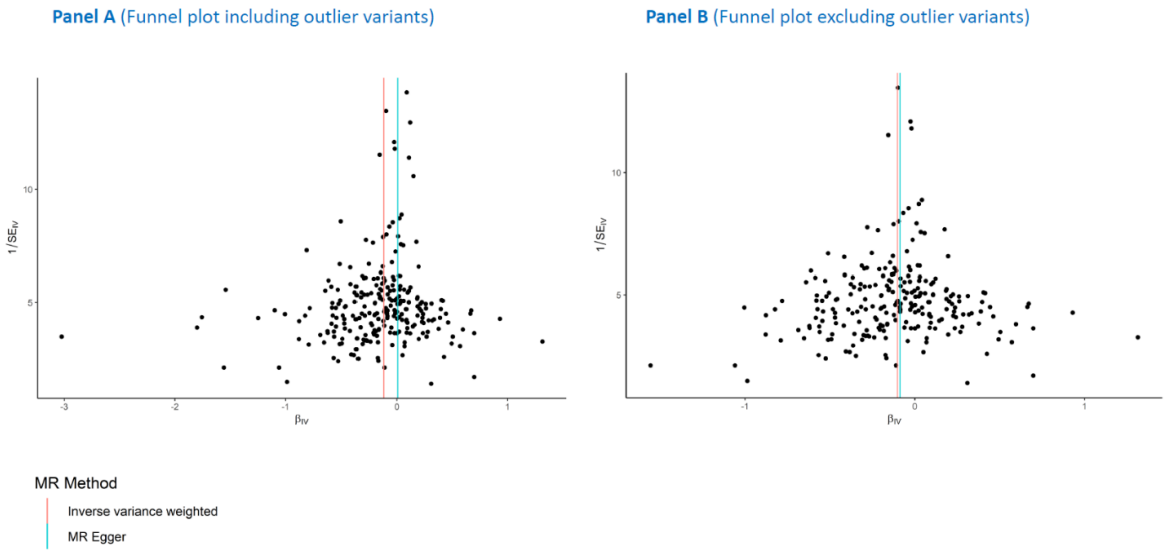


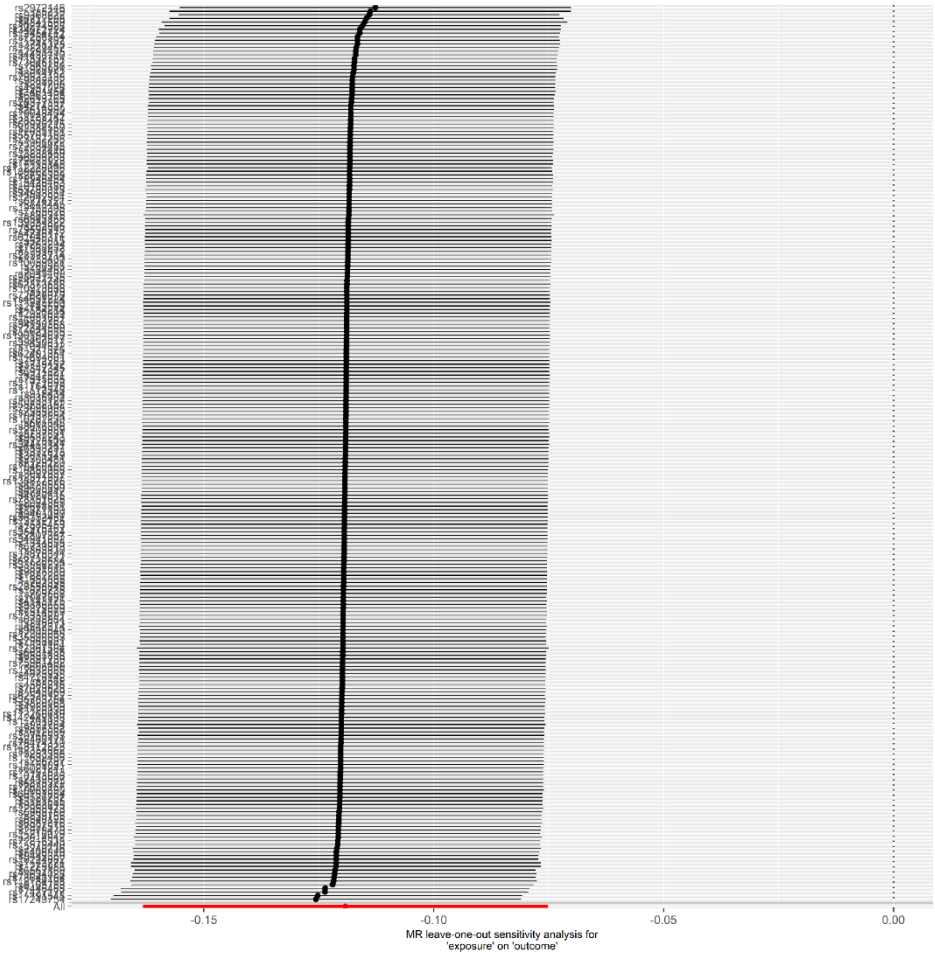
Figure 5-7. Funnel plot of variants, showing each variant causal estimate against instrument strength.

β: The causal effect of the exposure on the outcome, SE: standard error



There was evidence of directional pleiotropy based on MR-Egger intercept (beta = -0.008, P < 0.001). However, the exclusion of outlier genetic variants based on MR-PRESSO test had no observable effect on the main estimation (outlier-corrected OR 0.91 versus inverse-variance weighted OR 0.89; distortion test p = 0.13) and also the results of MR- RAPS and Steiger filtering methods suggested no material effect of pleiotropy on the effect estimation (**Figure 5-5**). Furthermore, In the leave-one-out analysis, I found that no single genetic variant was actively driving the overall effect of systolic blood pressure on diabetes (**Figure 5-8**).

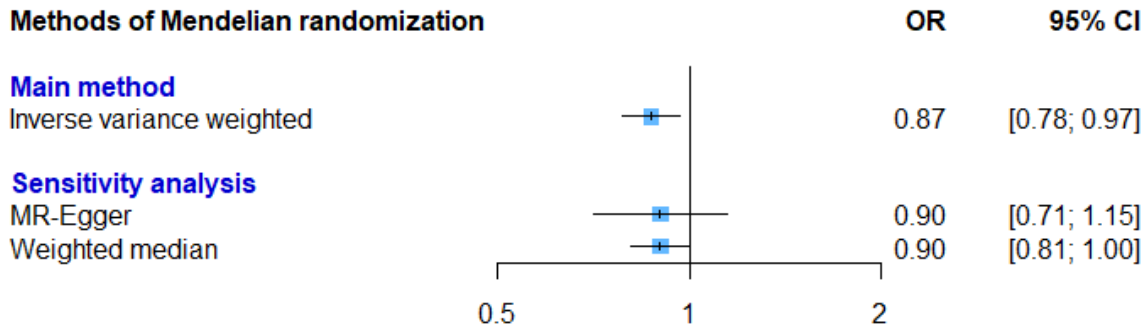
Figure 5-8. Leave-one-out plot to assess if a single variant is driving the association between systolic blood pressure and diabetes.



In addition, because I selected the candidate variants for exposure from Evangelou et al. GWAS study,[122] in which the investigator used UK Biobank data for the discovery phase of the GWAS, I performed a sensitivity analysis to assess the potential impact of using overlap data on the main estimation. **Figure 5-9** shows the result of the sensitivity analysis. In this analysis, the “variants-exposure” estimations were the same as the main analysis, but “variants-outcome” summary estimations were extracted from stage 1 of the DIAGRAM Consortium, which has no overlap with the UK Biobank. As the result shows, I did not find any material change in this sensitivity analysis that further supports the robustness of the main findings.

Figure 5-9. Sensitivity analysis to assess the impact of using an overlapping dataset for the discovery of candidate instrumental variables for exposure.

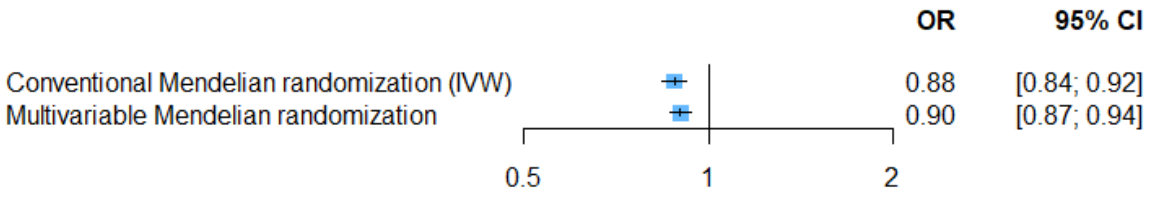
Blue squares represent the point estimation and the size of the squares is the same. The horizontal solid lines represent 95% confidence intervals. OR: odds ratio per 5- mmHg lower systolic blood pressure



On the other hand, I explored whether the identified association is driven by body mass index or other anthropometric measures, using unadjusted blood pressure estimations and by including anthropometric measures as a phenotype in multivariable Mendelian randomisation. The UK Biobank dataset was used to extract the unadjusted estimates. I used multivariable Mendelian randomisation through the IVW method to calculate direct effect estimation considering body mass index, waist circumference, hip circumference, and fat percentage versus conventional effect estimations using IVW (unadjusted). As **Figure 5-10** shows, the multivariable Mendelian randomisation analysis showed similar findings both before and after considering the anthropometric measures.

Figure 5-10. Multivariable Mendelian randomisation results unadjusted and adjusted for the anthropometric measures to check the possibility of collider bias in association between systolic blood pressure and diabetes.

In conventional Mendelian randomization analysis, I used the summary statistics not adjusted for body mass index or other anthropometric measures. Multivariable Mendelian randomization adjusted for body mass index, waist circumference, hip circumference, and fat percentage; OR: odds ratio per 5- mm Hg lower systolic blood pressure, IVW: inverse-variance weighted



Furthermore, I conducted a one-sample Mendelian randomisation to further explore the findings using individual-level data from the UK Biobank. The quality control and selection criteria for one-sample Mendelian randomisation were similar to my previous study.[147] The details of the initial inclusion and exclusion criteria are described in **Figure 5-11.** The result of the main one-sample Mendelian randomisation analysis, in which genetic variants were the same as in the two-sample study, was similar to the main findings (OR 0.87 [95% CI 0.84 to 0.90]) (**Figure 5-12**). In addition to this, I conducted a new one-sample Mendelian randomisation with a different genetic risk score. In this sensitivity analysis, to build a new genetic risk score, I selected 370 genetic variants from the final ICBP GWAS dataset including 77 cohorts (n = 299,024, no overlap with UK Biobank). The same criteria were used for the selection of genetic variants. The result of this sensitivity analysis using the newly built genetic risk score was in line with the previous one-sample analysis (OR 0.88 [95% CI 0.85 to 0.92]).

Figure 5-11. Flowchart for initial exclusion and inclusion criteria and genetic data quality control of UK Biobank for conducting one-sample Mendelian randomisation.

The flowchart is derived from my previous publication.[147]

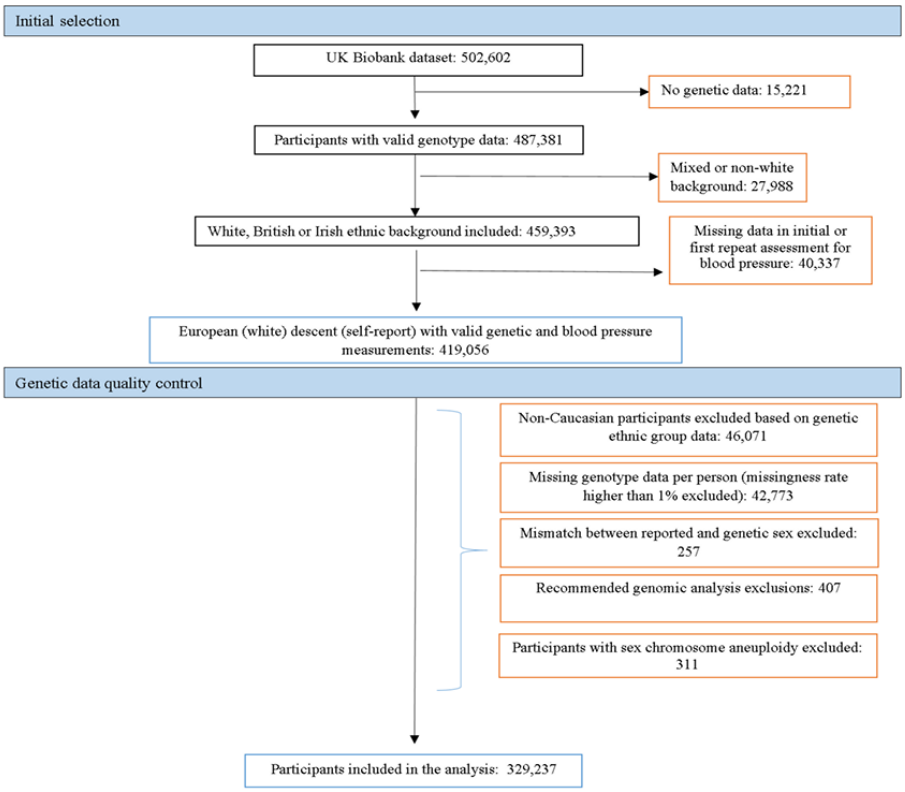
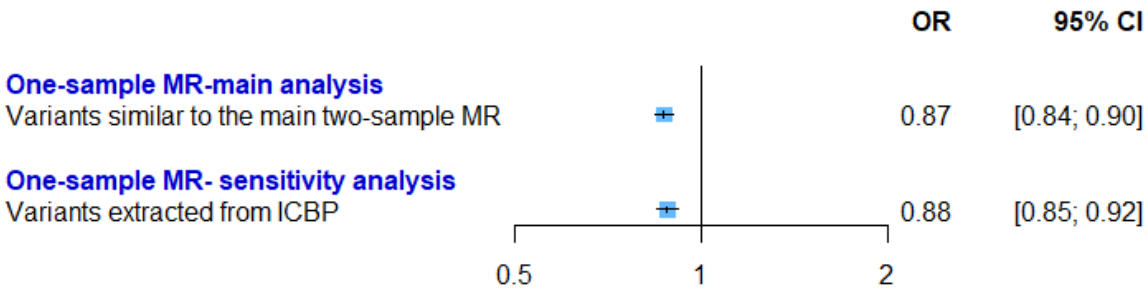


Figure 5-12. The association between systolic blood pressure and risk of diabetes replicated by one-sample Mendelian randomisation.

Blue squares represent the point estimation and size of squares is the same. The horizontal solid lines represent 95% confidence intervals. OR: odds ratio per 5 mmHg lower systolic blood pressure



5.5. Discussion

In this Chapter, analysis based on genetic data provides evidence for a possible causal link between elevated blood pressure and the risk of new-onset type 2 diabetes. Using the Mendelian randomisation approach and several sensitivity analyses that approved the robustness of findings, I found that each 5 mmHg reduction in systolic blood pressure, reduces the risk of diabetes by about 11 per cent. Although I cannot be certain that this estimated effect is not driven by the pleiotropy effect; but, after examining the effect from many angles, I could not discover any strong evidence for probable pleiotropy bias. Furthermore, since this finding is consistent with the effect estimate from the IPD meta-analysis of RCTs, we can be more confident that the result is robust and probably causative.

Several previous Mendelian randomisation studies tried to assess the causal relationship between blood pressure and the risk of type 2 diabetes. Sun et al., conducted Mendelian randomisation to assess the causal relationship between hypertension and type 2 diabetes and vice versa.[77] The investigators used UK Biobank individual-level data to conduct Mendelian randomisation. Totalling 318,664 unrelated people with validated genotyping data of European origin aged 37-73 from UK Biobank were included in the analysis, after excluding persons with a history of heart failure, cardiovascular disease, cardiac operations, and non-type 2 diabetes. Through the IVW method as the main method of Mendelian randomisation, they reported null findings for the effect of the genetically instrumented hypertension and risk of type 2 diabetes (OR: 0.98 [95% CI, 0.90 to 1.08]). Even if their results were not consistent with my

Mendelian randomisation, several differences and limitations should be taken into account. Because summary estimates for variants-exposure and variants-outcome were derived from the same population (i.e., UK biobank), the absence of a relationship in this research may be attributed to weak instrument bias.[148] In addition, the statistical power to identify a causal relationship is diminished when a continuous variable, such as blood pressure, is reduced to a binary variable, such as hypertension.

In another study, Aikens et al. applied a Mendelian randomisation study to evaluate the causal relationship between elevated systolic blood pressure and the risk of type 2 diabetes.[78] They investigated 28 genetic variants associated with systolic blood pressure and assessed their effect on type 2 diabetes as an outcome of interest using a European-centric meta-analysis consisting of 37,293 cases and 125,686 control participants. They reported that an increase in genetically determined systolic blood pressure levels by 1 mmHg was associated with a 2% increase in the risk of developing type 2 diabetes (OR 1.02 [95% CI 1.01 to 1.03]; equal to a 10% increase in risk by 5 mmHg increase in systolic blood pressure). Although the results of this research were consistent with my finding, only 28 genetic variants related to exposure were used. The area of GWAS research is steadily growing, and the larger GWAS was not accessible at the time this study was conducted. In my study, I used the largest ever GWAS on blood pressure and extracted more than 240 genetic variants to use as instrumental variables from a GWAS meta-analysis including about 1 million participants.

Alongside these findings, there are some results from more general Mendelian randomisation studies with null [79] and positive association.[149,150] However, in my study, I selected the candidate variants (n=246) from the most recent GWAS and also extracted the estimation from the non-overlap dataset, which provides the optimal level of statistical power. Also, sensitivity analyses using a wide range of approaches, including multivariable Mendelian randomisation, further supported the findings for the causal association between systolic blood pressure and diabetes. Furthermore, my Mendelian randomisation goes beyond the previous studies because I assessed the separate effects of each class of drugs using genetic variants validated through RCTs data (Next chapter).

This study has some limitations that should be discussed here. Mendelian randomisation analysis assumed that the genetic variants selected as a proxy for systolic blood pressure influence the outcome (i.e., diabetes) only through systolic blood pressure (i.e., exposure of interest). Although we cannot be sure that the genetic variants do not have pleiotropic effects, our sensitivity analyses through several methods, each with different assumptions, did not suggest substantial evidence in favour of the pleiotropy effect. On the other hand, consistent findings between Mendelian randomisation and IPD meta-analysis, further confirmed that the probability of the pleiotropy effect is minimal. Furthermore, since type 2 diabetics are often identified as the outcome of interest in GWAS studies using diagnostic codes, some degree of underestimation is probable. However, the effect of this possible underestimation is toward the null association. Additionally, in this analysis, I

only investigated the effect of systolic blood pressure on know type 2 diabetes, further studies are needed to assess this effect on the pre-diabetic condition and also diabetes biomarkers such as Hemoglobin A1C and blood glucose level. Moreover, additional genetic analysis in parallel with Mendelian randomisation could be conducted to further verify the validity of selected instrumental variables, such as colocalisation. Recently, colocalisation methods have been developed to evaluate the possibility of shared causal variants between interested outcomes and potential biological mediators.[151,152] Indeed, colocalisation considers the overlap of causal variants for two or more phenotypes and might be beneficial for checking the probable violation of the Mendelian randomisation technique. However, the use of colocalisation analyses in this thesis could pose challenges in terms of interpretation, owing to the intricate nature of blood pressure and diabetes. Consequently, it may be influenced by numerous biological mechanisms, or in the case of a molecular trait, it is probable that multiple distinct causal variants may be identified. The utilisation of colocalisation techniques that can integrate various causal variants is imperative for conducting a thorough investigation in the future.[153] Lastly, the present study was implemented using genetic data obtained from a population of European ancestry. While this approach offers the advantage of increased genetic homogeneity, it also restricts the generalisation of the current results to other ethnic groups.

Using Mendelian randomisation studies, and in line with the result of IPD meta-analysis, I found evidence to suggest that lower systolic blood pressure leads to a lower risk of type 2 diabetes. The results of this study have been

published in the Lancet, and parts of it were used for the writing of this chapter.[48]

Chapter 6. Effect of each class of blood pressure-lowering drugs on the risk of type 2 diabetes: A Mendelian randomisation study

6.1. Introduction

In Chapters 3 and 5, I showed that blood pressure lowering per se is a modifiable risk factor for new-onset type 2 diabetes, and this effect was observed both in analyses using RCT data and also using genetic data analysis. However, one important question remains: whether specific classes of blood pressure-lowering drugs have a similar protective effect on the risk of type 2 diabetes. This question was addressed in Chapter 4 utilising RCT data and a network meta-analysis approach. While renin-angiotensin-aldosterone system inhibitors can lower the chance of developing type 2 diabetes, I showed that beta-blockers and diuretics may have the opposite impact. However, RCT data are insufficient for a full and reliable evaluation of each drug class. Because, 1) the majority of trials in the field of blood pressure lowering compared a particular class of drug to another active treatment; hence, it is not straightforward to determine the pure effect of each class; 2) treatment and comparator arms are not consistent and uniform across trials, and the number of placebo-controlled trials is limited; 3) for some classes of drugs, such as beta-blockers, no direct evidence is available from trials, and we must rely on indirect comparisons using network meta-analysis, which is prone to bias and confounding similar to observational studies; 4) the length of follow-up in RCTs is usually short, and we cannot assess the long-term effect of each class of drug, for example, we showed that the median follow-up time across all trials included in BPLTTC is about 4 years.[54]

Recent advances in GWAS research have enabled the use of genetic information to predict the effect of a specific class of drugs, possible adverse effects and even drug repurposing opportunities.[154] Mendelian randomisation provides an innovative method for predicting medication effects. With the availability of genetic data, the use of Mendelian randomisation as pharmacovigilance has been enhanced in recent years. This is the first time a Mendelian randomisation study will be used to assess the effect of antihypertensive drugs on the risk of diabetes, and no genetic study has investigated this hypothesis until now, so no literature review is available. To date, observational analysis of cohort studies has provided the bulk of information about this important research question. The trustworthiness of this evidence is, however, being called into doubt. Also, as I discussed in Chapter 4, the findings from individual trials were mixed, with no consistent results. In this chapter, I will use genetic data to provide evidence for the effect of each class of blood pressure-lowering on the risk of developing type 2 diabetes, to complement the network meta-analysis results using RCTs.

6.2. Objective

- To assess the separate effect of blood pressure-lowering drug classes on the risk of type 2 diabetes using genetic data analysis.

6.3. Methods

Blood pressure-lowering drug class effects can be predicted through variants in genes that encode receptors related to their mechanism of action. By way of example, beta-blockers, as a sympatholytic class of drugs, work by

inhibiting the activation of the beta-adrenergic receptors with adrenaline and noradrenaline, thereby, reducing heart rate, myocardial contractility, and cardiac output.[155] In the same way, ADRB1 is a gene that encodes the beta-1 adrenergic receptor present in cardiomyocytes and in the heart conduction system, which plays a role in heart rate and myocardial contractility. Therefore, genetic variants in the ADRB1 gene associated with systolic blood pressure can be used as a proxy for the treatment effect of beta-blockers and thus help assess the effect of that drug class on the outcome of interest.[156] For this analysis, the genetic variants suggested by Gill et al.[157] and Walker et al.[158] were considered to estimate the effect of the blood pressure-lowering drug classes.

The approaches that Gill et al. and Walker et al. have taken for identifying druggable genes were similar, but the methods for the selection of genetic variants were different.[157,158] Gill et al. selected five classes of blood pressure lowering from the 2018 ESC/ESH guidelines for the management of arterial hypertension, including ACEIs, ARBs, beta-blockers, calcium channel blockers and thiazide diuretics.[159] The DrugBank (https://go.drugbank.com/) dataset was utilised to identify the genes encoding the drug targets. Then, they determined promoter and enhancer regions on the retrieved genes. Finally, their criteria for the selection of eligible genetic variants to use as a proxy for the effect of blood pressure-lowering drug classes were as follows: 1) single-nucleotide polymorphisms (SNPs) in corresponding genes, promoter regions, or enhancers, 2) SNPs associated with systolic blood pressure at the genome-wide level ($p<5\times10^{-8}$), 3) independent SNPs with linkage disequilibrium threshold of $r^2<0.1$. This method, according to the researchers, does not

differentiate between the selection of loss-of-function variants and those associated with gene expression.[157]

Walker et al, integrated gene expression data into their method to retrieve more valid genetic variants for each class.[158] In this method, based on British National Formulary (https://bnf.nice.org.uk/), they considered 12 blood pressure-lowering drug classes including ACEIs, adrenergic neurone blockers, alpha-adrenoceptor blockers, ARBs, beta-adrenoceptor blockers, centrally acting antihypertensive drugs, loop diuretics, potassium-sparing diuretics and aldosterone antagonists, calcium channel blockers, renin inhibitors, vasodilator antihypertensives and thiazides and related diuretics. From this list, I only included five classes of medications, comparable to my network meta-analysis, since trial data for other classes are scarce. Then, similar to Gill et al's approach, they extracted active protein targets and the associated genes in the DrugBank database. Finally, the ideal SNPs for each gene were identified using gene expression association data from the GTeX project (version 7).[160] The best SNPs are defined as "variants with the smallest nominal p-value for a variant-gene pair in any tissue".[158] They used two-sample Mendelian randomisation to determine the causal effect of chosen SNPs on systolic blood pressure. This was done in order to validate the SNPs as instruments for each class of blood pressure-lowering drugs. In my study, I chose to utilise reported SNPs from both approaches but validate them using RCT data. Further details on the selection of the candidate genetic variants for each class and my validation process using trial data are described in the section below.

I was interested in selecting the approach that has high statistical power and provides precise estimation for each drug class. The following steps were taken for selection:

I. For validation purposes and as a positive control outcome, I considered coronary heart disease as the outcome of interest throughout this analysis because there is strong evidence for the protective effect of major classes of antihypertensives on the risk of coronary heart disease.[51,106,161]

II. The effect of each class of antihypertensive drug was assessed first using the placebo-controlled trials (**Table 6-1**) to provide trial-based estimation for the effect of each class versus placebo and then using Mendelian randomisation analysis through genetic variants reported by Gill et al. and Walker et al. separately.[157,158]

Table 6-1. The features of placebo-controlled trials included for validation study of genetic variants to be used in Mendelian randomisation analysis for the effect of drug classes.

Trials	Intervention		Comparator		SBP reduction between arms (mmHg)	Class of drug
	CHD events	Total	CHD events	Total		
ACEIs/ARBs						
DIABHYCAR	61	2443	78	2469	2.05	ACEIs
EUROPA	320	6110	418	6108	4.83	ACEIs
HOPE	459	4645	570	4652	3.32	ACEIs
PART-2	24	308	35	282	5.91	ACEIs
PEACE	222	4158	220	4132	4.79	ACEIs
PREVEND IT	14	431	14	433	4.37	ACEIs
ACTIVE-I	143	4518	136	4498	2.64	ARBs
TRANSCEND	116	2954	147	2972	4.66	ARBs
PRoFESS	183	9873	179	9925	4.35	ARBs
Calcium channel blockers						
PREVENT	19	417	20	408	5.79	CCBs
SYSTEUR	63	2398	77	2297	9.44	CCBs
Beta-blockers						
DUTCH-TIA	45	732	40	741	4.42	BBs
UKPDS *	46	358	69	390	9.27	BBs
Diuretics						
ANBP	98	1721	109	1706	9.74	Diuretics
EWPHE	26	416	28	424	21.34	Diuretics
HYVET	9	1933	12	1912	12	Diuretics

* Subset of UKPDS trial with beta-blocker as treatment included in this analysis.
SBP: systolic blood pressure
ACEIs: Angiotensin converting enzyme inhibitors
ARBs: Angiotensin receptor blockers
CHD: Total coronary heart disease
CCBs: Calcium channel blockers
BBs: Beta-blockers

III. For each class of drug, I selected the better performing approach (Gill or Walker method) if the estimated effect size met the two predefined criteria: 1) the effect size (point estimation) should be in the same direction as the estimation from placebo-controlled trials; 2) the estimates to have higher precision (narrower confidence intervals or smaller standard error).

Two-sample Mendelian randomisation, through the random-effect IVW approach, was used for statistical analysis. I used coronary heart disease as a positive control to compare the estimates with an outcome in which there is well-established evidence from RCTs, particularly for the effect of each class of antihypertensive.[162] The same GWAS studies described in the previous Chapter were also utilised for this stage of the analysis.[122,123,125]

6.4. Results

Figure 6-1 presents the final selection. I selected the genetic variants for ACEIs/ARBs and thiazide diuretics from Walker et al. and beta-blockers and calcium channel blockers from Gill et al. The characteristics of genetic variants selected for each class of drugs are shown in **Table 6-2**.

Figure 6-1. Comparison of the effect of major antihypertensive drug classes on coronary heart disease as positive control outcome, using individual participant data meta-analysis of randomised placebo-controlled clinical trials and Mendelian randomisation.

Blue squares represent the point estimation and the size of the squares is the same. The horizontal solid lines represent 95% confidence intervals. RR indicate hazard ratio in individual participant data meta-analysis and odds ratio in Mendelian randomisation; ACEIs/ARBs: angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers

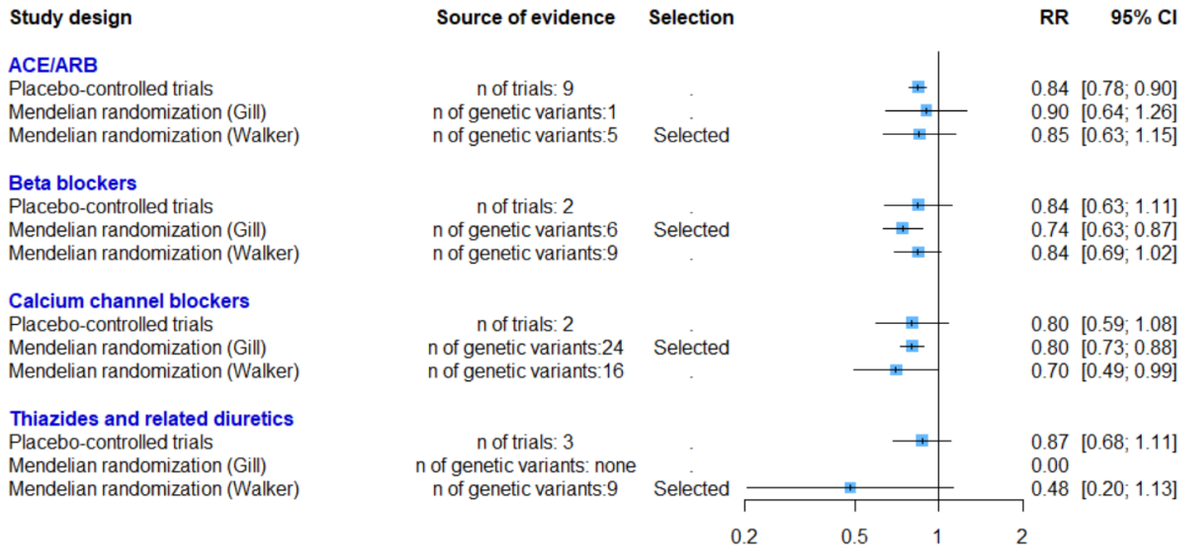


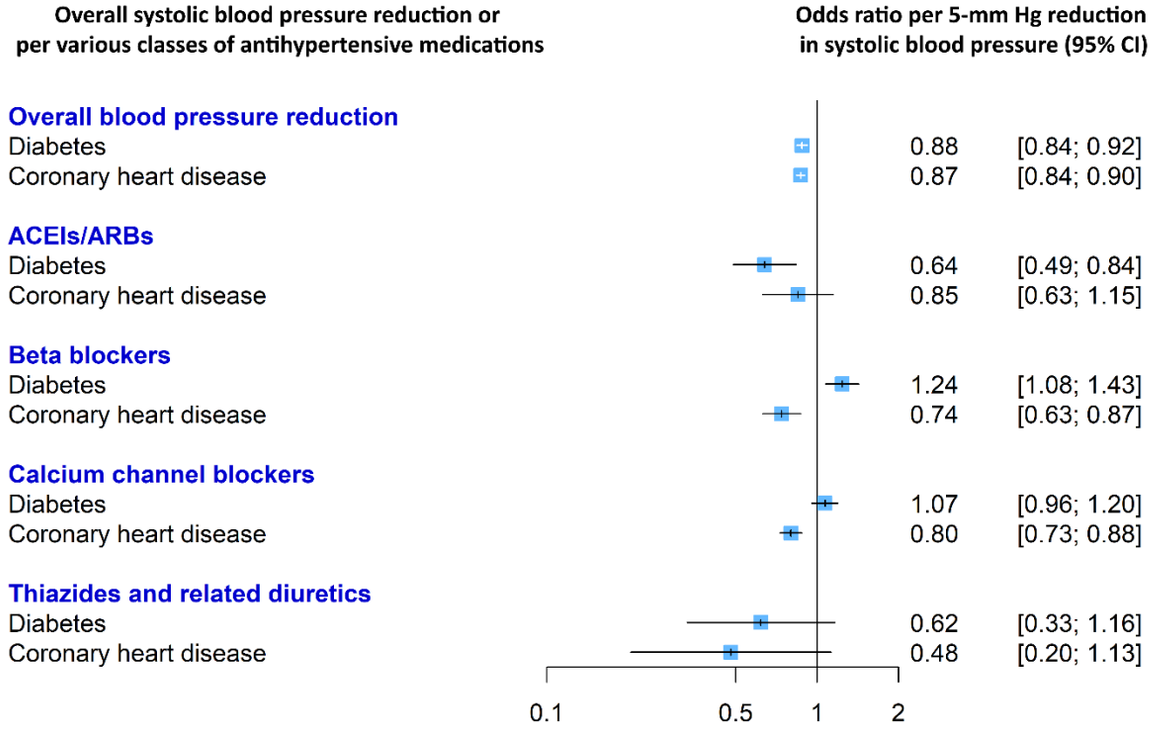
Table 6-2. The characteristics of genetic variants selected to assess the effect of major antihypertensive drug classes on the risk of new-onset type 2 diabetes, using the Mendelian randomisation approach.

Chromosome (GRCh37)	SNP	allele1	allele2	freq1	Effect	SE	p-value	Total sample size
ACEIs/ARBs								
3:148913426	rs118123032	t	c	0.0332	-0.153	0.1381	0.2677	273115
3:149370293	rs79387447	a	g	0.0145	0.0148	0.2263	0.9477	273295
3:148485148	rs80350379	t	c	0.0207	0.0982	0.1937	0.6121	272143
3:11652673	rs9829399	t	c	0.1172	0.1489	0.0716	0.03745	278480
17:61550729	rs4968783	a	c	0.6091	-0.2927	0.0472	5.54E-10	285133
Beta-blockers								
10:115707298	rs11196549	a	g	0.0421	0.7923	0.1255	2.75E-10	279594
10:115721364	rs460718	a	g	0.3222	-0.1832	0.0497	0.000226	279594
10:115788094	rs11196597	a	g	0.1379	0.2439	0.0716	0.000664	278589
10:115800294	rs17875473	t	c	0.0944	0.2345	0.0844	0.005472	279595
10:115805056	rs1801253	c	g	0.7305	0.4394	0.0524	5.08E-17	279594
10:115826508	rs4359161	a	g	0.1822	-0.2376	0.0592	6.09E-05	279593
Calcium channel blockers								
12:2434419	rs2239046	a	g	0.6788	0.2237	0.0483	3.61E-06	287243
12:2514270	rs714277	t	c	0.2837	0.2199	0.0503	1.24E-05	287245
10:18334521	rs2488136	a	g	0.2803	0.1453	0.0513	0.004658	279594
10:18440444	rs1888693	a	g	0.3472	0.3351	0.0482	3.57E-12	277475
10:18457722	rs16916914	t	c	0.9604	-0.4955	0.1192	3.22E-05	278849
10:18459450	rs7076319	a	g	0.7277	-0.2133	0.0516	3.56E-05	278479
10:18481737	rs61278674	a	g	0.911	-0.1719	0.0865	0.0469	278588
10:18514561	rs1779209	t	c	0.296	0.1356	0.0514	0.00836	270873
10:18553968	rs10828399	a	g	0.5323	-0.1507	0.0459	0.001027	279593
10:18592450	rs10828452	a	t	0.7949	0.2464	0.061	5.32E-05	278589
10:18627285	rs10828542	a	g	0.6157	0.143	0.0475	0.002622	279595
10:18678987	rs12780039	c	g	0.1201	0.1752	0.072	0.01497	279592
10:18695681	rs112133583	t	c	0.0266	-0.4123	0.1724	0.0168	278594
10:18710991	rs11014170	a	g	0.0234	-0.6098	0.1781	0.000618	273573
10:18727901	rs7923191	a	g	0.7884	-0.3403	0.0572	2.64E-09	278479
10:18727959	rs12258967	c	g	0.7104	0.5426	0.0529	1.06E-24	278590
10:18729855	rs72786098	a	g	0.0291	-0.3976	0.1472	0.006913	278480
10:18755664	rs1998822	a	g	0.7272	-0.1349	0.0529	0.01072	268756
10:18790727	rs4748474	a	g	0.5273	0.1149	0.0467	0.0138	271333
12:49209340	rs150857355	c	g	0.0213	1.0616	0.1906	2.56E-08	272725
3:53558012	rs3821843	a	g	0.6838	0.331	0.0524	2.61E-10	277474
3:53605712	rs114987861	a	g	0.0305	0.395	0.1472	0.007298	278479
3:53612327	rs113210396	t	g	0.0445	-0.3563	0.1293	0.005856	278589
3:53734443	rs7340705	t	c	0.6684	-0.1929	0.0485	7.08E-05	279594
Thiazide diuretics								
10:78695467	rs10762738	a	g	0.5005	0.0565	0.0469	0.2278	261609
15:26818362	rs8030011	a	g	0.1342	0.0187	0.0665	0.779	287242
15:27722954	rs140443467	a	g	0.9691	-0.0779	0.1645	0.6357	269648
15:47906718	rs12914000	t	c	0.8366	0.0882	0.0636	0.1658	286240
4:45844166	rs139787011	a	g	0.9834	0.1814	0.2274	0.425	257608
4:45956676	rs7699135	t	c	0.8659	-0.0591	0.0684	0.3876	279594
5:160335398	rs13188637	a	g	0.5052	-0.0181	0.0461	0.694	276577
5:161908897	rs10076365	a	g	0.8301	-0.0443	0.0616	0.4717	275462
8:87064009	rs62509890	a	g	0.8927	-0.134	0.0757	0.07688	279595

Each 5 mm Hg decrement in systolic blood pressure determined through genetic variants for ACEIs/ARBs, beta-blockers, calcium channel blockers, and thiazide diuretics was associated with a lower risk of coronary heart disease as an established evidence-based target for preventive blood pressure-lowering treatment, supporting the validity of selected variants (**Figure 6-2**).

Figure 6-2. Association of genetically influenced systolic blood pressure reduction overall and for each major class of antihypertensive medications, with type 2 diabetes as the main outcome, and coronary heart disease as the positive control.

Blue squares represent the point estimation and the size of the squares is the same. The horizontal solid lines represent 95% confidence intervals. Odds ratio: estimated using the inverse-variance weighted method. Effect of blood pressure-lowering class of drugs estimated using genetic variants in gene encodes drug targets. ACEIs/ARBs: angiotensin-converting enzyme inhibitors/ angiotensin receptor blockers. CI: confidence interval



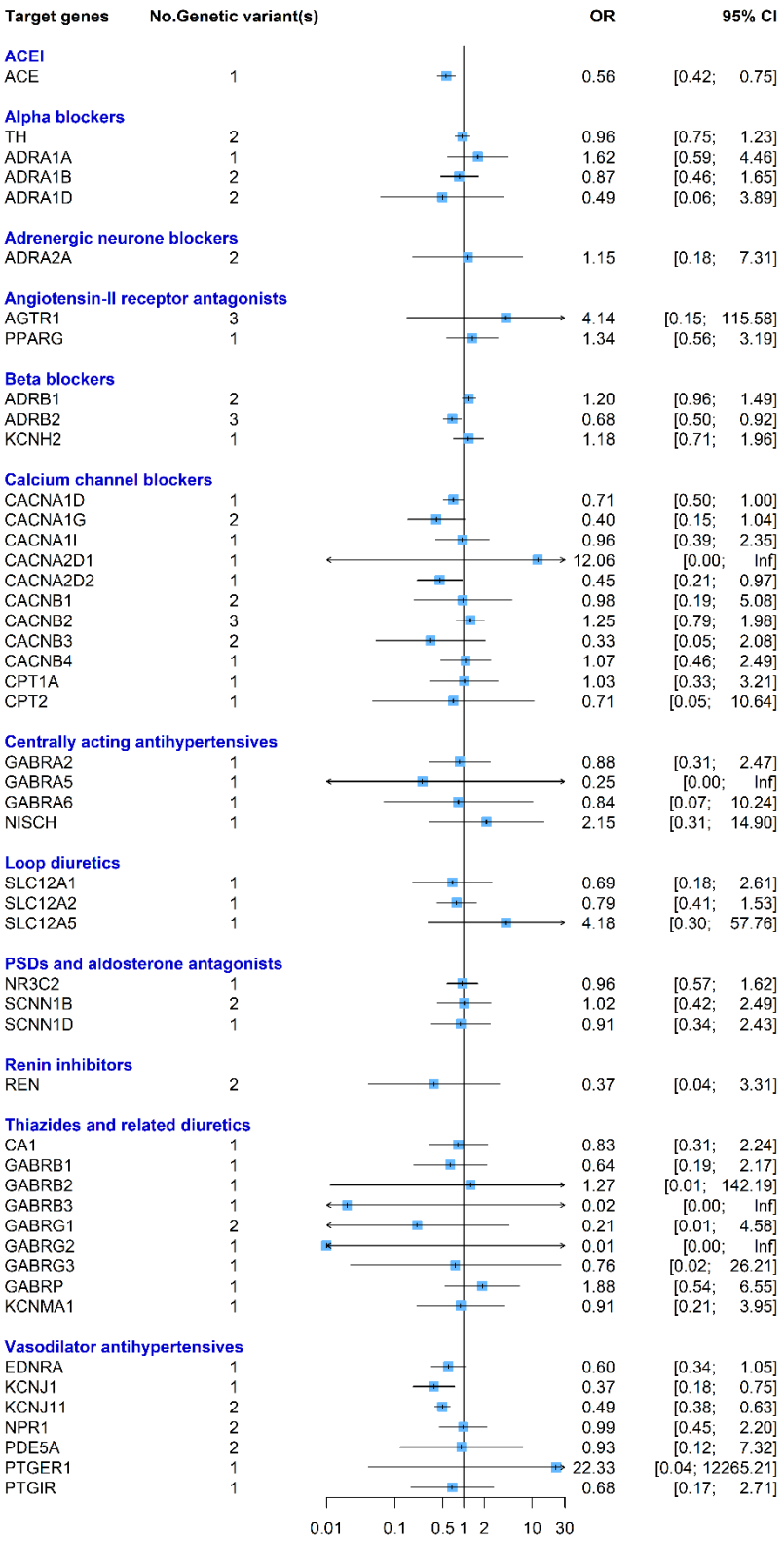
In line with analysis for coronary heart disease, we found an observable decrease in the risk of type 2 diabetes determined through genetic variants for ACEIs/ARBs (OR 0.64 [95% CI 0.49 to 0.84]) (**Figure 6-2**). While I did not find any association between the calcium channel blockers and the risk of diabetes (OR 1.07 [95% CI 0.96 to 1.20]), I observed an increase in the risk of diabetes with beta-blockers (OR 1.24 [95% CI 1.08 to 1.43]) (**Figure 6-2**). However, genetic evidence for thiazide diuretics did not provide adequate statistical power for estimation (OR 0.62 [95% CI 0.33 to 1.16]), and the findings both for coronary heart disease and type 2 diabetes were null with wide confidence intervals.

Additionally, I conducted a post-hoc analysis repeating the analyses with a small set of SNPs more closely linked to the drug mechanism (**Figure 6-3).** Although focusing on a specific gene that encodes drug target is becoming a popular method for drug discovery and also for shortening times to approval for repurposed drugs, the current project aimed to use genetic data to boost the result of clinical trials, particularly in comparison to each class of drug with placebo. Therefore, given the fact that only 5.7% of blood pressure variance is explained by the discovered genetic variant (until today),[122,123] we could not expect to have precise estimation using only a specific gene, particularly for blood pressure as a multigenic phenotype. Consequently, I combined genetic variants in all genes related to each drug class to have a more precise estimation for confirmation of network-meta-analysis findings. It can be a separate project for the future to find further druggable targets for antihypertensive drugs. In addition, in this analysis, I found a strong protective effect for three targets related to vasodilator antihypertensive drugs (**Figure 6-3)**. This finding is interesting, but I can not confirm the validity of this estimation as there is no

trial on the effect of vasodilator antihypertensive drugs on the risk of cardiovascular disease. Therefore, I prefer to keep it as a supplementary analysis, which could be considered a hypothesis-generating result for future studies.

Figure 6-3. Mendelian randomisation for the effect of systolic blood pressure on the risk of diabetes estimated using genetic variants in target genes for each drug class.

OR: odds ratio per 5 mm Hg lower systolic blood pressure



6.5. Discussion

In the previous Chapter using the Mendelian randomisation study, I showed that each 5 mmHg reduction in systolic blood pressure, on average, reduces the risk of diabetes by about 10 per cent. In a separate analysis in the current Chapter, I found that effects were not consistent across different classes of antihypertensives. While I found strong evidence that ACEIs and ARBs reduce the risk of diabetes, the use of beta-blockers showed an increase in risk. Calcium channel blockers had no material influence on diabetes risk and the effect of thiazide diuretics was null but with a wide confidence interval.

This study utilised large-scale GWAS data to assess the effect of blood pressure-lowering drug classes on diabetes risk using genetic proxies for ACEIs, beta-blockers, calcium channel blockers, and diuretics, four of the most commonly used drugs globally. Mendelian randomisation estimates for the risk of coronary heart disease were equivalent to those reported in placebo-controlled RCTs, indicating the validity of my chosen method for variant selection. In terms of biological mechanisms of action for each class, I have previously explained all potential mechanisms in Chapter 4, which are also relevant here. The application of genetic data as a complementary analysis to investigate the effect of antihypertensive drugs utilising current data from large-scale studies is a key strength of this work. Using this approach, I was able to avoid the budget and time restrictions associated with such research through RCTs, especially in the context of a DPhil thesis, as well as fill a knowledge gap regarding the limitations of possible confounding and reverse causation from observational studies.

The Mendelian randomisation findings assess the cumulative impact of lifetime exposure to genetic variations, rather than the outcome of a therapeutic intervention, which is one of the limitations of the research.[157] This potential weakness of Mendelian randomisation may be seen as an advantage in other contexts. For instance, there is a lack of data on the effects of prolonged exposure to pharmacological interventions, and the average follow-up duration in RCTs is often rather short. As a result, we can get a more accurate assessment of the long-term impact of each drug class by using Mendelian randomisation. Given that Mendelian randomisation captures the life-long exposure effect, some scientists argue that this is not a realistic estimation, particularly in the case of drug effects, as in clinical trial settings, drug compliance is not 100% and patients take drugs for a shorter period, not from conception until death. If the interpretation is only based on genetic data, this argument may be legitimate. To address both the limitations of genetic data analysis and the evidence from RCTs, I attempted to evaluate both of these randomised data and make more realistic interpretations while taking into account the limitations and strengths of both approaches in this thesis. In Chapter 8, I provided a detailed explanation of all findings in this thesis and interpreted the conclusions while taking into account all aspects of evidence and analysis.

Furthermore, this research, as with any Mendelian randomisation study, is vulnerable to pleiotropic effects, in which chosen genetic variations affect the outcome through a pathway unrelated to blood pressure. There is no definite way to ensure that pleiotropy does not affect the results of Mendelian

randomisation; however, because the selection of variants in this part of the analysis was limited to specific genes, and I interpreted the results alongside evidence from a network meta-analysis of RCTs, I expected the likelihood of pleiotropy to be minimal. Furthermore, with the exception of diuretics, the findings of the other pharmacological classes were consistent with the network meta-analysis estimates, ensuring the robustness of the estimates. The approach taken by Gill et al., on the other hand, did not include gene expression data. To deal with the difference in variants selection pipelines, I did not rely on a central approach for variant selection, and I validated the estimates from each approach using both positive control analysis and comparison to placebo-controlled studies.

In conclusion, using genetic data analysis and the Mendelian randomisation design, I found that various classes of antihypertensive drugs have a different effect on the risk of type 2 diabetes, and these results were consistent with evidence from my RCTs analysis, except for the diuretic class. The results of this study have been published in the Lancet, and parts of it were used for the writing of this chapter.[48]

Chapter 7. Blood pressure-lowering for prevention of major cardiovascular disease in persons with and without type 2 diabetes

7.1. Introduction

Diabetes is a leading cause of mortality, vascular diseases, and healthcare costs worldwide.[163] People with type 2 diabetes who have high blood pressure are more likely to experience morbidity and mortality from major cardiovascular events.[164] However, there is insufficient randomised evidence to establish if the efficacy of blood pressure-lowering treatment differs between people with type 2 diabetes and those who do not have this metabolic disease. Similarly, there is disagreement about starting blood pressure reduction treatment at a certain blood pressure threshold, especially in patients with normal or high-normal blood pressure readings.

These uncertainties originate mostly from the differential results of the ACCORD and SPRINT trials in people with and without type 2 diabetes. The SPRINT trial demonstrated that aiming for a systolic blood pressure of less than 120 mmHg versus less than 140 mmHg, substantially reduced the risk of cardiovascular disease among those who did not have known diabetes at the trial baseline.[165] In contrast, the ACCORD trial, which had comparable blood pressure-lowering targets and similar interventions, revealed no obvious preventative effect in people with known type 2 diabetes at baseline.[166] These findings highlighted the possibility of an interaction between blood pressure reduction and diabetes on the risk of cardiovascular diseases. A subsequent aggregate data meta-analysis of randomised trials that included 100,354 people

with type 2 diabetes reported that blood pressure-lowering treatment reduces the risk of major cardiovascular disease and all-cause death overall, but with a stronger relative effect among those with baseline systolic blood pressure of 140 mmHg and greater.[69] Based on the findings of these studies, it seems that treating type 2 diabetes patients with antihypertensive medication at lower blood pressure thresholds or to lower targets may not be beneficial.

Even though data from randomised controlled studies generally support the recommendation for blood pressure-lowering treatment in individuals with type 2 diabetes, there is much ambiguity about the differential effects of drug classes (**Table 7-1**).

Table 7-1. A comparison of clinical guidelines differences regarding blood pressure management in persons with type 2 diabetes mellitus.

	ESC/ESH 2018[159]	NICE 2019[167]	ACC/AHA 2018[168]	ISH 2020[169]
The threshold for initiation of pharmacotherapy	BP ≥ 140/90 mmHg	-	BP ≥130/80 mmHg	BP ≥140/90 mmHg
Recommended drug classes	ACEIs or ARBs in combination with CCBs or thiazide diuretics.	Step 1: ACEIs/ARBs Step 2: ACEIs/ARBs + CCBs/ thiazide diuretic Step 3: A combination of ACEIs/ARBs + CCBs+thiazide diuretics	All first-line classes of antihypertensive agents (i.e., diuretics, ACEIs, ARBs, and CCBs)	The treatment strategy should include ACEIs/ARBs (and a CCBs and/or thiazide diuretics

BP: blood pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, ACEI: Angiotensin-converting enzyme inhibitors, ARB: Angiotensin receptor blockers, CCB: calcium channel blockers

An IPD meta-analysis of the BPLTTC from the first cycle found that the effects of various classes of blood pressure-lowering drugs are generally comparable, with major cardiovascular events being reduced to a comparable

level amongst individuals with and without type 2 diabetes.[170] However, the lack of interaction between treatment and diabetes status and the similar effect across specific blood pressure-lowering drug classes may stem, in part, from the lack of statistical power both by dint of study design and the relatively small sample size, particularly for identifying effects across baseline blood pressure levels and drug classes. Although most guidelines recommend renin-angiotensin-aldosterone system inhibitors as the first-line drug class, the choice of antihypertensive agents is more complicated in persons with type 2 diabetes than in those without, necessitating a comparative evaluation of the effects of specific blood pressure-lowering drug classes on cardiovascular disease prevention in this group. Such uncertainties are evident in the inconsistent nature of current clinical guideline recommendations (**Table 7-1**).

The third cycle of the BPLTTC includes more than 358,000 participants. This allows for the simultaneous investigation of heterogeneity of effect by type 2 diabetes status and systolic blood pressure categories at baseline, making use of the largest known dataset of randomly assigned participants with type 2 diabetes. In this Chapter, I examined the effect of blood pressure-lowering treatment on the risk of major cardiovascular events in adults with and without type 2 diabetes, as well as by baseline levels of systolic blood pressure and specific drug class, using IPD from large randomised trials.

7.2. Objectives

- To investigate the heterogeneity of effects of blood pressure-lowering on the risk of major cardiovascular events and death (cardiovascular and all-cause) in persons with and without type 2 diabetes at baseline.
- To investigate the heterogeneity of effects of blood pressure-lowering on the risk of major cardiovascular events and death (cardiovascular and all-cause) in persons with and without type 2 diabetes at baseline, stratified by baseline systolic blood pressure categories.
- To compare the effect of each blood pressure-lowering drug class with placebo on the risk of major cardiovascular events and death (cardiovascular and all-cause) in persons with and without type 2 diabetes at baseline.

7.3. Methods

In this Chapter, I used the BPLTTC dataset to test the objectives. However, the inclusion and exclusion criteria were different from the previous Chapters.

Using the BPLTTC dataset, I conducted an IPD meta-analysis. Investigators from major pharmacological blood pressure reduction trials have joined together to form the BPLTTC, which includes 52 randomised studies and individual-level data on 363,684 individuals (December 2021). Specifics on the current round of cooperation have been provided elsewhere.[44,85] All trials that provided individual-level data to the collaboration and shared information on type 2 diabetes diagnosis at baseline, blood pressure levels at randomisation and during follow-up, and outcome data for cardiovascular events were included

in this analysis. The minimum number of person-years of follow-up required in each randomly assigned arm was 1000. Trials that were only conducted on patients with heart failure, those that used short-term treatments for patients with acute myocardial infarction, and those that evaluated the effect of blood pressure reduction in acute settings were all excluded from the analysis. Before providing a dataset available for statistical analysis, a research protocol was developed and revised with input from several international partners and the BPLTTC steering committee.

Based on trial designs, I determined the treatment and comparison groups in each trial. In placebo-controlled trials, the placebo arm served as the comparator, whilst the active treatment arm served as the intervention. In head-to-head trials comparing two or more classes of drugs (active comparison group), the arm with the greatest systolic blood pressure decrease was labelled the treatment arm, and the other arm(s) were considered the comparator. In trials comparing two blood pressure-lowering strategies, intensive versus standard, the intensive arm served as the treatment and the standard arm served as the comparator. The comparison groups, participant profiles, trial designs, and amount of blood pressure reduction for each trial have already been published.[44,54]

The primary outcome was the development of major cardiovascular events, which were defined as the first incidence of fatal or non-fatal stroke, cerebrovascular diseases (both ischemic and haemorrhagic), fatal or non-fatal ischaemic heart disease, or heart failure resulting in death or hospitalisation.

The individual components of the primary outcome were included as secondary outcomes, along with cardiovascular causes of death (such as myocardial infarction, sudden cardiac, coronary heart disease, stroke, and heart failure), and all-cause mortality. The outcomes were determined using the diagnostic data each trial supplied.

I classified individuals according to the treatment assignment they received in each trial (intention-to-treat). I used a one-stage IPD meta-analysis with a fixed effect model that incorporates individual-level data from all trials concurrently by fitting a single statistical model.[84] Using a Cox proportional hazard model stratified by trial, the HR was estimated. A Poisson regression model with an identity link was used to calculate the absolute risk reduction over the follow-up duration. Using Kaplan-Meier estimates of cumulative incidence, event rates were estimated and shown separately for the group with and without type 2 diabetes at baseline. I conducted a meta-regression analysis to assess the effect of lowering blood pressure on the proportionate risk reduction of major cardiovascular disease at the trial level for individuals with and without type 2 diabetes. In meta-regression analysis, decreases in systolic blood pressure across comparison groups and estimated HRs for each trial were used, both stratified by baseline type 2 diabetes status.

I standardised the effect sizes for a 5 mmHg reduction in systolic blood pressure, which was a reasonable approximation to the mean reduction in systolic blood pressure that was attained throughout all of the blood pressure-lowering intensity trials and the placebo-controlled trials (in the BPLTTC).[44,54,56]

To evaluate the heterogeneity in blood pressure lowering treatment by baseline diabetes status, I included a binary interaction term for type 2 diabetes status and treatment into the model. In addition, I analysed data for those with and without type 2 diabetes separately, and I also evaluated the interaction by baseline categories of systolic BP in 10 mmHg increments from 120 mmHg to 170 mmHg. Secondary outcomes were also examined to evaluate the consistency of patterns. To check for interaction, I utilised multiple comparison-adjusted likelihood-ratio tests. P-values were corrected for multiple comparisons using Hommel's approach to minimise the likelihood of false positive results.[171,172]

I examined drug-class effects stratified by the presence or absence of type 2 diabetes at baseline to see whether any observed heterogeneity of effects could be explained by differential drug use in these two groups. Using a network meta-analysis framework, the effect of each of the five major blood pressure-lowering drug classes was estimated, including ACEIs, ARBs, beta-blockers, calcium channel blockers, and thiazide diuretics.[48,109] The approaches were similar to those described in Chapter 4. In summary, the logistic regression model was used to estimate the relative risk (RR) for each available comparison, separately for individuals with type 2 diabetes and those who did not have the condition at baseline. The estimates were based on individual-level data from each trial. For fitting the network meta-analysis model, a Markov chain Monte-Carlo simulation with four chains, 100,000 iterations, and a burn-in of 10,000 was performed.[109]

I performed several sensitivity analyses to further check the robustness of my findings. To check whether the standardisation method had any impact on the overall findings, I performed analyses without standardisation for blood pressure reduction across trials. I restricted the analysis to trials that utilised at least one laboratory measurement for the diagnosis of type 2 diabetes at baseline in order to examine the validity of type 2 diabetes ascertainment. I also conducted additional analyses without head-to-head trials to determine their impact on the main findings.

7.4. Results

I excluded one trial from the study since there was insufficient time-to-event data (E-COST trial).[173] The analysis included data from 358,533 individuals from 51 randomised clinical trials (**Table 7-2**). Of the 51 trials, three trials included only patients without a history of type 2 diabetes, 41 trials included both participants with and without type 2 diabetes, and seven trials had only persons with type 2 diabetes at the time of enrolment (**Table 7-2**). At randomisation, the mean age and proportion of women were comparable across groups with and without type 2 diabetes (**Table 7-3**). In patients with type 2 diabetes, the mean systolic/diastolic blood pressure at baseline was 149/84 mmHg, whereas it was 153/88 mmHg in persons without type 2 diabetes.

Table 7-2. General characteristics of trials included in the analysis.

Trial name	Inclusion criteria	Mean of follow-up duration (year)	Intervention (n)	Comparator (n)	Number of participants with and without diabetes at baseline (with diabetes/without diabetes)	Definition of diabetes at baseline	SBP difference (mmHg) excluding first 12 months *
AASK	Age 18-70 years, African American, hypertension, renal disease (GFR=20-65 ml/min per 1.73m^2)	4.8	More intensive (540)	Less intensive (554)	0/1094	Fasting glucose level ≥ 140 mg/dl (7.8 mmol/L), a random glucose level ≥ 200 mg/dl (11.1 mmol/L), or pharmacological glucose-lowering therapy	13.0
ABCD	Age 40-74 years, type 2 diabetes mellitus, DBP ≥80 mmHg, not on antihypertensive treatment	4.7	More intensive (474)	Less intensive (476)	950/0	Diagnosis/history of type 2 diabetes mellitus	7.7
ACCORD	Age ≥40 years with CVD or ≥50 years with substantial atherosclerosis, diagnosis/history of type 2 diabetes mellitus, HbA1c ≥7.5%, albuminuria, LVH or ≥2 CVD risk factors (dyslipidaemia, hypertension, smoking, obesity); SBP 130-180 mmHg and taking ≤3 antihypertensive drugs, 24-hour protein excretion rate <1g	4.7	More intensive (2362)	Less intensive (2371)	4733/0	Diagnosis/history of type 2 diabetes mellitus, or HbA1c ≥ 7.5%	13.9
ACTIVE I	Atrial fibrillation, ≥1 risk factor (age ≥75 years, on antihypertensive treatment, history of stroke, TIA or non-CNS embolism, LVEF <45%, PVD, or age 55-74 years with either CAD or diabetes)	4.1	ARB (3058)	Placebo (3076)	1120/5014	Diagnosis/history of type 2 diabetes mellitus, or pharmacological glucose-lowering therapy	2.6
ADVANCE	Age ≥55 years, type 2 diabetes mellitus (diagnosed aged ≥30 years), ≥1 major CVD or ≥1 CVD risk factor (microvascular disease, smoking, dyslipidaemia, microalbuminuria, Diagnosis of type 2 diabetes mellitus for ≥10 years, age ≥65 years)	4.2	ACEI and Diuretic (5569)	Placebo (5571)	11140/0	Diagnosis of type 2 diabetes mellitus at ≥ 30 years old, or previous diagnosis of type 2 diabetes mellitus for ≥ 10 years	5.4
ALLHAT	Age ≥55 years, stage 1 or 2 hypertension plus ≥1 risk factor (MI or stroke >6 months, LVH, diagnosis/history of type 2 diabetes mellitus, smoking, HDL <0.91 mmol/l), other atherosclerotic CVD	4.8	Diuretic (15255)	ACEI, CCB and Alpha-blockers (27163)	16575/25843	Diagnosis/history of type 2 diabetes mellitus, or baseline fasting glucose level of ≥126 mg/dL (7 mmol/L)	2.0
ANBP	Age 30-69 years with mild hypertension (DBP 95-110 mmHg and SBP <200 mmHg)	3.6	Diuretic (1721)	Placebo (1706)	0/3427	Diagnosis/history of type 2 diabetes mellitus	7.5
ANBP2	Age 65-84 years, SBP ≥160 mmHg or DBP ≥90 mmHg (if SBP ≥140 mmHg), no recent CVD	4.1	Diuretic (3039)	ACEI (3044)	402/5681	Diagnosis/history of type 2 diabetes mellitus	0.9
ASCOT-BPLA	Age 40-79 years, untreated (SBP ≥160 or DBP ≥100 mmHg) or treated hypertension (SBP ≥140 or DBP ≥90 mmHg), ≥3 CVD risk factors (documented LVH, abnormal ECG, type 2 diabetes mellitus, PAD, previous stroke or TIA, male sex, age ≥55 years, microalbuminuria or proteinuria, smoking, TC:HDL ≥6, family history of premature coronary heart disease	5.3	CCB-based (9639)	Beta-blocker based (9618)	5145/14112	Fasting glucose level ≥ 126 mg/dL (7 mmol/L), or a 2-h post-load plasma of 199.8 mg/dL (11.1 mmol/l), or pharmacological and non-pharmacological glucose-lowering therapy, or diagnosis/history of type 2 diabetes mellitus	2.2

Trial name	Inclusion criteria	Mean of follow-up duration (year)	Intervention (n)	Comparator (n)	Number of participants with and without diabetes at baseline (with diabetes/without diabetes)	Definition of diabetes at baseline	SBP difference (mmHg) excluding first 12 months *
BENEDICT	Age ≥40 years, untreated SBP ≥130 / DBP ≥85 mmHg or needing treatment to attain below these levels, type 2 diabetes mellitus for <25 years, urinary albumin excretion rate <20 mcg/min, serum creatinine ≤133 μmol/l	3.1	ACEI, CCB and ACEI/CCB (907)	Placebo (302)	1209/0	History of type 2 diabetes mellitus not exceeding 25 years	2
CAMELOT	Age 30-79 years, coronary artery stenosis >20% by angiography, DBP <100 mmHg	1.6	CCB and ACEI (1340)	Placebo (657)	439/1542	Diagnosis/history of type 2 diabetes mellitus, or fasting glucose level ≥ 126 mg/dL (7 mmol/L)	5.3
CAPPP	Age 25-66 years, DBP ≥100 mmHg on two occasions	5.8	Beta-blocker and/or Diuretic (5493)	ACEI (5492)	572/10413	At least two abnormal fasting glucose values that were unequivocal (i.e., between 99 mg/dL [5.5 mmol/L] and 120.6 mg/dL [6.7 mmol/L]). If they were not unequivocal, diagnosis was confirmed by an oral glucose tolerance test.	2.2
CARDIO-SIS	Age ≥55 years, SBP ≥150 mmHg, taking antihypertensive drug ≥12 weeks, ≥1 CV risk factor (smoking, dyslipidaemia, family history of premature CVD, prior TIA or stroke, established CAD or PAD	4.7	More intensive (558)	Less intensive (553)	0/1111	Fasting blood glucose ≥126 mg/dL (≥7 mmol/l), diagnosis/history of diabetes	3.8
CASE-J	Age 20-85 years, ≥1 high-risk factor: SBP ≥180 or DBP ≥110 mmHg, type 2 diabetes mellitus, history of angina pectoris, MI, stroke, TIA >6 months	3.1	CCB (2349)	ARB (2354)	2018/2685	Fasting blood glucose ≥126 mg/dL [7 mmol/l], casual blood glucose ≥200 mg/dL [11.1 mmol/l], haemoglobin A1c ≥ 6.5%, 2-h blood glucose on 75 g oral glucose tolerance test ≥200 mg/dL [11.1 mmol/l], or pharmacological glucose-lowering therapy	1.7
COLM	Age 65-84 years, hypertension (treated: blood pressure ≥140/90 mmHg; untreated: blood pressure ≥160/100 mmHg), CVD history or CVD risk factors including diabetes and dyslipidaemia	3.0	ARB and Diuretic (2573)	ARB and CCB (2568)	1362/3779	Diagnosis/history of type 2 diabetes mellitus, fasting blood glucose ≥110 mg/dL [6.1 mmol/l] or postprandial blood glucose≥140 mg/dL [7.8 mmol/l]	0.3
CONVINCE	Age ≥55 years, hypertension, ≥1 CVD risk factor (e.g., diabetes, smoking)	2.8	CCB (8179)	Beta-blocker or Diuretic (8297)	3239/13144	Diagnosis/history of type 2 diabetes mellitus	0.0
COPE	Age 40-85 years, blood pressure ≥140/90 mmHg	3.6	CCB/Diuretic and CCB/ Beta-blocker (2183)	CCB and ARB (1110)	498/2795	Diagnosis/history of type 2 diabetes mellitus (excluding patients required insulin treatment)	0.4

Trial name	Inclusion criteria	Mean of follow-up duration (year)	Intervention (n)	Comparator (n)	Number of participants with and without diabetes at baseline (with diabetes/without diabetes)	Definition of diabetes at baseline	SBP difference (mmHg) excluding first 12 months *
DIABHYCAR	Age ≥50 years, type 2 diabetes mellitus, urinary albumin excretion ≥20 mg/l in two consecutive urine samples	3.9	ACEI (2443)	Placebo (2469)	4912/0	Treatment with at least one oral antidiabetic agent	0.9
Dutch TIA Trial	TIA or non-disabling ischaemic stroke (Rankin Scale ≤3) in past 3 months	2.3	Beta-blocker (732)	Placebo (741)	97/1376	Diagnosis/history of diabetes, the use of oral antidiabetic drugs or insulin, or a nonfasting plasma glucose level of ≥ 199.8 mg/dl [11.1 mmol/l]	3.1
ELSA	Age 45-79 years, blood pressure 150-210/95-115 mmHg	3.4	CCB (1177)	Beta-blocker (1157)	156/2178	Fasting plasma glucose ≥126 mg/dl [7 mmol/l], or report of current drug treatment for diabetes	0.8
EUROPA	Age ≥18 years, documented MI >3 months before screening, revascularisation >6 months before screening, >70% coronary obstruction	4.2	ACEI (6110)	Placebo (6108)	1502/10716	Diagnosis/history of diabetes, or taking antidiabetic agents	4.6
EWPHE	Age ≥60 years, blood pressure 160-239/90-119 mmHg	4.6	Diuretic (416)	Placebo (424)	91/734	Fasting plasma glucose ≥126 mg/dl [7 mmol/l], or diagnosis/history of type 2 diabetes mellitus	22.4
HIJ-CREATE	Age 20-80 years, CAD hospitalisation and hypertension (blood pressure ≥140/90 mmHg or antihypertensive treatment use)	4.0	ARB (1024)	non-ARB (1025)	1009/1040	Fasting blood glucose ≥126 mg/dL [7 mmol/l] or treatment with hypoglycaemic agents at the time of enrolment.	0.4
HOMED-BP	Self-measured SBP 135-179 mmHg or DBP 85-119 mmHg, but not if DBP <65 mmHg or SBP <110 mmHg (clinic SBP <220 mmHg and DBP <125 mmHg)	4.9	More intensive (1759)	Less intensive (1759)	531/2915	Fasting blood glucose ≥126 mg/dL [7 mmol/l], or an HbA1c ≥ 6.5%, or treatment with oral antidiabetic drugs or insulin	2.0
HOPE	Age ≥55 years, CAD, stroke, PVD or diabetes, plus ≥1 risk factor (hypertension, dyslipidaemia, smoking, or documented microalbuminuria)	4.5	ACEI (4645)	Placebo (4652)	3577/5720	Diagnosis/history of type 2 diabetes mellitus	3.0
HYVET	Age ≥80y years, sustained SBP ≥160 mmHg	2.1	Diuretic (1933)	Placebo (1912)	388/3457	Diagnosis/history of type 2 diabetes mellitus, the receipt of antidiabetic treatment, or a random blood glucose > 200 mg/dl [11.1 mmol/l]	13.1
IDNT	Age 30-70 years, type 2 diabetes, hypertension (blood pressure ≥135/85 mmHg or taking anti-hypertensive drug), proteinuria, serum creatinine (µmol/l): 88 to 265 (women) or 106 to 265 (men)	2.6	ARB and CCB (1143)	Placebo (568)	1711/0	Diagnosis/history of type 2 diabetes mellitus	2.8
INSIGHT	Age 55-80 years, hypertensive (SBP ≥150 or DBP ≥95 mmHg, or SBP ≥160 mmHg), ≥1 other risk factor (TC ≥6.43 mmol/l, smoking, family history of premature MI, CAD, other CVD	2.8	Diuretic (3164)	CCB (3157)	1302/5019	Diagnosis/history of diabetes mellitus	1.1

Trial name	Inclusion criteria	Mean of follow-up duration (year)	Intervention (n)	Comparator (n)	Number of participants with and without diabetes at baseline (with diabetes/without diabetes)	Definition of diabetes at baseline	SBP difference (mmHg) excluding first 12 months *
INVEST	Age ≥50 years, documented CAD, essential hypertension requiring drug therapy, heart failure Class I-III	2.8	CCB (10648)	non-CCB (10672)	5879/15441	History of or currently taking antidiabetic medications	0.1
JMIC-B	Age <75 years, hypertension (blood pressure ≥160/≥95 mmHg or both SBP ≥150 and DBP ≥90 mmHg, or antihypertensive treatment), CAD or meeting both criteria: history of >2 anginal attacks per week with stable frequency and ST-segment depression of ≥1 mm on stress test (or detection of MI with myocardial scintigraphy)	2.3	CCB (828)	ACEI (822)	372/1278	Diagnosis/history of diabetes mellitus	2.0
LIFE	Age 55-80 years, hypertension (SBP 160-200 mmHg; DBP 95-115 mmHg), electrocardiogram signs of LVH	4.9	ARB (4605)	Beta-blocker (4588)	1195/7998	Diagnosis/history of diabetes mellitus	1.2
MOSES	Hypertension requiring treatment, documented TIA, ischaemic stroke or cerebral haemorrhage	3.3	CCB (671)	ARB (681)	498/854	Diagnosis/history of diabetes mellitus	1.5
NICS-EH	Age ≥60 years, SBP 160-220 mmHg and DBP <115 mmHg and no cardiovascular complications	3.2	Diuretic (214)	CCB (215)	17/412	Diagnosis/history of diabetes mellitus	0.3
NORDIL	Age 50-74 years, untreated hypertension (DBP ≥100 mmHg on two occasions); if previously treated, DBP ≥100 mmHg on two consecutive visits at one week apart during run-in period and no treatment was given	4.2	Beta-blocker and/or Diuretic (5471)	CCB (5410)	727/10154	Diagnosis/history of type 2 (non-insulin dependent) diabetes mellitus	3.3
ONTARGET	CAD, PAD, CeVD or diabetes with end-organ damage	4.8	ARB/ACEI (8502)	ACEI and ARB (17118)	9612/16001	Diagnosis/history of type 2 diabetes mellitus with end-organ damage	1.9
PART 2	Age ≤75 years, diagnosis (in past 5 year) of MI, documented CAD, TIA or intermittent claudication	4.6	ACEI (308)	Placebo (309)	51/566	Diagnosis/history of diabetes mellitus	6.5
PEACE	Age ≥50 years, documented CAD	4.7	ACEI (4158)	Placebo (4132)	1380/6910	Diagnosis/history of diabetes mellitus	3.0
PREVEND IT	Microalbuminuria, SBP <160/100 mmHg (no previous antihypertension treatment)	3.8	ACEI (431)	Placebo (433)	43/821	Diagnosis/history of diabetes mellitus, or fasting blood glucose ≥126 mg/dL [7 mmol/l]	5.6
PREVENT	Age 30-80 years, documented CAD, DBP <95 mmHg, cholesterol <325 mg/dl, fasting blood glucose <200 mg/dl	3.0	CCB (417)	Placebo (408)	98/727	Diagnosis/history of diabetes mellitus	6.1
PROFESS	Age ≥55 years with ischaemic stroke <90 days before randomization (later modified to include age 50 to 54 years or had stroke 90 to 120 days before randomisation if with ≥2 additional risk factors: diabetes, hypertension, smoker, obesity previous CVD, end-organ damage or hyperlipidaemia) and remained stable[a]	2.5	ARB (9873)	Placebo (9925)	5587/14211	Diagnosis/history of diabetes mellitus	3.4

Trial name	Inclusion criteria	Mean of follow-up duration (year)	Intervention (n)	Comparator (n)	Number of participants with and without diabetes at baseline (with diabetes/without diabetes)	Definition of diabetes at baseline	SBP difference (mmHg) excluding first 12 months *
PROGRESS	Stroke or TIA in past 5 years	3.9	ACEI and/or Diuretic (3051)	Placebo (3054)	761/5344	Diagnosis/history of diabetes mellitus	9.2
SHEP	Age ≥60 years, isolated systolic hypertension (BP 160-219/<90 mmHg, not on treatment)	5.0	Beta-blocker and Diuretic (2365)	Placebo (2371)	476/4238	Diagnosis/history of type 2 diabetes mellitus	12.8
SPRINT	Age ≥50 y years, SBP 130-180 mmHg, increased CVD risk (clinical/subclinical CVD other than stroke, CKD excluding polycystic kidney disease and with eGFR of 20-60 ml/min/1.73m^2 body surface area, 10-year Framingham CVD risk ≥15%, age ≥75y)	3.0	More intensive (4678)	Less intensive (4683)	394/8967	Diagnosis/history of diabetes mellitus, fasting glucose at randomization ≥126 mg/dL (7 mmol/L), treatment with hypoglycaemic agents	14.9
STOP Hypertension-2	Aged 70-84 years, SBP ≥180 mmHg and/or DBP ≥105 mmHg	4.5	Beta-blocker and/or Diuretic (2213)	ACEI and CCB (4401)	719/5895	Diagnosis/history of diabetes mellitus	2.1
SYST-EUR	Age ≥60 years, sitting SBP 160-219 mmHg, sitting DBP <95 mmHg, and standing SBP ≥140 mmHg	2.6	CCB (2398)	Placebo (2297)	584/4111	Diagnosis/history of diabetes mellitus, or fasting glucose at randomization ≥ 126 mg/dL (7 mmol/L)	10.1
TRANSCEND	Intolerant to ACEI and with established CAD, PVD, CeVD or diabetes with end-organ damage	4.9	ARB (2954)	Placebo (2972)	2284/3642	Diagnosis/history of diabetes mellitus, or fasting glucose at randomization ≥ 126 mg/dL (7 mmol/l)	4.5
UKPDS	Age 25-65 years, newly-diagnosed diabetes, and hypertension (untreated: SBP ≥160 mmHg and/or DBP ≥90 mmHg; treated: SBP ≥150 mmHg and/or DBP ≥85 mmHg)	7.9	More intensive (758)	Less intensive (390)	1148/0	Fasting plasma glucose concentration > 108 mg/dl (6 mmol/l) on two mornings	11.2
VALISH	Age ≥70 to <85 years, isolated hypertension (SBP >160 mmHg and DBP <90 mmHg)	2.6	More intensive (1545)	Less intensive (1534)	418/2661	Diagnosis/history of diabetes mellitus, or fasting glucose at randomization ≥126 mg/dL (7 mmol/L)	5.0
VALUE	Age ≥50 years, hypertension, CVD, CVD risk factors (male sex, age >50 years, diabetes, current smoking, high cholesterol, LVH, proteinuria, serum creatinine 150 to 265 μmol/l)	4.2	CCB-based (7596)	ARB-based (7649)	5376/9869	Diagnosis/history of diabetes mellitus, or fasting glucose at randomization ≥126 mg/dl (7 mmol/L)	1.6
VHAS	Age 40-65 years, BP ≥160/95 mmHg	1.7	Diuretic (707)	CCB (707)	135/1279	Diagnosis/history of diabetes mellitus	1.7
HDFP	Ages 30-69 years, hypertension, DBP home readings and clinic readings ≥ 95 mmHg and 90 mmHg, respectively	7.2	More intensive (5553)	Less intensive (5387)	894/10034	Diagnosis/history of diabetes mellitus, or random blood glucose > 200 mg/dl (11.1 mmol/L)	9.9

* Estimated through a one-stage individual patient data meta-analysis approach, and applied linear mixed models to estimate the effect of treatment on blood pressure between comparison arms, SD: standard deviation, SBP: systolic blood pressure, DBP: diastolic blood pressure, eGFR: estimated glomerular filtration rate, CVD: cardiovascular diseases, LVH: left ventricular hypertrophy, TIA: transient ischaemic attack, CNS: central nervous system, LVEF: left ventricular ejection fraction, PVD: peripheral vascular disease, CAD: coronary artery disease, MI: myocardial infarction, HDL: high-density lipoprotein, ECG: electrocardiography, TC: total cholesterol, CeVD: cerebrovascular disease, CHD: coronary artery disease, CKD: coronary artery disease, ACEI: Angiotensin-converting enzyme inhibitors, ARB: angiotensin receptor blockers, CCB: calcium channel blocker

Table 7-3. Baseline characteristics of participants stratified by type 2 diabetes at baseline.

Characteristics	Diabetes (103325)	No diabetes (255208)
Women, n (%)	43276 (41.9)	105832 (41.5)
Age, mean (SD)	65.4 (8.2)	64.8 (10.2)
Systolic blood pressure (mmHg), mean (SD)	149.5 (19.9)	153.5 (21.7)
Diastolic blood pressure (mmHg), mean (SD)	84.10 (11.5)	88.67 (12.5)
Body-mass index (kg/m^2), mean (SD)	29.33 (5.5)	27.3 (8.0)
Ethnicity, n (%)		
White/Caucasian/European	51276 (60.8)	118206 (66.5)
Black	8916 (10.6)	16403 (9.2)
Hispanic	7661 (9.1)	13631 (7.7)
Asians	13089 (15.5)	25337 (14.3)
Other	3446 (4.1)	4046 (2.3)
Categories of systolic blood pressure (mmHg), n (%)		
<120	5133 (5.1)	11583 (4.5)
120 to 129	9188 (9.1)	20936 (8.2)
130 to 139	15686 (15.5)	32408 (12.7)
140 to 149	21500 (21.2)	44582 (17.5)
150 to 159	18951 (18.7)	41911 (16.4)
160 to 169	15228 (15.0)	45667 (17.9)
≥170	15828 (15.6)	57974 (22.7)
Categories of diastolic blood pressure (mmHg), n (%)		
<70	9209 (9.1)	14207 (5.6)
70 to 79	22440 (22.1)	39841 (15.6)
80 to 89	35267 (34.7)	72893 (28.6)
90 to 99	24718 (24.3)	73059 (28.6)
100 to 109	8073 (8.0)	42248 (16.6)
≥110	1805 (1.8)	12799 (5.0)
Comorbidity, n (%)		
Peripheral vascular disease	4433 (13.3)	8462 (8.4)
Arial fibrillation	2942 (2.9)	7548 (3.0)
Chronic kidney disease	6936 (24.4)	17125 (14.7)
Cerebrovascular disease	14056 (19.2)	36627 (17.1)
Ischaemic heart disease	32537 (32.4)	87440 (34.3)
Previous use of non-study medications, n (%)		
Diuretic	14864 (26.8)	19554 (17.7)
Alpha-blocker	1674 (4.7)	3176 (3.9)
Beta-blocker	18231 (32.2)	41697 (35.2)
ACEIs	22160 (41.6)	26198 (27.1)
Angiotensin receptor blocker	3759 (10.1)	4818 (7.6)
Calcium channel blocker	19265 (34.0)	36770 (31.1)
Anti-platelet	22438 (45.1)	28584 (41.9)
Anti-coagulant	1821 (5.4)	4748 (9.2)
Lipid-lowering treatment	20653 (41.8)	33811 (34.3)
Follow-up (years), median (IQR)	4.33 (1.88)	4.13 (2.07)

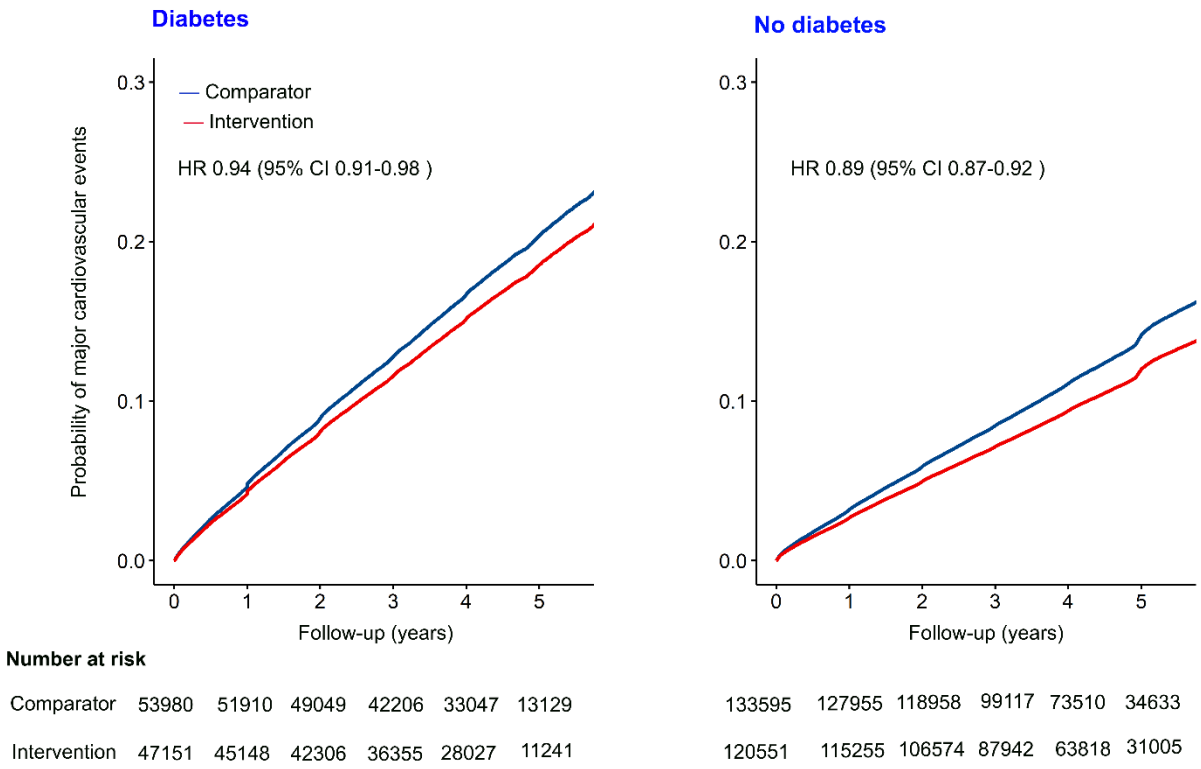
SD: standard deviation, IQR: interquartile range, ACEIs: Angiotensin-converting enzyme inhibitors

Peripheral vascular disease and chronic renal disease were the two most prevalent comorbid diseases among type 2 diabetes patients at baseline, but the distribution of other comorbid disorders was similar across the two groups. At baseline, more people in the diabetes group than those without diabetes had a history of taking diuretics, ACEIs, and ARBs (**Table 7-3**).

During the median follow-up time of 4.2 years, 43,461 major cardiovascular disease events occurred. The numbers of events that occurred for each component of major cardiovascular diseases were as follows: 14,866 for stroke, 21,417 for ischemic heart disease, 8204 for heart failure, 11,765 for cardiovascular causes of death, and 30,792 for mortality from all causes. Incidence rates for the primary outcome were 14.3 (95% CI 14.1 to 14.5) and 8.51 (95% CI 8.4 to 8.6) per 100,000 person-years of follow-up, respectively, for persons with and without type 2 diabetes. In participants with type 2 diabetes at baseline, the incidence rates of primary outcomes per 100,000 person-years of follow-up between comparator and treatment groups were 15.5 (95% CI 15.2 to 15.9) and 13.9 (95% CI 13.6 to 14.3), respectively. In those without type 2 diabetes at baseline, the corresponding incidence rates were 9.3 (95% CI 9.2 to 9.5) and 7.8 (95% CI 7.7 to 8.0), respectively (**Figure 7-1**).

Figure 7-1. Cumulative probability of major cardiovascular events by treatment allocation per 5 mmHg reduction in systolic blood pressure, stratified by type 2 diabetes status at baseline.

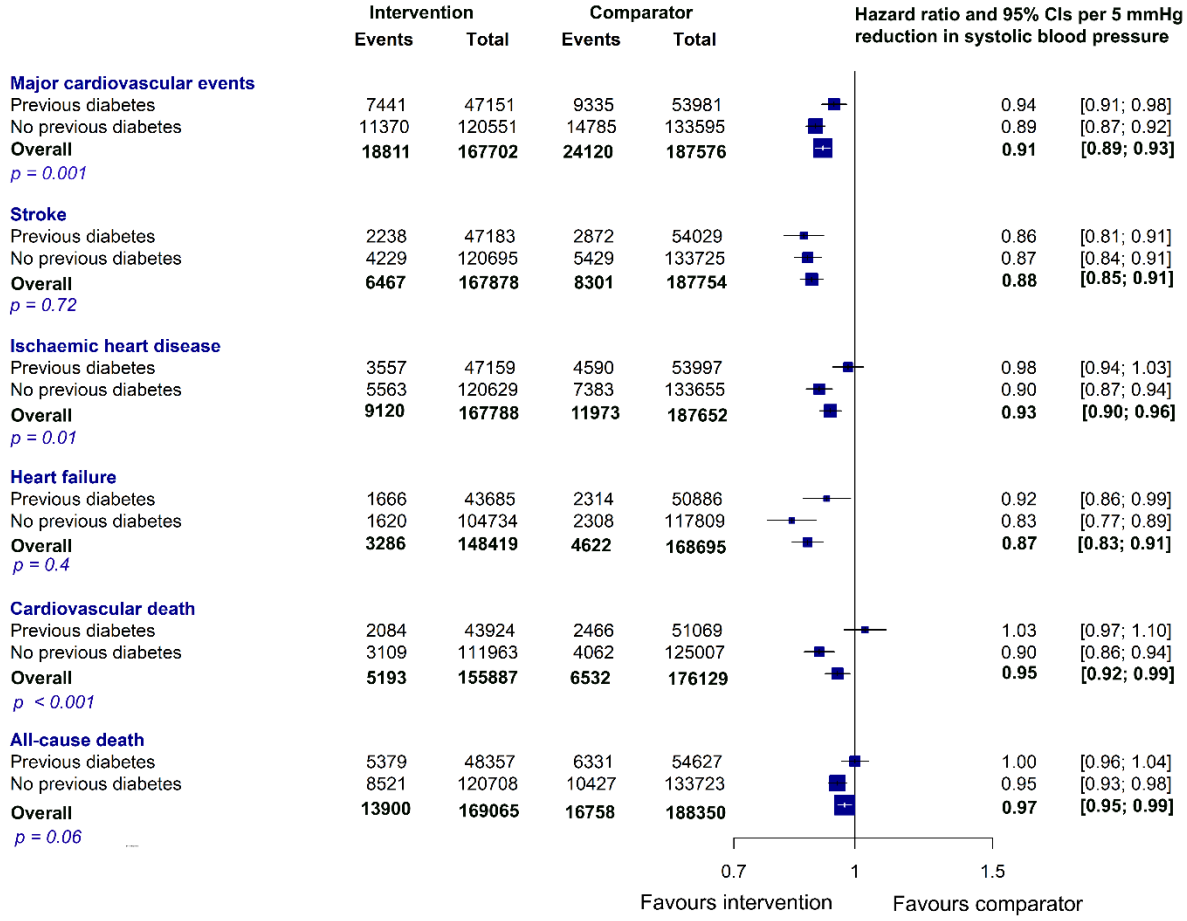
Major cardiovascular events are defined as a composition of fatal or non-fatal stroke, fatal or non-fatal ischaemic heart disease, or heart failure causing death or requiring hospitalisation. HR: hazard ratio, CI: confidence interval



A 5-mmHg reduction in systolic blood pressure reduced the risk of developing primary outcome both in participants with and without type 2 diabetes, with a weaker relative risk reduction in diabetes participants (HR 0.94 [95% CI 0.91 to 0.98]) than those without a history of diabetes (HR 0.89 [95% CI 0.87 to 0.92], p for interaction=0.001). The heterogeneity of effects identified for the primary outcome was mostly driven by cardiac events, where effects were smaller in those with diabetes compared to those without diabetes (**Figure 7-2**).

Figure 7-2. Effects of blood pressure-lowering treatment on primary and secondary outcomes, by type 2 diabetes status at baseline.

Hazard ratios were standardised for blood pressure reduction across trials and rescaled to a 5 mmHg reduction in systolic blood pressure. *p:* p-value for interaction adjusted for multiple comparisons, CI: confidence interval



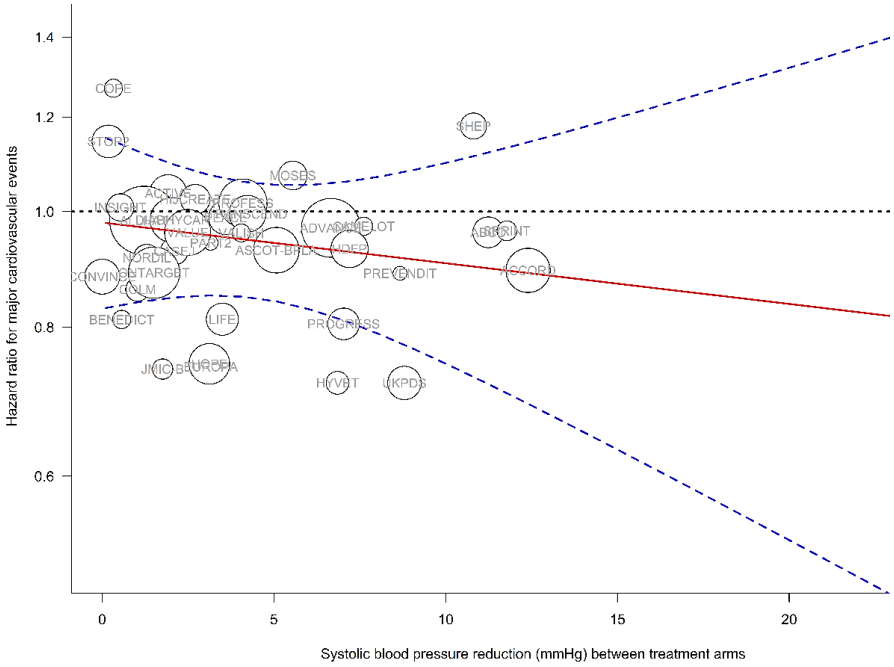
For stroke, the relative risk reduction appeared equivalent across diabetes status at baseline (all p for interaction = 0.72), however for heart failure, despite a trend of weaker effect size in the diabetes group, there was no statistical evidence of an interaction (p for interaction = 0.40). There was suggestive evidence of heterogeneous treatment effect for all-cause mortality (p for interaction=0.06) (**Figure 7-2**).

The relative risk reduction for major cardiovascular events was shown to be proportional to the amount of trial-level systolic blood pressure reductions, with a shallower slope among those with diabetes (**Figure 7-3**). When results were compared on an absolute risk scale, the observed heterogeneous relative treatment effects mostly disappeared, or at least diminished, mainly because of higher absolute baseline risk in diabetes participants (**Figure 7-4**). However, absolute risk reductions for cardiovascular mortality remained lower among those with diabetes compared to those without (**Figure 7-4**). We found no compelling evidence of heterogeneity of treatment effects by baseline systolic blood pressure level in persons with or without diabetes, for either primary or secondary outcomes, in stratified analyses (**Figure 7-5**).

Figure 7-3. Meta-regression of the intensity of blood pressure reduction and hazard ratio of major cardiovascular events, by type 2 diabetes status at baseline.

The hazard ratio for each trial is shown by the centre of the bubbles, with the size of the bubble inversely related to the respective standard error. The solid red line is the fitted regression line; the dotted blue lines represent the 95 per cent confidence intervals; the dashed grey line represents hazard ratio=1.

Diabetes



No diabetes

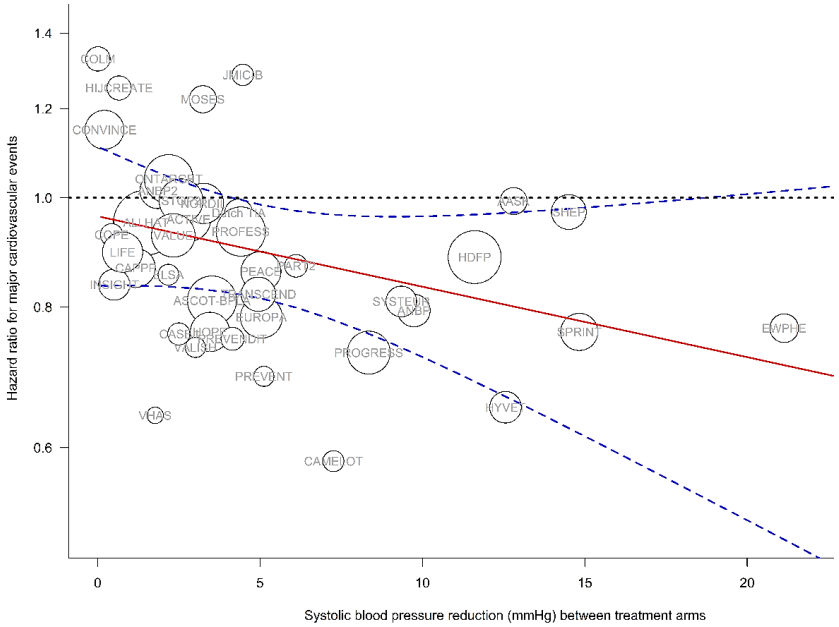


Figure 7-4. Percentage absolute risk reductions for the effect of blood pressure-lowering treatment on primary and secondary outcomes, by type 2 diabetes status at baseline.

Absolute risk reduction was estimated using a Poisson regression model with an identity link. The unit is the percentage of absolute risk difference, treatment versus comparator groups, over follow-up time and reflects the mean of blood pressure reduction across all trials. *p*: p-value for interaction adjusted for multiple comparisons

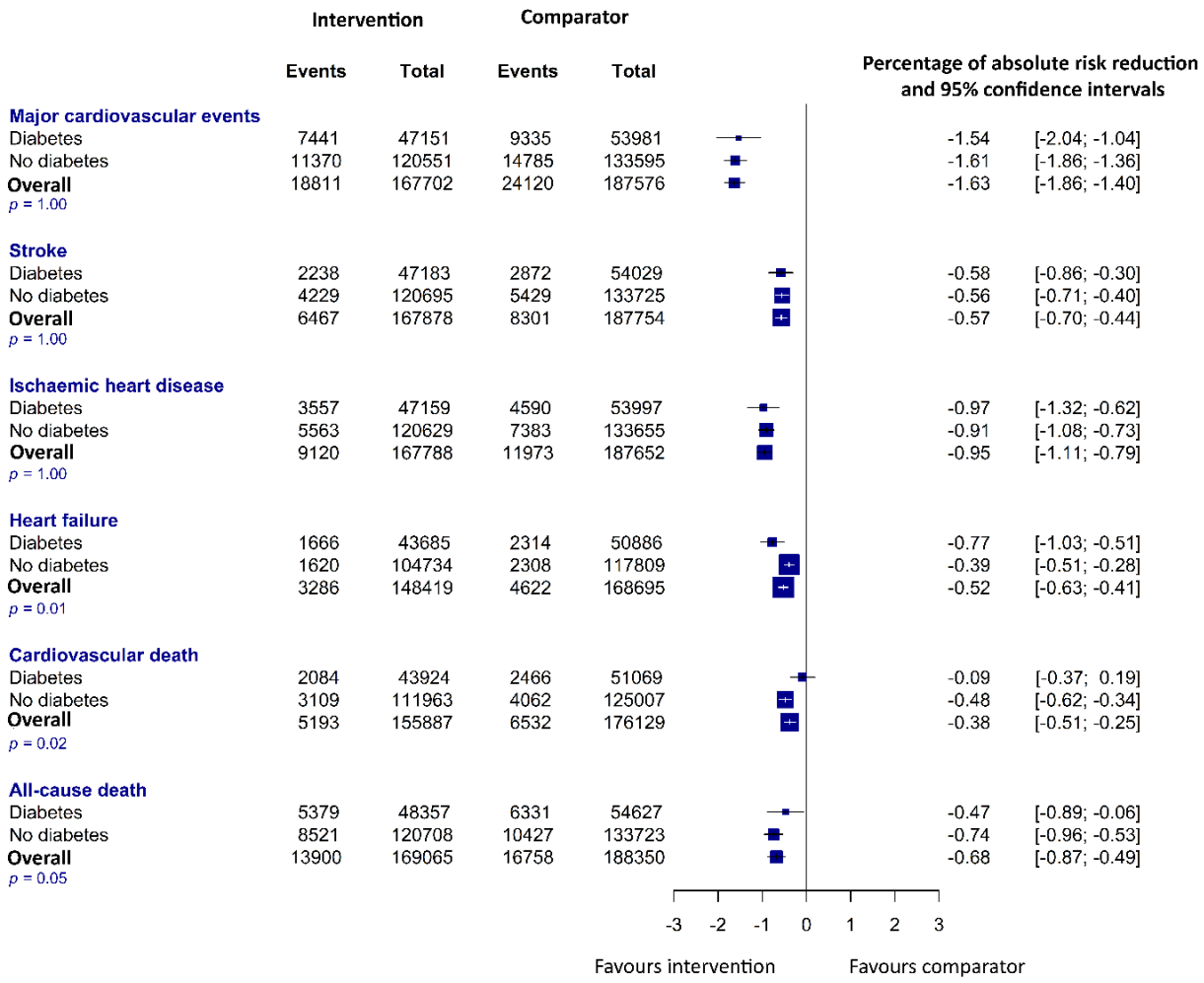
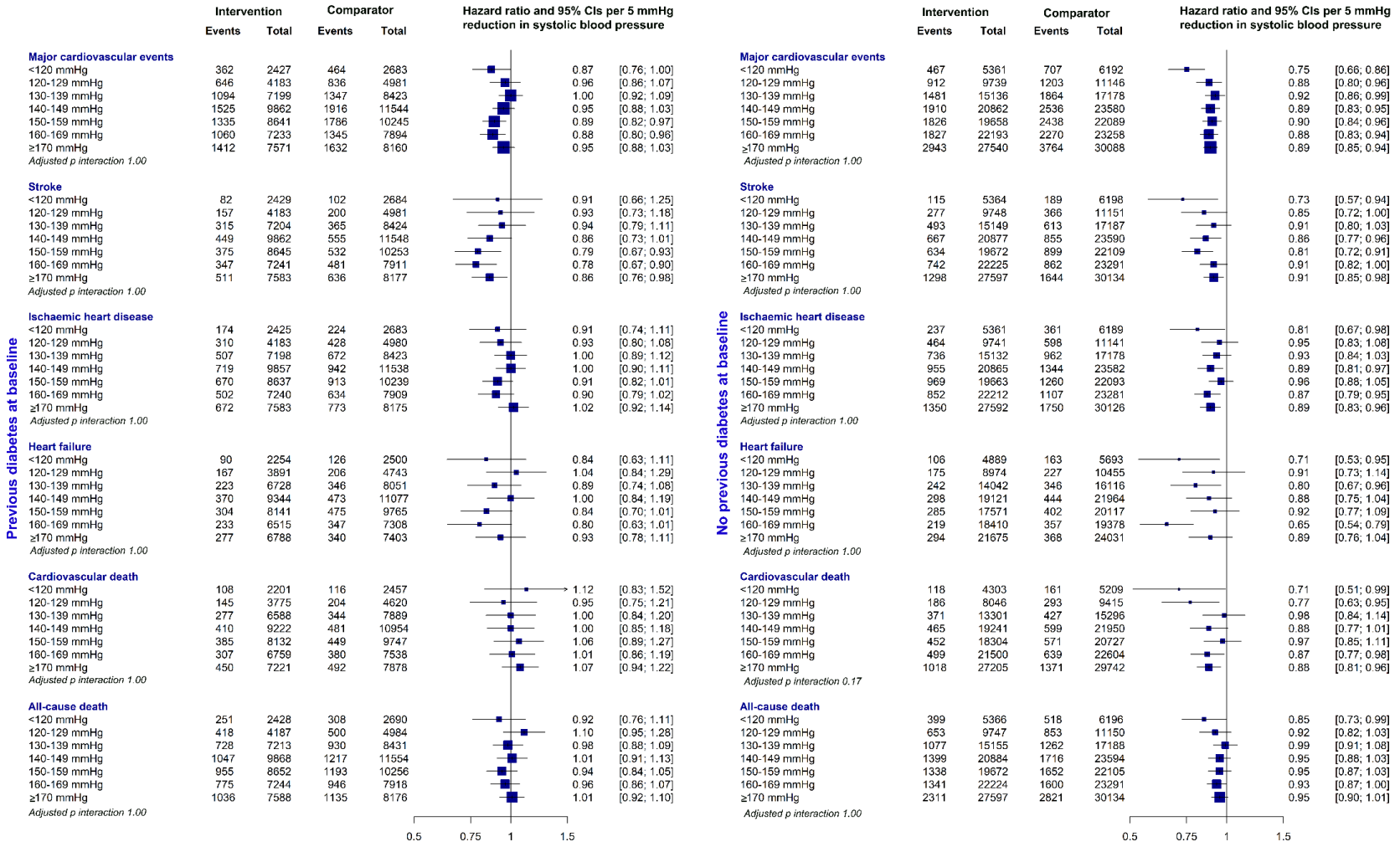


Figure 7-5. Effects of blood pressure-lowering treatment on primary and secondary outcomes stratified by baseline systolic blood pressure and type 2 diabetes at baseline.

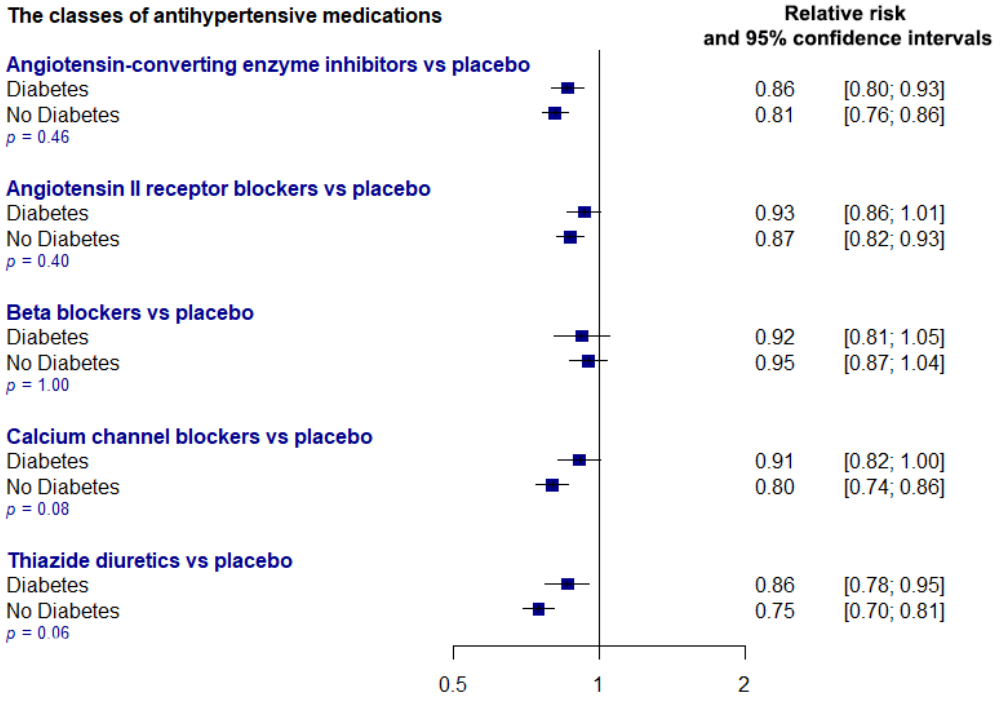
Hazard ratios standardised for blood pressure reduction across trials and rescaled to a fixed amount of 5 mmHg reduction in systolic blood pressure.



In line with the primary findings, stratified network meta-analysis revealed no indication that relative treatment effects differed between people with type 2 diabetes and those without type 2 diabetes for any of the drug classes studied (**Figure 7-6**).

Figure 7-6. Effect of major antihypertensive drug classes on the risk of major cardiovascular outcomes, by type 2 diabetes at baseline.

The relative risk for each trial was estimated using the binary logistic regression model. p for interaction calculated using the Chi-square test for heterogeneity of effect and adjusted for multiple comparisons.



Furthermore, the findings in sensitivity analysis were consistent with the main results in the analysis without standardisation for blood pressure reduction across trials (**Figure 7-7**). Furthermore, when we restricted the analysis to trials that utilised a laboratory test to diagnose type 2 diabetes at baseline, we found no material

changes in treatment effects (**Figure 7-8**). There was also no difference in effect sizes when head-to-head trials were excluded (**Table 7-4**).

Figure 7-7. The unstandardised effects of blood pressure-lowering treatment on primary and secondary outcomes, by type 2 diabetes status at baseline.

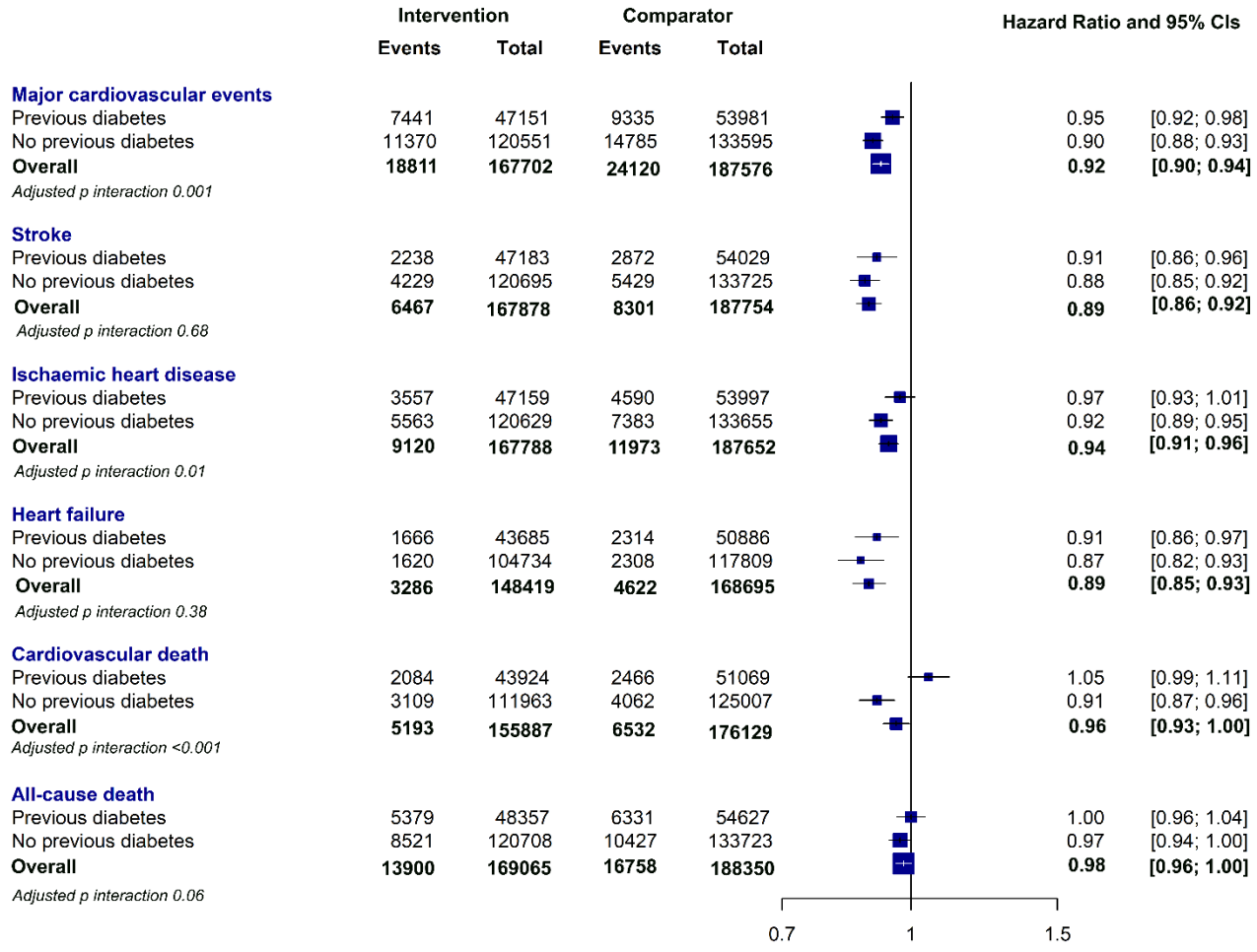


Figure 7-8. Sensitivity analysis restricted to trials that used a laboratory test for diagnosis of type 2 diabetes at baseline.

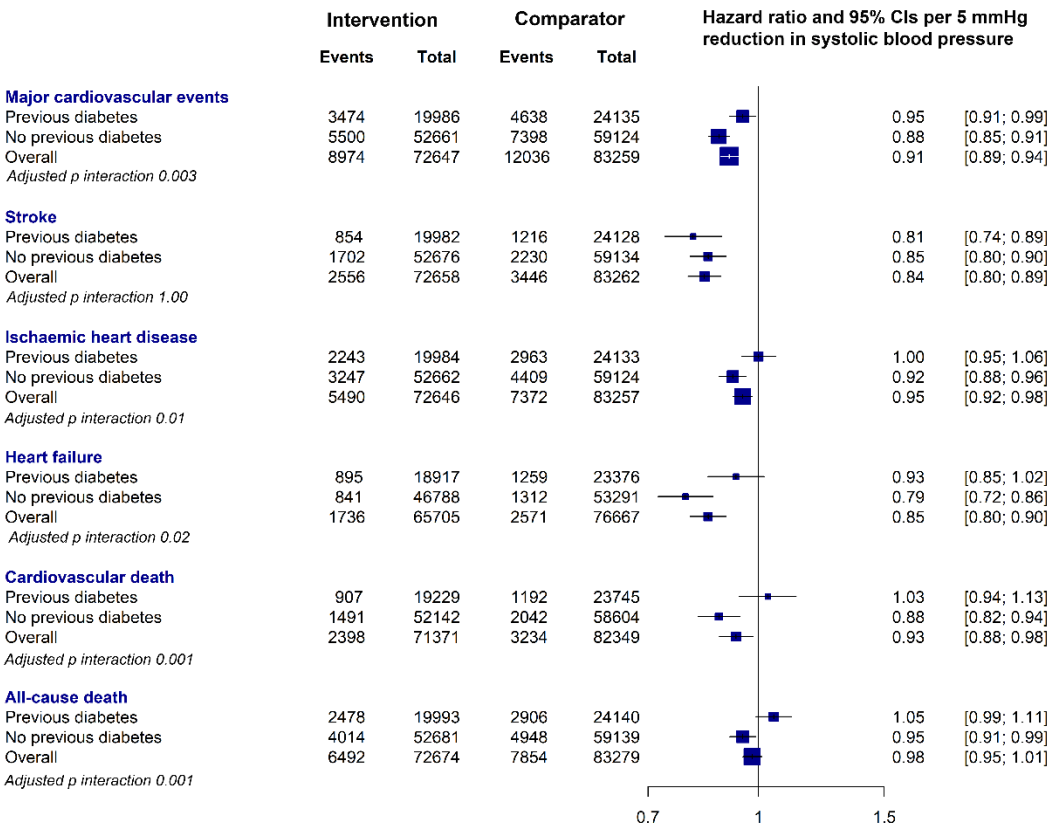


Table 7-4. Sensitivity analysis excluding head-to-head trials for the effect of blood pressure-lowering treatment on primary and secondary outcomes, by type 2 diabetes status at baseline.

Diabetes status	Intervention		Comparator					
	Events	Total	Events	Total	Hazard ratio	95% confidence interval		Adjusted p for interaction
Major cardiovascular events								
Previous diabetes	3639	22787	3821	21722	0.94	0.89	0.98	
No previous diabetes	5016	49827	5871	49066	0.86	0.83	0.90	
Overall	8655	72614	9692	70788	0.89	0.87	0.92	0.003
Stroke								
Previous diabetes	1139	22805	1305	21744	0.87	0.80	0.94	
No previous diabetes	2076	49896	2474	49133	0.86	0.80	0.92	
Overall	3215	72701	3779	70877	0.88	0.83	0.91	1.00
Ischaemic heart disease								
Previous diabetes	1376	22785	1434	21720	0.90	0.83	0.97	
No previous diabetes	2111	49837	2433	49075	0.85	0.79	0.91	
Overall	3487	72622	3867	70795	0.87	0.82	0.91	0.28
Heart failure								
Previous diabetes	680	19966	732	19301	0.93	0.84	1.03	
No previous diabetes	659	44281	808	43459	0.84	0.75	0.94	
Overall	1339	64247	1540	62760	0.88	0.82	0.95	0.10
Cardiovascular death								
Previous diabetes	948	19541	925	18826	1.05	0.96	1.15	
No previous diabetes	1368	41155	1556	40406	0.93	0.86	1.02	
Overall	2316	60696	2481	59232	0.98	0.92	1.04	0.007
All-cause death								
Previous diabetes	2447	23975	2428	22335	0.95	0.90	1	
No previous diabetes	3650	49905	3944	49129	0.95	0.90	1	
Overall	6097	73880	6372	71464	0.95	0.91	0.98	1.00

Hazard ratios standardised for blood pressure reduction across trials and rescaled to a fixed amount of 5 mmHg reduction in systolic blood pressure.

7.5. Discussion

In this IPD meta-analysis of major pharmacological blood pressure lowering trials including 103,325 people with type 2 diabetes and 255,208 participants without type 2 diabetes at baseline, pharmacological blood pressure lowering treatment reduced the risk of major cardiovascular events in both groups. However, the relative benefits were smaller in people with established type 2 diabetes compared to those without diabetes. Nevertheless, since patients with type 2 diabetes were at a greater risk for major cardiovascular events, the absolute risk reductions were identical across the two groups. Further investigation into the underlying causes of the heterogeneous relative effects showed that the differences were not significantly influenced by baseline systolic blood pressure levels or drug type.

Blood pressure reduction is a proven strategy for decreasing the risk of macro- and microvascular complications in type 2 diabetic patients. Significant decreases in the risk of cardiovascular events were shown in the UKPDS trial, one of the first large-scale trials of antihypertensive therapy in individuals with type 2 diabetes.[174] Several subsequent trials examined the effect of specific blood pressure-lowering drugs or different strategies of blood pressure control on people with type 2 diabetes. The ADVANCE trial, for instance, indicates that following a fixed regimen of perindopril–indapamide decreases all-cause mortality and major cardiovascular events in adults with type 2 diabetes, independent of baseline blood pressure or 10-year cardiovascular risk.[175] In 2005, the previous circle of BPLTTC published the findings of an IPD meta-analysis of these trials.[170] The use of antihypertensive therapy was shown to

lower the risk of major cardiovascular events to roughly the same level in adults with and without type 2 diabetes, according to the findings of this IPD meta-analysis that included 158,700 participants, of whom 33,395 had type 2 diabetes. However, the study lacked statistical power, the analysis was not standardised to account for different degrees of blood pressure reduction, and there was no attempt made to divide participants into groups according to their baseline blood pressure levels.

Several trials conducted in more recent years tried to compare the extent of the cardioprotective impact of blood pressure decreasing by baseline blood pressure. The SPRINT and ACCORD trials have reported some of the most unexpected results since they used essentially the same methodology but arrived at opposite conclusions.[165,166] This led to the notion that intensive blood pressure lowering may not be beneficial for type 2 diabetes patients.[159,168,169] This idea was supported by conventional meta-analyses of published data on individuals with type 2 diabetes. There were positive benefits on the risk of major cardiovascular events (HR 0.89 [95% CI 0.83 to 0.95] per 10 mmHg lower systolic blood pressure) in a meta-analysis that included 100,354 people with type 2 diabetes.[69] But, significant heterogeneity of effect was detected when trials were classified into two groups of baseline systolic blood pressure with a cut-off of 140 mmHg; in the group with a baseline systolic blood pressure less than 140 mmHg, there was no obvious decrease in risk of cardiovascular events (HR 0.96 [95% CI 0.88 to 1.05]).[69] Another traditional meta-analysis using data from 73,738 participants with type 2 diabetes revealed that antihypertensive treatment decreased the risk of

mortality and cardiovascular morbidity in people with diabetes and a systolic blood pressure greater than 140 mmHg. However, when the baseline systolic blood pressure was lower than 140 mmHg, treatment was more likely to have negative effects than positive ones, primarily because the treated group had an increased risk of cardiovascular death.[176] Nevertheless, these meta-analyses had a number of limitations. Importantly, they lacked individual-level data, thus their results may have been susceptible to ecological bias.[177] To fill this knowledge gap, I directly compared the effect of a fixed amount of blood pressure reduction in persons with and without type 2 diabetes by using a comprehensive database of randomised clinical trials with individual-level data. In addition to this, I was able to stratify the results according to more detailed categories of baseline blood pressure.

When comparing relative risks, the effects of a 5 mmHg systolic blood pressure reduction in type 2 diabetes were almost half those of individuals without diabetes. Because patients with diabetes had a greater risk of cardiovascular disease, absolute risk reductions were comparable in both groups. Assuming that patients with diabetes in clinical practice are at high risk for cardiovascular disease, our findings show that, despite smaller relative risk reductions, people with diabetes benefit more from even minor blood pressure reductions. However, trial data are seldom representative of the target population. The risk of the cardiovascular disease relies on a variety of variables and may vary considerably among type 2 diabetes patients. For example, the implementation of screening programmes has resulted in a rise in the number of patients identified with diabetes, but with far lower average

risks of cardiovascular disease than previously observed.[178] As a result, we advise against overgeneralising the absolute risk reductions from the RCTs and advocate including risk stratification at the stage of clinical decision-making for a more realistic calculation of the absolute benefits from treatments and the selection of people who are most likely to benefit from treatment.

My research also revealed that there was no heterogeneity in effects based on the baseline categories of systolic blood pressure. Even though the relative benefits on cardiovascular outcomes per unit reduction in blood pressure were shown to be reduced in diabetic patients, it was not observed that lowering blood pressure became ineffective or even harmful at a certain blood pressure threshold. This research thus casts doubt on the validity of prior recommendations for the establishment of fixed thresholds for the use of antihypertensive medication.[159,168,169] Clinicians caring for individuals with type 2 diabetes should inform their patients that antihypertensive treatments reduce the risk of cardiovascular disease proportionate to the degree of blood pressure decrease, regardless of their measured blood pressure.

While this pattern has not been seen in other disease phenotypes, the finding that relative risk reductions were smaller in persons with diabetes is remarkable. For example, in two recent BPLTTC investigations, the relative effects were unaffected by the presence or absence of cardiovascular disease or atrial fibrillation.[54,56] Consequently, this brings up the question of biological or even statistical causes driving diabetes' heterogeneous impact. To study

the results further, I did multiple complementary analyses. I conducted a meta-regression stratified by type 2 diabetes as well as a network meta-analysis by pharmacological class. These analyses supported the robustness of my results and showed no indication that the observed heterogeneity could be explained by different antihypertensive drug classes. Another potential explanation is that a greater average risk of diabetes may have diminished the proportionate effect of a reduction in blood pressure. However, considering that in an earlier BPLTTC research, stratification by baseline cardiovascular clinical risk did not change relative effects, this also appears implausible.[179] Patients with cardiovascular disease and atrial fibrillation were likewise at higher average risk in previous investigations, but no heterogeneous treatment effects were detected.[54,56]

Different pathophysiological pathways might explain the difference in effects between those with and without diabetes. There is substantial evidence that diabetes itself is a major risk factor for cardiovascular disease.[180] In previous chapters, I reported consistent evidence that lowering blood pressure is associated with a lower risk of new-onset type 2 diabetes using data from randomised trials and genetic information.[48] As a consequence, the benefits of blood pressure reduction on the risk of cardiovascular events may work in part via a decrease in diabetes risk. If this is genuine, the diluted magnitude of the effect in people with diabetes may be explained in part by diabetes's mediator role, which does not work in individuals with established diabetes.

Some considerations should be taken into account when interpreting and generalising these findings. I acknowledge that diabetes is a complex metabolic condition. Diabetes was diagnosed using a variety of criteria in the trials that were included in my analysis. In sensitivity analysis, when the main findings were stratified based on different diabetes ascertainment strategies, no significant changes were detected. Whether our results extend to other phases of the disease, however, needs more investigation. A post-hoc analysis of the SPRINT trial, in which individuals without known diabetes were stratified according to their baseline fasting serum glucose level, revealed that intensive blood pressure reduction may have a comparable favourable impact on major cardiovascular events and all-cause mortality among people with prediabetes versus those with normal blood glucose and that this benefit might be consistent throughout the spectrum of fasting serum glucose at baseline; however, the confidence intervals were wide and the results remained inconclusive.[181] More research is needed to examine the effects in a wider range of glucose intolerance and diabetes, as well as in a variety of glycemia management strategies for patients with diabetes. Similarly, the length of diabetes and associated comorbidities, such as nephropathy, may play a role in or explain the heterogeneous effects. Future BPLTTC investigations will study the role of these characteristics, which may assist in refining patient identification and treatment recommendations. Previous research has shown that lowering blood pressure in individuals with type 2 diabetes decreases the risk of retinopathy and chronic kidney disease.[69] Future research is needed to evaluate the degree to which the effects of blood pressure reduction on these outcomes are attributable to diabetes or other

comorbidities and to quantify the total advantages of blood pressure reduction.

In this chapter, I showed that the relative benefit of blood pressure reduction on major cardiovascular events is weaker in individuals with diabetes compared to those without diabetes. This was not because reducing blood pressure below a specific threshold was ineffective or hazardous. Indeed, no subgroup was shown to have harmful effects on major cardiovascular outcomes throughout the whole spectrum of blood pressure at baseline. These results highlight the importance of blood pressure reduction for cardioprotection prior to the development of diabetes. In individuals who already have type 2 diabetes, the existing blood pressure thresholds for the initiation of blood pressure therapy do not seem to be reasonable. On the other hand, classifying all patients with type 2 diabetes as being at a sufficiently high risk of cardiovascular diseases to merit decreasing their blood pressure does not seem to be warranted either, given that the relative risk reduction was not as strong.

Based on my findings in this Chapter, precise blood pressure thresholds should be eliminated from the procedure for selecting eligible persons with type 2 diabetes for antihypertensive treatment. The results of this study have been published in The Lancet Diabetes & Endocrinology, and parts of it were used for the writing of this chapter.[49]

Chapter 8. Discussion

8.1. Results in the context of objectives

The purpose of this thesis was to integrate genetic and trial data in order to obtain high-quality randomised evidence for the effect of blood pressure on the risk of type 2 diabetes, as well as to investigate the effect of blood pressure lowering in individuals with and without diabetes at baseline. Although these research questions are intimately familiar to many scientists in the field, to date no individual study has provided a conclusive answer to them. One probable explanation is that a single design or set of data cannot adequately address the potential association between two complex diseases, such as hypertension and diabetes. Therefore, as suggested in the literature "robust research needs many lines of evidence".[182] Before I began working on this thesis, I was well aware that it would not be simple to answer the aformentioned questions by relying just on IPD meta-analysis or genetic analysis alone.[70] As a result, in order to give the most compelling evidence possible, I made use of a concept known as the triangulation of evidence. Triangulation is a method used in navigation for pinpointing a position by measuring the angle between two or three points.[183] In medical research, it refers to the use of multiple methods or datasets, each with independent assumptions, to study identical research questions. The goal is to boost confidence in the findings by confirming estimates with two or more independent designs. This might be a very relevant idea when the data from the scientific literature remain disputed for an extended period of time and when we are dealing with complicated diseases and exposures that include

many known and unknown biological pathways. This approach may bring us closer to the truth, despite the fact that we cannot provide a definitive and universal answer to a medical research question.

In terms of the research question, this thesis sought to examine five objectives:

(1) To investigate the effect of pharmacological blood pressure-lowering on the risk of new-onset type 2 diabetes using data from randomised clinical trials.

(2) To investigate the separate effects of blood pressure-lowering drug classes on the risk of new-onset type 2 diabetes using randomised clinical trial data.

(3) To assess the causal association between blood pressure reduction and risk of type 2 diabetes using genetic data analysis.

(4) To assess the separate effect of blood pressure-lowering drug classes on the risk of type 2 diabetes using genetic data analysis.

(5) To investigate the effect of pharmacological blood pressure-lowering treatment for the prevention of major cardiovascular disease in persons with and without type 2 diabetes, using randomised clinical trials.

With regard to the effect of blood pressure-lowering on the risk of new-onset type 2 diabetes using trial data, the HR and 95% CIs for diagnosis of new-onset type 2 diabetes during 4.4 years of median follow-up for a 5 mmHg reduction in systolic blood pressure were 0.89 (95% CI 0.84 to 0.95). This effect did not appear to differ by body mass index categories at baseline.

Furthermore, the effect did not change in several sensitivity analyses including stratification by various diabetes diagnostic methods, adjustment for several further confounders, replicating analysis using two-stages meta-analysis, and assessment of acquisition bias. Because the overall idea in this thesis was the triangulation of evidence from randomised data, therefore I tested the same hypothesis in a separate study using Mendelian randomisation (Objective 3). Each 5 mmHg genetically predicted lower systolic blood pressure was associated with an 11% lower risk of type 2 diabetes (OR: 0.89 [95% CI 0.86 to 0.93]). After removing outlier variants, the size and direction of estimated ORs based on various Mendelian randomisation approaches were comparable. These findings provided compelling evidence that reducing blood pressure may be used as a preventative strategy to lower the risk of new-onset type 2 diabetes.

For the second objective, investigation of the effects of five major classes of antihypertensive drugs showed that in comparison to placebo, ACEIs (RR 0.84 [95% 0.76 to 0.93]) and ARBs (RR 0.84 [0.76 to 0.92]) reduced the risk of new-onset type 2 diabetes; however, the use of beta-blockers (RR 1.48 [1.27 to1.72]) and thiazide diuretics (RR 1.20 [1.07 to 1.35]) increased this risk, and no material effect was found for calcium channel blockers (RR 1.02 [0.92 to 1.13]). However, majority of evidence in this network meta-analysis comes from indirect comparison, therefore I used Mendelian randomisation approach to test a similar objective. In Objective 4, in line with results from RCTs, Mendelian randomisation showed a strong protective effect for ACEIs and ARBs (OR 0.64 [0.49 to 0.84]). Likewise, an

increase in risk and null effect approved for beta-blockers and calcium channel blockers respectively (OR for beta-blockers 1.24 [95% CI 1.08 to 1.43]; OR for calcium channel blockers 1.07 [95% CI 0.96 to 1.20). The genetic evidence for thiazide diuretics did not provide sufficient statistical power for analysis, and the Mendelian randomisation result remains inconclusive. Overall, based on consistent findings from RCTs and genetic analysis, I established substantial support for the preventive benefit of ACEIs and ARBs, no effect for calcium channel blockers, and an increase in diabetes risk by beta-blockers. In light of the fact that genetic analysis did not provide any findings that could be considered trustworthy for diuretics, I continue to consider the observed increase in the risk of type 2 diabetes to be the best available evidence for diuretics.

I could only study the fifth objective using IPD data from RCTs. Because the purpose was to compare the effect of lowering blood pressure in persons with and without diabetes, therefore we cannot answer this question using a Mendelian randomisation design. According to the findings of the IPD meta-analysis, there is no justification for differing blood pressure thresholds, intensities of blood pressure reduction, or medication classes based on diabetes status. I found that while the relative risk reduction of blood pressure lowering for major cardiovascular diseases is weaker in individuals with type 2 diabetes, the absolute risk reduction is roughly equal in those with and without type 2 diabetes.

8.2. Importance of this thesis for future research

Even though the data, methodologies, and designs used in this thesis were the strongest and most extensive available, there are still some significant questions that should be taken into consideration in further research. I provided compelling evidence supporting the effect of decreasing blood pressure on its own, as well as data about each individual class of medications. It is not well understood whether or to what extent the combination or co-prescribing of blood pressure medications from different classes can result in drug-drug interactions. For example, we know that beta-blockers and diuretics increase the risk of diabetes, while ACEIs decrease the risk. Consequently, what is the effect on diabetes if clinicians prescribe a combination of ACEIs and diuretics? This possible drug-drug interaction is an important current knowledge gap that cannot be answered easily using the currently available data. A series of large-scale RCTs with a factorial design comparing a combination of drug versus arms with single drug treatment and also a placebo are required to provide a precise answer to this question. We are not aware of any trials or ongoing studies that aim to assess such an effect. Also, some observational studies tried to answer this question, however, observational data with conventional designs are not ideal to assess drug class effects.[184] Therefore, apart from conducting an original RCT, two approaches can be taken in the future to answer this question. The first one is using AI on electronic health record (EHR) data. When conducting an RCT is unfeasible or costly, observational causal inference is an important alternative to assess research questions. The most recent advancements in machine learning (particularly deep learning) provide an excellent chance to fulfil this

unmet requirement. Scientists in the DeepMedicine group at the University of Oxford developed a ‘transformer-based model, targeted bidirectional EHR transformer (T-BEHRT) coupled with doubly robust estimation to estimate the causal average risk ratio’.[185] T-BEHRT has shown successful performance in tests using semi-realistic data with confounding, and it is now possible to use it for assessing treatment effects in observational studies using routine clinical data.[185] However, currently this model is optimised to compare the effect of one class of drug versus another drug. Therefore, in the future, it is recommended that this model be further evaluated and extended to study additional concerns about causal inference, in particular for drug-drug interaction effects on different outcomes.

Another alternative is using genetic data and Mendelian randomisation. There is a new subtype of Mendelian randomisation, so-called "factorial Mendelian randomisation" that provides this opportunity to investigate the drug-drug interaction effect.[186] Although the design has been used in several research projects, there is a dearth of methodological guidance about how to design or carry out a factorial Mendelian randomisation study. Previous studies have utilised the pooling of individual data from genetic biobanks by means of one-sample Mendelian randomisation and have constructed a 2×2 design by making use of dichotomised genetic risk scores in order to divide the population into four subgroups in the same manner as a factorial randomised trial. However, this approach, which requires pooling individual data from large-scale biobanks and dividing the population into four subgroups, usually generates a new

issue of weak statistical power. Recently methodologists suggested a new approach for using continuous genetic scores rather than dichotomised scores to enhance statistical power.[186] Although this improvement may be useful in some circumstances, this field of research still needs to develop a new extension of factorial Mendelian randomisation that can be conducted using summary statistics from independent GWAS studies in order to have much better statistical power for the assessment of interaction.

I investigated the effect of blood pressure lowering on the risk of major cardiovascular diseases in patients with and without diabetes at baseline, and the results revealed that blood pressure treatment is effective for cardiovascular disease prevention in both groups, regardless of baseline blood pressure or type of drug classes. However, some unanswered questions remain in this area of research. Major cardiovascular disorders, their components, and mortality were the focus of the final section of the thesis. Microvascular disease is a well-known and serious complication for persons with diabetes. To provide one example, retinopathy is a microvascular problem that may damage either the macula or the peripheral retina, or both. It is the major cause of vision impairment and blindness in persons who have diabetes.[187] We need further one-stage meta-analyses of RCTs to investigate the effect of blood pressure-lowering treatment on different microvascular outcomes and compare the effect between people with and without diabetes. Also, re-assessment of this effect by subgroups of blood pressure at baseline and with different characteristics of patients, including age, sex, ethnicity, and body mass index, is of high clinical importance.

On the other hand, the treatment and management of type 2 diabetes are greatly influenced by the existence of various comorbid diseases. Still, little is known about the management of blood pressure in type 2 diabetes, in particular in the presence of chronic comorbidities. For example, chronic kidney disease is one of the more prevalent co-morbid conditions in diabetes, with 50% prevalence in diabetic patients.[188] Previous studies showed that the combination of chronic kidney disease and diabetes has a considerable negative influence on the quality of life, and individuals with both conditions had a much worse quality of life than those with a single ailment.[189] Despite the common co-occurrence of diabetes and renal disease, little is known about the preventive effect of blood pressure lowering in such a condition, or the effect of specific blood pressure-lowering drug classes on the risk of microvascular and macrovascular complications in diabetics with chronic kidney disease and other comorbidities. Perhaps future IPD meta-analyses with the new extension of BPLTTC, which will provide a larger sample size and more information about co-morbidities at baseline, could answer this important question in more detail. Furthermore, causal inference using deep learning approaches, which have been shown to be particularly efficient in subgroups of high-risk patients with multiple co-morbidities, could be used as a complementary analysis to apply triangulation of evidence to assess this question from various perspectives.[185]

The pathophysiology of cardiovascular disease differs between genders, in part because women and men are exposed to different

physiological and environmental factors. While it has been demonstrated conclusively that pharmacological treatments to lower blood pressure reduce cardiovascular morbidity and mortality, there has been a growing demand for research to better understand the gender-specific effects of blood pressure management and cardiovascular disease.[190,191] There are clinically important questions regarding whether distinct blood pressure-lowering strategies should be used to prevent cardiovascular disease in women and men, as well as individuals of different ethnicities. The BPLTTC research team is presently investigating these research queries, and the results will be published in the near future. Aside from this, more stratified analyses employing multiple risk factors or a clinical risk score will be required in the future to provide more precise evidence, especially for less representative patient groups.

Finally, in this thesis, I found that blood pressure-lowering drugs could have possible off-target effects on the risk of diabetes, as a complex cardiometabolic condition. This may pave the way for the development of novel treatments for type 2 diabetes. Apart from causal inference for risk factors and prediction of known drugs' effects, Mendelian randomisation provides a strong framework for drug discovery.[192,193] Particularly with the availability of proteome data in recent years, it is feasible to use proteome data in a two-sample Mendelian randomisation framework to discover and validate new drug targets for different diseases.[194]

8.3. Methodological considerations

The main study design in this thesis was a one-stage IPD meta-analysis. Due to the scarcity of IPD data, particularly from trials, some methodological aspects and the correct way of interpreting results are less known to the scientific communities. In this part of my thesis, I will discuss some important methodological points that could be important for a valid interpretation of one-stage meta-analysis results.

Generalisability and representativeness: We commend those who choose principle above pragmatism, but what is the philosophy that "representativeness" epitomise? Representativeness is crucial for election prediction and selecting well-represented audiences for polling businesses, but it is not essential for causal inference in scientific research. The strength of randomised comparisons of medical interventions lies in their ability to provide unbiased estimates of relative treatment effects. Several prerequisites, including internal and external validity, must be satisfied in order to attain this goal. Internal validity is the accuracy of the inferences made in relation to the individuals of the sample chosen. Likewise, the accuracy of the inferences as they apply to others outside of that study sample is known as external validity. In the context of RCTs, internal validity corresponds to accurate measurement of effects apart from random variation, bias and confounding. Pursuant to such a concept, it is considered a prerequisite for external validity. The valid evaluation of the causal link may be broadly generalisable if the internal validity is high, and the participants do not have to be representative of those to whom the new findings would be

applied. Unlike population-based national surveys that aim to capture the incidence, prevalence or other measures of absolute risk in a particular representative population, RCTs are rarely sampled for population representativeness. Thus, in order to select the best therapies, clinicians rely on the findings of internally valid investigations, often RCTs conducted in different nations on patients who have different characteristics, comorbidities, ethnicities and lifestyles. Indeed, there are good reasons why RCTs should actively deviate from a nonrepresentative sample and focus on particular risk groups or oversample individuals with particular features, however, the intricacies do not fit in this research. Of course, the price for achieving a high internal validity in RCTs is their limited external validity or generalisability to heavily underrepresented groups. Nevertheless, meta-analyses are one efficient way of investigating or extending the generalisability of trial findings. Even when trials are individually underrepresentation of particular groups of individuals, collectively they might have gathered enough information from marginalised part of the population. With a sufficiently large sample of individuals from such trials, one can compare effects across various groups of interest. In absence of any meaningful variation in outcomes, one can then conclude that the trial findings collectively apply to those groups investigated. If there are meaningful differences, then one can formulate more stratified treatment recommendations. If information is insufficient to make an inference, then a potential area in need of more research is identified. Thus, in general, representativeness is not a big issue in the context of IPD meta-analysis, and given the fact that trials included in this analysis come from all

around the world regardless of nationality or ethnicity, we could even expect greater generalisability of results in comparison to a single trial finding.

Trial selection and inclusion in the BPLTTC: IPD meta-analyses, in particular at the scale of BPLTTC, are resource intensive. In an overview protocol paper, we described the approach taken for trial selection, data collection and harmonisation, as well as analyses and reporting.[44] Eligible large-scale trials meeting the pre-specified definitions are identified and investigators and sponsors are contacted to join the collaboration. To avoid selection bias, no eligible study has been excluded from this stage. All analyses are pre-specified (with the exception of some sensitivity or supplementary analyses that become necessary during peer review). Data collection has been an ongoing process in the BPLTTC but despite our best efforts some data have been inaccessible, some investigators uncontactable, or others unable or unwilling to share data. This of course is inevitable in any large-scale collaborative project. Some scientists may argue that the recent BPLTTC IPD meta-analyses missed to include more than half of RCTs with blood pressure-lowering drugs that would have been eligible. They then may conclude that because of the exclusion of certain types of trials or participants, the key findings from the reports are unreliable and clinically not useful. On the other hand, they may raise concerns that certain types of studies that were included should not have been. Fortunately, the issues can be addressed through established methods that check for random errors and biases in IPD meta-analyses. These were followed by us and reported in the overall BPLTTC protocol paper and all subsequent studies.[43,44,49,53-56] To

clarify these points further, I expand on the issue of statistical power and then bias.

Statistical power in comparison to aggregate data meta-analysis: One key concern about missing a large fraction of potentially eligible trials is that this could have reduced statistical power, leading to type 2 error. A superficial comparison of tabular meta-analyses of published information with the IPD meta-analyses by BPLTTC would lead to such an impression but such concerns are unfounded. As mentioned earlier, IPD meta-analyses are much more efficient in making use of information than meta-analyses of published reports. For instance, a large-scale tabular meta-analysis based on 123 trials and 613,815 participants ranked only 4 trials with 8,428 participants into its lowest baseline blood pressure category (e.g., systolic blood pressure <130 mmHg).[51] No trial had a mean blood pressure category of 120 mmHg, which caused a lot of ambiguity and hampered the comprehensive categorising of blood pressure. By contrast, the BPLTTC included 45,849 participants in groups with systolic blood pressure <130 mmHg. The total number of primary events (the key measure of statistical power) in the groups with normal or mildly elevated blood pressure was 5,702 in BPLTTC and 1,072 in the tabular meta-analyses.[51] Thus, despite the apparent smaller number of trials included, the statistical power of the IPD meta-analyses to examine stratified treatment effects is unsurpassed, thanks to the availability of individual-level data and the capacity to accurately define blood pressure categories. For the first time, we were able to make meaningful assessments of effects not only across such a wide range of baseline blood pressure but also through

simultaneous assessment of detailed blood pressure categories and cardiovascular diseases, atrial fibrillation, age at baseline, and diabetes.[43,49,53,54,56]

Risk of bias due to missing trials: But could missing information from some trials have biased study findings in a particular direction? This seems also highly unlikely for several reasons. First, the identification of trials was not biased and contacting investigators and seeking data were not related to testing a particular hypothesis. Second, the overall findings of the effects of blood pressure lowering per unit reduction in the main BPLTTC study were similar to 'larger' tabular meta-analyses where only published information was used.[51,54] Third, the potential of data acquisition bias can be investigated more formally. This was done by me using a funnel plot and Egger's regression test and as reported previously, no evidence of such bias was found.

Head-to-head trials: My IPD meta-analyses aimed to investigate stratified effects of blood pressure-lowering as well as the effects of specific drug classes on a range of outcomes including diabetes and major cardiovascular endpoints. As per the overview protocol, trials are eligible if they compare one drug against a placebo, different intensities of pharmacological blood pressure reduction, or a drug vs another drug(head-to-head trials).[44] Therefore, eligible studies would fall into one of the three categories: placebo-controlled trials, intensity-comparison trials, or head-to-head drug comparison trials. Some scientists may argue that head-to-head trials should have been excluded from the analyses because of the low

achieved and potentially unintentional blood pressure reduction in those trials. This would, however, create additional methodological challenges that in our view outweigh any benefits that it might offer. For instance, take a look at the HOPE trial few would disagree that this was a blood pressure-lowering trial but when designed, was intended to assess whether the inhibition of the angiotensin-converting enzyme (not blood pressure reduction) would prevent events related to ischaemia and atherosclerosis. They in fact reported that 'only a small part of the benefit could be attributed to a reduction in blood pressure'.[195] In this context, was the 3 mmHg blood pressure reduction observed in HOPE intentional or unintentional? Then, there is the related challenge of defining what would be a worthwhile blood pressure reduction. For instance, if a minimum level of blood pressure reduction is necessary for inclusion of trials into BPLTTC, then what would that minimum threshold be? A difference in systolic blood pressure between treatment arms of 1 mmHg, 5 mmHg or another value? Should the threshold apply to all types of trials or only those that compared a single drug vs another one? For instance, the DIABHYCAR trial was a placebo-controlled trial and achieved a 0.9 mmHg (95% CI -0.2 to 1.6) systolic blood pressure reduction over the course of the study.[196] Should this trial have been excluded? More importantly, what would be the biological or clinical justification for setting any threshold? Observational epidemiology, Mendelian randomisation studies and meta-regression of RCTs are all in agreement that no threshold exists above which the effects of blood pressure reduction are materialised.[51,197,198] Therefore, it seems that the inclusion of trials based on an arbitrary threshold of blood pressure reduction or intention in blood pressure-lowering would have been

far less defensible and more prone to selection bias than including all trials a priori. The inclusion of head-to-head trials also offers the advantage of being able to investigate the effect of drug classes where at least some of the effect could be mediated through pathways other than blood pressure-lowering (as shown in this thesis). The trade-off that such an inclusive approach has is that studies with very little blood pressure reduction could introduce random error and hence bias the overall findings towards the null. But instead of excluding them from the outset, we have adopted several strategies to mitigate this risk and quantify impacts. First, our main analyses were additionally weighted by the achieved intensity of blood pressure reduction in each trial. Assuming that treatment effects are proportional to the intensity of blood pressure reduction, one can re-scale the effects from the different studies and express them as a per unit change in blood pressure reduction. This 'standardisation' enables comparison of like-with-like when primary hypotheses are concerned with the blood pressure-mediated effects. In practical terms, this means that everything else being equal, trials with very little blood pressure reduction are given a proportionately lower weight than when no standardisation is applied. Second, to quantify whether the findings could have been affected by inclusion of such low-information studies, we report sensitivity analyses excluding them. We find that this does not affect the observed heterogeneity of effects.

Impact of specific trials on estimations: one may argue that some specific trials should be excluded from the analysis. Of course, the value of the systematic approach taken by BPLTTC is that any 'unique' features of

individual trials become less relevant and the tyranny of selecting single trials for comparison with the collective evidence is made obsolete. But let's assume that there are particular studies that have escaped the scrutiny of bias assessment by researchers, reviewers and editors. How can readers be reassured that no particular study is driving the study findings? In the main BPLTTC studies, we have reported an extensive sensitivity analysis where one trial was excluded at a time.[54] This showed no evidence that any particular trial dominated the study findings. This of course would be statistically unlikely anyway, given the substantially stronger power of the BPLTTC dataset in comparison to any single trial. However, if there were some residual concerns, those should have been dealt with unequivocally with this sensitivity analysis. For instance, some people are particularly concerned about the SPRINT trial and feel that a 'non-marginal fraction of the findings' belonged to this potentially biased study. A glance at Table S4 from a previously published paper reveals that the exclusion of this trial did not have any material impact on the study findings. The pattern remained the same and there was no evidence that effects varied by baseline categories of blood pressure.[54]

Statistical free rider: Some may raise issues about the way patients were grouped. They may argue that given the greatest uncertainty of blood pressure-mediating effects in patients with blood pressure <140 mmHg, participants with blood pressure greater than that value should have been excluded. As explained earlier, a priori exclusion of any group would be highly subjective and could increase the risk of bias. In this context, observational

studies and RCTs that have generated the hypothesis of the existence of optimal blood pressure have mostly been inconsistent about that optimum (ie, the nadir of the J-shape association).[51,197,198] From the statistical point of view, it makes more sense to investigate the variation of treatment effects across the full range of blood pressure available than a truncated section of it. Indeed, one could argue that the likelihood of detecting heterogeneous treatment effects would only increase when more patients and categories are introduced. Thus, the absence of such evidence without an arbitrary and potentially biased exclusion of certain participants is a key strength and not a weakness of the BPLTTC studies. The notion that the inclusion of patients with higher blood pressure will have 'inflated the statistical analysis of the treatment effects at lower systolic blood pressure values' is statistically wrong.[199]

Interpretation of subgroup analysis: A mistake that is very common in the interpretation of subgroup analysis is ignoring the p for interaction and making inferences about subgroup effects in the absence of evidence for effect modification or by focusing only on a significant effect in a specific subgroup. This goes against statistical recommendations where in the absence of heterogeneous treatment effect, one should accept the overall effect across all groups as the best estimate of effect in any subgroup investigated.[200]

Inclusion of trials restricted to those only with or without diabetes: Inclusion of trials restricted to those only with or without diabetes can be an

issue when using conventional regression models without accounting for the clustering of subjects within the data. As I described in the method sections, I used a one-stage meta-analysis approach using a stratified Cox regression model which assumes a different baseline hazard for each trial. The robustness of this approach was investigated in a previous BPLTTC study through sensitivity analysis.[56]

Blood pressure variability as a potential treatment modifier: The idea that larger blood pressure variability in patients with diabetes could explain the observed heterogeneity could be subject to future studies. In the meantime, traditional blood pressure measures should be used when targeting cardiovascular risk, since they are an important risk factor and the ones that can be altered most effectively by means of both lifestyle changes and medications.

8.4. Conclusions and clinical implications

To reduce the risk of a fatal heart attack or stroke, clinicians often prescribe affordable blood pressure drugs. The issue of whether or not these medications may also benefit in preventing type 2 diabetes was previously unresolved. Now I have found that the benefits of these drugs are far broader than we previously believed. This thesis used randomised evidence from large pharmacological blood pressure lowering trials and genetic biobanks to show consistent evidence that the preventive effect of blood pressure reduction on type 2 diabetes risk is causal, and that lowering blood pressure is therefore prevent new-onset type 2 diabetes. This data also lends credence

to the use of some types of antihypertensive medications for the prevention of type 2 diabetes, which might lead to an even more nuanced selection of pharmaceuticals based on an individual's risk profile. In particular, if the clinical risk of diabetes is a concern, ACEIs and ARBs should be regarded as having the most favourable results. The simplest method to prevent the risk of type 2 diabetes is now to maintain a healthy weight and follow a healthy lifestyle. Existing medications, notably ACEIs and ARBs, should now be considered for a subset of individuals who have a greater diabetes risk. Furthermore, based on my results, blood pressure reduction is an effective strategy for the prevention of major cardiovascular diseases in both people with and without type 2 diabetes. Additionally, the previous threshold for initiation of blood pressure-lowering treatment or using different drug classes in people with diabetes that is recommended by clinical guidelines is not supported by my results from this thesis. Indeed, blood pressure level is not a justifiable indicator for making a decision about the start of blood pressure-lowering treatment, not only in people with diabetes but also in people without this condition.

References

1. Jeffrey DS, Ashkan A, Emmanuela G, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 2018; **392**(10159): 1923-94.

2. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nature Reviews Nephrology* 2020; **16**(4): 223-37.

3. Mills KT, Bundy JD, Kelly TN, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. *Circulation* 2016; **134**(6): 441-50.

4. Danaei G, Finucane MM, Lin JK, et al. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5·4 million participants. *Lancet* 2011; **377**(9765): 568-77.

5. Collaboration NCDRF. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. *Lancet* 2017; **389**(10064): 37-55.

6. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol* 2020; **16**(4): 223-37.

7. Chow CK, Teo KK, Rangarajan S, et al. Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. *JAMA* 2013; **310**(9): 959-68.

8. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline

for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* 2018; **138**(17): e484-e594.

9. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. *New England Journal of Medicine* 2015; **373**(22): 2103-16.

10. Wang Z, Chen Z, Zhang L, et al. Status of Hypertension in China: Results From the China Hypertension Survey, 2012-2015. *Circulation* 2018; **137**(22): 2344-56.

11. Fleming S, Atherton H, McCartney D, et al. Self-Screening and Non-Physician Screening for Hypertension in Communities: A Systematic Review. *Am J Hypertens* 2015; **28**(11): 1316-24.

12. Mahdi A, Armitage LC, Tarassenko L, Watkinson P. Estimated Prevalence of Hypertension and Undiagnosed Hypertension in a Large Inpatient Population: A Cross-sectional Observational Study. *American Journal of Hypertension* 2021; **34**(9): 963-72.

13. Hypertension prevalence estimates in England , 2017. Public Health England https://www.gov.uk/government/publications/hypertension-prevalence-estimates-for-local-populations.

14. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet* 2015; **385**(9963): 117-71.

15. Roglic G, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. *Diabetes Res Clin Pract* 2010; **87**(1): 15-9.

16. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; **386**(9995): 743-800.

17. Forouzanfar MH, Afshin A, Alexander LT, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet* 2016; **388**(10053): 1659-724.

18. Xu Y, Wang L, He J, et al. Prevalence and control of diabetes in Chinese adults. *JAMA* 2013; **310**(9): 948-59.

19. Anjana RM, Pradeepa R, Deepa M, et al. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. *Diabetologia* 2011; **54**(12): 3022-7.

20. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology* 2018; **14**(2): 88-98.

21. Al-Siyabi H, Al-Anquodi Z, Al-Hinai H, Al-Hinai S. Nizwa Healthy Lifestyle Project Evaluation Report 2010. *Directorate General of Health Services, Ad Dakhliyah Region, Ministry of Health, Oman* 2010.

22. Al-Rubeaan K, Al-Manaa HA, Khoja TA, et al. Epidemiology of abnormal glucose metabolism in a country facing its epidemic: SAUDI-DM study. *J Diabetes* 2015; **7**(5): 622-32.

23. Haghdoost AA, Rezazadeh-Kermani M, Sadghirad B, Baradaran HR. Prevalence of type 2 diabetes in the Islamic Republic of Iran: systematic review and meta-analysis. *East Mediterr Health J* 2009; **15**(3): 591-9.

24. Type 2 diabetes in adults: management, NICE guideline [NG28], URL: https://www.nice.org.uk/guidance/ng28, Published: 02 December 2015 Last updated: 29 June 2022.

25. Ryan R, Newnham A, Khunti K, Majeed A. New cases of diabetes mellitus in England and Wales, 1994-1998: database study. *Public Health* 2005; **119**(10): 892-9.

26. Holman N, Young B, Gadsby R. Current prevalence of Type 1 and Type 2 diabetes in adults and children in the UK. *Diabet Med* 2015; **32**(9): 1119-20.

27. Hoff HE, Geddes LA, McCrady JD. The contributions of the horse to knowledge of the heart and circulation. 1. Stephen Hales and the measurement of blood pressure. *Conn Med* 1965; **29**(11): 795-800.

28. Roguin A. Scipione Riva-Rocci and the men behind the mercury sphygmomanometer. *Int J Clin Pract* 2006; **60**(1): 73-9.

29. Shevchenko YL, Tsitlik JE. 90th Anniversary of the Development by Nikolai S. Korotkoff of the Auscultatory Method of Measuring Blood Pressure. *Circulation* 1996; **94**(2): 116-8.

30. Ward M, Langton JA. Blood pressure measurement. *Continuing Education in Anaesthesia Critical Care & Pain* 2007; **7**(4): 122-6.

31. Kuwabara M, Harada K, Hishiki Y, Ohkubo T, Kario K, Imai Y. Validation of a wrist-type home nocturnal blood pressure monitor in the sitting

and supine position according to the ANSI/AAMI/ISO81060-2:2013 guidelines: Omron HEM-9601T. *J Clin Hypertens (Greenwich)* 2020; **22**(6): 970-8.

32. Han M, Lee YR, Park T, et al. Feasibility and measurement stability of smartwatch-based cuffless blood pressure monitoring: A real-world prospective observational study. *Hypertens Res* 2023; **46**(4): 922-31.

33. Kotchen TA. Historical Trends and Milestones in Hypertension Research. *Hypertension* 2011; **58**(4): 522-38.

34. Mahmood SS, Levy D, Vasan RS, Wang TJ. The Framingham Heart Study and the epidemiology of cardiovascular disease: a historical perspective. *Lancet* 2014; **383**(9921): 999-1008.

35. Oppenheimer GM. Framingham Heart Study: the first 20 years. *Prog Cardiovasc Dis* 2010; **53**(1): 55-61.

36. Multiple risk factor intervention trial. Risk factor changes and mortality results. Multiple Risk Factor Intervention Trial Research Group. *JAMA* 1982; **248**(12): 1465-77.

37. Fineberg MH. POTASSIUM THIOCYANATE IN THE TREATMENT OF PATIENTS WITH HYPERTENSION. *JAMA: The Journal of the American Medical Association* 1930; **94**(23): 1822.

38. Piepho RW, Beal J. An Overview of Antihypertensive Therapy in the 20th Century. *The Journal of Clinical Pharmacology* 2000; **40**(9): 967-77.

39. Rosenthal T. Contemplating the history of drug therapy for hypertension. *Blood Pressure* 2004; **13**(5): 262-71.

40. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. *JAMA* 1967; **202**(11): 1028-34.

41. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP Cooperative Research Group. *Jama* 1991; **265**(24): 3255-64.

42. Staessen JA, Fagard R, Thijs L, et al. Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (Syst-Eur) Trial Investigators. *Lancet* 1997; **350**(9080): 757-64.

43. Nazarzadeh M, Canoy D, Bidel Z, et al. The Blood Pressure Lowering Treatment Trialists' Collaboration: methodological clarifications of recent reports. *J Hypertens* 2022; **40**(5): 847-52.

44. Rahimi K, Canoy D, Nazarzadeh M, et al. Investigating the stratified efficacy and safety of pharmacological blood pressure-lowering: an overall protocol for individual patient-level data meta-analyses of over 300 000 randomised participants in the new phase of the Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTTC). *BMJ Open* 2019; **9**(5): e028698.

45. Burke DL, Ensor J, Riley RD. Meta-analysis using individual participant data: one-stage and two-stage approaches, and why they may differ. *Stat Med* 2017; **36**(5): 855-75.

46. Riley RD, Legha A, Jackson D, et al. One-stage individual participant data meta-analysis models for continuous and binary outcomes: Comparison of treatment coding options and estimation methods. *Statistics in Medicine* 2020; **39**(19): 2536-55.

47. Abo-Zaid G, Guo B, Deeks JJ, et al. Individual participant data meta-analyses should not ignore clustering. *J Clin Epidemiol* 2013; **66**(8): 865-73.e4.

48. Nazarzadeh M, Bidel Z, Canoy D, et al. Blood pressure lowering and risk of new-onset type 2 diabetes: an individual participant data meta-analysis. *Lancet* 2021; **398**(10313): 1803-10.

49. Nazarzadeh M, Bidel Z, Canoy D, et al. Blood pressure-lowering treatment for prevention of major cardiovascular diseases in people with and without type 2 diabetes: an individual participant-level data meta-analysis. *Lancet Diabetes Endocrinol* 2022; **10**(9): 645-54.

50. Cholesterol Treatment Trialists C, Fulcher J, O'Connell R, et al. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials. *Lancet* 2015; **385**(9976): 1397-405.

51. Ettehad D, Emdin CA, Kiran A, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Lancet* 2016; **387**(10022): 957-67.

52. Canoy D, Copland E, Nazarzadeh M, et al. Antihypertensive drug effects on long-term blood pressure: an individual-level data meta-analysis of randomised clinical trials. *Heart* 2022; **108**(16): 1281-9.

53. Blood Pressure Lowering Treatment Trialists C. Age-stratified and blood-pressure-stratified effects of blood-pressure-lowering pharmacotherapy for the prevention of cardiovascular disease and death: an individual participant-level data meta-analysis. *Lancet* 2021; **398**(10305): 1053-64.

54. Blood Pressure Lowering Treatment Trialists C. Pharmacological blood pressure lowering for primary and secondary prevention of cardiovascular disease across different levels of blood pressure: an individual participant-level data meta-analysis. *Lancet* 2021; **397**(10285): 1625-36.

55. Copland E, Canoy D, Nazarzadeh M, et al. Antihypertensive treatment and risk of cancer: an individual participant data meta-analysis. *Lancet Oncol* 2021; **22**(4): 558-70.

56. Pinho-Gomes AC, Azevedo L, Copland E, et al. Blood pressure-lowering treatment for the prevention of cardiovascular events in patients with atrial fibrillation: An individual participant data meta-analysis. *PLoS Med* 2021; **18**(6): e1003599.

57. Li T, Puhan MA, Vedula SS, Singh S, Dickersin K. Network meta-analysis-highly attractive but more methodological research is needed. *BMC Medicine* 2011; **9**(1): 79.

58. Singh J, Gsteiger S, Wheaton L, et al. Bayesian network meta-analysis methods for combining individual participant data and aggregate data from single arm trials and randomised controlled trials. *BMC Medical Research Methodology* 2022; **22**(1).

59. Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. *Nature Reviews Methods Primers* 2022; **2**(1): 6.

60. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ* 2018: k601.

61. Angrist JD, Imbens GW, Rubin DB. Identification of Causal Effects Using Instrumental Variables. *Journal of the American Statistical Association* 1996; **91**(434): 444-55.

62. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?*. *International Journal of Epidemiology* 2003; **32**(1): 1-22.

63. Nazarzadeh M, Pinho-Gomes A-C, Bidel Z, et al. Plasma lipids and risk of aortic valve stenosis: a Mendelian randomization study. *European Heart Journal* 2020; **41**(40): 3913-20.

64. Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018; **562**(7726): 203-9.

65. Zhou B, Lu Y, Hajifathalian K, et al. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4·4 million participants. *The Lancet* 2016; **387**(10027): 1513-30.

66. Hu G, Jousilahti P, Tuomilehto J. Joint effects of history of hypertension at baseline and type 2 diabetes at baseline and during follow-up on the risk of coronary heart disease. *Eur Heart J* 2007; **28**(24): 3059-66.

67. Zafari N, Asgari S, Lotfaliany M, Hadaegh A, Azizi F, Hadaegh F. Impact Of Hypertension versus Diabetes on Cardiovascular and All-cause Mortality in Iranian Older Adults: Results of 14 Years of Follow-up. *Scientific Reports* 2017; **7**(1): 14220.

68. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation* 1979; **59**(1): 8-13.

69. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood Pressure Lowering in Type 2 Diabetes: A Systematic Review and Meta-analysis. *JAMA* 2015; **313**(6): 603-15.

70. Nazarzadeh M, Rahimi K. Intensive Blood Pressure Lowering and Risk of Diabetes. *Hypertension* 2020; **75**(2): 293-4.

71. Chamarthi B, Williams GH, Ricchiuti V, et al. Inflammation and hypertension: the interplay of interleukin-6, dietary sodium, and the renin-angiotensin system in humans. *Am J Hypertens* 2011; **24**(10): 1143-8.

72. Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A. Antihypertensive drugs and reversing of endothelial dysfunction in hypertension. *Curr Hypertens Rep* 2000; **2**(1): 64-70.

73. Emdin CA, Anderson SG, Woodward M, Rahimi K. Usual Blood Pressure and Risk of New-Onset Diabetes: Evidence From 4.1 Million Adults and a Meta-Analysis of Prospective Studies. *J Am Coll Cardiol* 2015; **66**(14): 1552-62.

74. Fletcher AE. Adverse treatment effects in the trial of the European Working Party on High Blood Pressure in the Elderly. *Am J Med* 1991; **90**(3a): 42s-4s.

75. Roumie CL, Hung AM, Russell GB, et al. Blood Pressure Control and the Association With Diabetes Mellitus Incidence: Results From SPRINT Randomized Trial. *Hypertension* 2020; **75**(2): 331-8.

76. Savage PJ, Pressel SL, Curb JD, et al. Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension:

The Systolic Hypertension in the Elderly Program. SHEP Cooperative Research Group. *Arch Intern Med* 1998; **158**(7): 741-51.

77. Sun D, Zhou T, Heianza Y, et al. Type 2 Diabetes and Hypertension. *Circ Res* 2019; **124**(6): 930-7.

78. Aikens RC, Zhao W, Saleheen D, et al. Systolic Blood Pressure and Risk of Type 2 Diabetes: A Mendelian Randomization Study. *Diabetes* 2017; **66**(2): 543-50.

79. Zhu Z, Zheng Z, Zhang F, et al. Causal associations between risk factors and common diseases inferred from GWAS summary data. *Nat Commun* 2018; **9**(1): 224.

80. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. *N Engl J Med* 2000; **342**(13): 905-12.

81. Fletcher A, Amery A, Birkenhäger W, et al. Risks and benefits in the trial of the European Working Party on High Blood Pressure in the Elderly. *J Hypertens* 1991; **9**(3): 225-30.

82. Cooper-Dehoff R, Cohen JD, Bakris GL, et al. Predictors of development of diabetes mellitus in patients with coronary artery disease taking antihypertensive medications (findings from the INternational VErapamil SR-Trandolapril STudy [INVEST]). *Am J Cardiol* 2006; **98**(7): 890-4.

83. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. *Lancet* 2007; **369**(9557): 201-7.

84. Tudur Smith C, Williamson PR. A comparison of methods for fixed effects meta-analysis of individual patient data with time to event outcomes. *Clin Trials* 2007; **4**(6): 621-30.

85. Canoy D, Copland E, Nazarzadeh M, et al. Antihypertensive drug effects on long-term blood pressure: an individual-level data meta-analysis of randomised clinical trials. *Heart* 2022; **108**(16): 1281-9.

86. Perry IJ, Wannamethee SG, Walker MK, Thomson AG, Whincup PH, Shaper AG. Prospective study of risk factors for development of non-insulin dependent diabetes in middle aged British men. *BMJ* 1995; **310**(6979): 560-4.

87. Kumari M, Head J, Marmot M. Prospective study of social and other risk factors for incidence of type 2 diabetes in the Whitehall II study. *Arch Intern Med* 2004; **164**(17): 1873-80.

88. Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: The Task Force for diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD). *European Heart Journal* 2019; **41**(2): 255-323.

89. Jones NR, McCormack T, Constanti M, McManus RJ. Diagnosis and management of hypertension in adults: NICE guideline update 2019. *British Journal of General Practice* 2020; **70**(691): 90-1.

90. Barone Gibbs B, Hivert M-F, Jerome GJ, et al. Physical Activity as a Critical Component of First-Line Treatment for Elevated Blood Pressure or Cholesterol: Who, What, and How?: A Scientific Statement From the American Heart Association. *Hypertension* 2021; **78**(2).

91. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ* 2013; **346**(apr03 3): f1325-f.

92. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005; **366**(9493): 1267-78.

93. Sattar N, Preiss D, Murray HM, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet* 2010; **375**(9716): 735-42.

94. Wang S, Cai R, Yuan Y, Varghese Z, Moorhead J, Ruan XZ. Association between reductions in low-density lipoprotein cholesterol with statin therapy and the risk of new-onset diabetes: a meta-analysis. *Sci Rep* 2017; **7**: 39982.

95. Ference BA, Ray KK, Catapano AL, et al. Mendelian Randomization Study of ACLY and Cardiovascular Disease. *N Engl J Med* 2019; **380**(11): 1033-42.

96. Swerdlow DI, Preiss D, Kuchenbaecker KB, et al. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet* 2015; **385**(9965): 351-61.

97. Collins R, Reith C, Emberson J, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 2016; **388**(10059): 2532-61.

98. Gillespie EL, White CM, Kardas M, Lindberg M, Coleman CI. The impact of ACE inhibitors or angiotensin II type 1 receptor blockers on the

development of new-onset type 2 diabetes. *Diabetes Care* 2005; **28**(9): 2261-6.

99. Jandeleit-Dahm KA, Tikellis C, Reid CM, Johnston CI, Cooper ME. Why blockade of the renin-angiotensin system reduces the incidence of new-onset diabetes. *J Hypertens* 2005; **23**(3): 463-73.

100. Scheen AJ. Renin-angiotensin system inhibition prevents type 2 diabetes mellitus. Part 1. A meta-analysis of randomised clinical trials. *Diabetes Metab* 2004; **30**(6): 487-96.

101. Zhang X, Zhao Q. Association of Thiazide-Type Diuretics With Glycemic Changes in Hypertensive Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. *J Clin Hypertens (Greenwich)* 2016; **18**(4): 342-51.

102. Gurwitz JH, Bohn RL, Glynn RJ, Monane M, Mogun H, Avorn J. Antihypertensive drug therapy and the initiation of treatment for diabetes mellitus. *Ann Intern Med* 1993; **118**(4): 273-8.

103. Gurwitz JH, Field TS, Glynn RJ, et al. Risk factors for non-insulin-dependent diabetes mellitus requiring treatment in the elderly. *J Am Geriatr Soc* 1994; **42**(12): 1235-40.

104. Rajala U, Qiao Q, Laakso M, Keinänen-Kiukaanniemi S. Antihypertensive drugs as predictors of type 2 diabetes among subjects with impaired glucose tolerance. *Diabetes Res Clin Pract* 2000; **50**(3): 231-9.

105. Samuelsson O, Hedner T, Berglund G, Persson B, Andersson OK, Wilhelmsen L. Diabetes mellitus in treated hypertension: incidence, predictive factors and the impact of non-selective beta-blockers and thiazide diuretics

during 15 years treatment of middle-aged hypertensive men in the Primary Prevention Trial Göteborg, Sweden. *J Hum Hypertens* 1994; **8**(4): 257-63.

106. Officers A, Coordinators for the ACRGTA, Lipid-Lowering Treatment to Prevent Heart Attack T. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 2002; **288**(23): 2981-97.

107. Dahlöf B, Sever PS, Poulter NR, et al. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. *Lancet* 2005; **366**(9489): 895-906.

108. Ades AE, Sculpher M, Sutton A, et al. Bayesian methods for evidence synthesis in cost-effectiveness analysis. *Pharmacoeconomics* 2006; **24**(1): 1-19.

109. van Valkenhoef G, Lu G, de Brock B, Hillege H, Ades AE, Welton NJ. Automating network meta-analysis. *Res Synth Methods* 2012; **3**(4): 285-99.

110. Abuissa H, Jones PG, Marso SP, O'Keefe JH, Jr. Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: a meta-analysis of randomized clinical trials. *J Am Coll Cardiol* 2005; **46**(5): 821-6.

111. Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J Hum Hypertens* 2007; **21**(1): 20-7.

112. Noto H, Goto A, Tsujimoto T, Noda M. Effect of calcium channel blockers on incidence of diabetes: a meta-analysis. *Diabetes Metab Syndr Obes* 2013; **6**: 257-61.

113. Xu G, Chen J, Jing G, Shalev A. Preventing β-cell loss and diabetes with calcium channel blockers. *Diabetes* 2012; **61**(4): 848-56.

114. Sarafidis PA, Bakris GL. Antihypertensive treatment with beta-blockers and the spectrum of glycaemic control. *QJM* 2006; **99**(7): 431-6.

115. Dahlöf B, Devereux RB, Kjeldsen SE, et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 2002; **359**(9311): 995-1003.

116. de Boer IH, Bangalore S, Benetos A, et al. Diabetes and Hypertension: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**(9): 1273-84.

117. Helderman JH, Elahi D, Andersen DK, et al. Prevention of the glucose intolerance of thiazide diuretics by maintenance of body potassium. *Diabetes* 1983; **32**(2): 106-11.

118. Zillich AJ, Garg J, Basu S, Bakris GL, Carter BL. Thiazide Diuretics, Potassium, and the Development of Diabetes. *Hypertension* 2006; **48**(2): 219-24.

119. Carter BL, Einhorn PT, Brands M, et al. Thiazide-Induced Dysglycemia. *Hypertension* 2008; **52**(1): 30-6.

120. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Human Molecular Genetics* 2014; **23**(R1): R89-R98.

121. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *International Journal of Epidemiology* 2017; **45**(6): 1866-86.

122. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet* 2018; **50**(10): 1412-25.

123. Ehret GB, Munroe PB, Rice KM, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 2011; **478**(7367): 103-9.

124. Hartwig FP, Davies NM, Hemani G, Davey Smith G. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. *Int J Epidemiol* 2016; **45**(6): 1717-26.

125. Xue A, Wu Y, Zhu Z, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun* 2018; **9**(1): 2941.

126. Fortier I, Raina P, Van den Heuvel ER, et al. Maelstrom Research guidelines for rigorous retrospective data harmonization. *Int J Epidemiol* 2017; **46**(1): 103-5.

127. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol* 2017; **46**(6): 1985-98.

128. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Human Molecular Genetics* 2018; **27**(R2): R195-R208.

129. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016; **40**(4): 304-14.

130. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nature Genetics* 2018; **50**(5): 693-8.

131. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015; **44**(2): 512-25.

132. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol* 2017; **32**(5): 377-89.

133. Cook RD. Detection of Influential Observation in Linear-Regression. *Technometrics* 1977; **19**(1): 15-8.

134. Zhao Q, Wang J, Hemani G, Bowden J, Small DS. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. *The Annals of Statistics* 2020; **48**(3): 1742-69, 28.

135. Qi G, Chatterjee N. Mendelian randomization analysis using mixture models for robust and efficient estimation of causal effects. *Nature Communications* 2019; **10**(1).

136. Hemani G, Tilling K, Davey Smith G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLOS Genetics* 2017; **13**(11): e1007081.

137. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity Analyses for Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic Variants. *Epidemiology* 2017; **28**(1): 30-42.

138. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 2018; **7**: e34408 , citation = eLife 2018;7 e.

139. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. *Int J Epidemiol* 2017; **46**(6): 1734-9.

140. Neale B. Rapid GWAS of thousands of phenotypes in the UK Biobank 2020. 2020. http://www.nealelab.is/blog/2017/7/19/rapid-gwas-of-thousands-of-phenotypes-for-337000-samples-in-the-uk-biobank (accessed April 19, 2020).

141. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol* 2015; **181**(4): 251-60.

142. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol* 2019; **48**(3): 713-27.

143. Malik R, Traylor M, Pulit SL, et al. Low-frequency and common genetic variation in ischemic stroke: The METASTROKE collaboration. *Neurology* 2016; **86**(13): 1217-26.

144. Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015; **47**(10): 1121-30.

145. Morris AP, Voight BF, Teslovich TM, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nature Genetics* 2012; **44**(9): 981-90.

146. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLOS Medicine* 2015; **12**(3): e1001779.

147. Nazarzadeh M, Pinho-Gomes AC, Smith Byrne K, et al. Systolic Blood Pressure and Risk of Valvular Heart Disease: A Mendelian Randomization Study. *JAMA Cardiol* 2019; **4**(8): 788-95.

148. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. *International Journal of Epidemiology* 2011; **40**(3): 755-64.

149. Carslake D, Fraser A, May MT, et al. Associations of mortality with own blood pressure usingson's blood pressure as an instrumental variable. *Scientific Reports* 2019; **9**(1): 8986.

150. Wade KH, Carslake D, Ivar Nilsen T, Timpson NJ, Davey Smith G, Romundstad P. Blood pressure and mortality: using offspring blood pressure as an instrument for own blood pressure in the HUNT study. *Scientific Reports* 2015; **5**(1): 12399.

151. Zuber V, Grinberg NF, Gill D, et al. Combining evidence from Mendelian randomization and colocalization: Review and comparison of approaches. *Am J Hum Genet* 2022; **109**(5): 767-82.

152. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet* 2014; **10**(5): e1004383.

153. Wallace C. A more accurate method for colocalisation analysis allowing for multiple causal variants. *PLoS Genet* 2021; **17**(9): e1009440.

154. Walker VM, Davey Smith G, Davies NM, Martin RM. Mendelian randomization: a novel approach for the prediction of adverse drug events and drug repurposing opportunities. *Int J Epidemiol* 2017; **46**(6): 2078-89.

155. Lewis CJ, Gong H, Brown MJ, Harding SE. Overexpression of beta 1-adrenoceptors in adult rat ventricular myocytes enhances CGP 12177A cardiostimulation: implications for 'putative' beta 4-adrenoceptor pharmacology. *Br J Pharmacol* 2004; **141**(5): 813-24.

156. Nazarzadeh M, Pinho-Gomes A-C, Bidel Z, et al. Genetic susceptibility, elevated blood pressure, and risk of atrial fibrillation: a Mendelian randomization study. *Genome Medicine* 2021; **13**(1): 38.

157. Gill D, Georgakis MK, Koskeridis F, et al. Use of Genetic Variants Related to Antihypertensive Drugs to Inform on Efficacy and Side Effects. *Circulation* 2019; **140**(4): 270-9.

158. Walker VM, Kehoe PG, Martin RM, Davies NM. Repurposing antihypertensive drugs for the prevention of Alzheimer's disease: a Mendelian randomization study. *Int J Epidemiol* 2020; **49**(4): 1132-40.

159. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). *European Heart Journal* 2018; **39**(33): 3021-104.

160. Battle A, Brown CD, Engelhardt BE, Montgomery SB. Genetic effects on gene expression across human tissues. *Nature* 2017; **550**(7675): 204-13.

161. Jamerson K, Weber MA, Bakris GL, et al. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med* 2008; **359**(23): 2417-28.

162. Law MR, Morris JK, Wald NJ. 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* 2009; **338**: b1665.

163. Lin X, Xu Y, Pan X, et al. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Sci Rep* 2020; **10**(1): 14790.

164. Shah AD, Langenberg C, Rapsomaniki E, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. *Lancet Diabetes Endocrinol* 2015; **3**(2): 105-13.

165. Group SR, Wright JT, Jr., Williamson JD, et al. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. *N Engl J Med* 2015; **373**(22): 2103-16.

166. Group AS, Cushman WC, Evans GW, et al. Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med* 2010; **362**(17): 1575-85.

167. Hypertension in adults: diagnosis and management|NICE guideline [NG136]. UK: National Institute for Health and Clinical Excellence. 2019.

168. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of

Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018; **71**(6): 1269-324.

169. Unger T, Borghi C, Charchar F, et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension* 2020; **75**(6): 1334-57.

170. Turnbull F, Neal B, Algert C, et al. Effects of different blood pressure-lowering regimens on major cardiovascular events in individuals with and without diabetes mellitus: results of prospectively designed overviews of randomized trials. *Arch Intern Med* 2005; **165**(12): 1410-9.

171. Hommel G. A Stagewise Rejective Multiple Test Procedure Based on a Modified Bonferroni Test. *Biometrika* 1988; **75**(2): 383-6.

172. Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in medicine--reporting of subgroup analyses in clinical trials. *N Engl J Med* 2007; **357**(21): 2189-94.

173. Suzuki H, Kanno Y. Effects of candesartan on cardiovascular outcomes in Japanese hypertensive patients. *Hypertens Res* 2005; **28**(4): 307-14.

174. Group UKPDS. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ* 1998; **317**(7160): 703-13.

175. Rahman F, McEvoy JW, Ohkuma T, et al. Effects of Blood Pressure Lowering on Clinical Outcomes According to Baseline Blood Pressure and Cardiovascular Risk in Patients With Type 2 Diabetes Mellitus. *Hypertension* 2019; **73**(6): 1291-9.

176. Brunström M, Carlberg B. Effect of antihypertensive treatment at different blood pressure levels in patients with diabetes mellitus: systematic review and meta-analyses. *BMJ* 2016: i717.

177. Riley RD, Steyerberg EW. Meta-analysis of a binary outcome using individual participant data and aggregate data. *Res Synth Methods* 2010; **1**(1): 2-19.

178. Pylypchuk R, Wells S, Kerr A, et al. Cardiovascular risk prediction in type 2 diabetes before and after widespread screening: a derivation and validation study. *Lancet* 2021; **397**(10291): 2264-74.

179. Karmali KN, Lloyd-Jones DM, Van Der Leeuw J, et al. Blood pressure-lowering treatment strategies based on cardiovascular risk versus blood pressure: A meta-analysis of individual participant data. *PLOS Medicine* 2018; **15**(3): e1002538.

180. Sarwar N, Gao P, Seshasai SR, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; **375**(9733): 2215-22.

181. Bress AP, King JB, Kreider KE, et al. Effect of Intensive Versus Standard Blood Pressure Treatment According to Baseline Prediabetes Status: A Post Hoc Analysis of a Randomized Trial. *Diabetes Care* 2017; **40**(10): 1401-8.

182. Munafò MR, Davey Smith G. Robust research needs many lines of evidence. *Nature* 2018; **553**(7689): 399-401.

183. Heale R, Forbes D. Understanding triangulation in research. *Evidence Based Nursing* 2013; **16**(4): 98-.

184. Cooper--DeHoff RM, Bird ST, Nichols GA, Delaney JA, Winterstein AG. Antihypertensive drug class interactions and risk for incident diabetes: a nested case-control study. *J Am Heart Assoc* 2013; **2**(3): e000125.

185. Rao S, Mamouei M, Salimi-Khorshidi G, et al. Targeted-BEHRT: Deep Learning for Observational Causal Inference on Longitudinal Electronic Health Records. *IEEE Trans Neural Netw Learn Syst* 2022; **Pp**.

186. Rees JMB, Foley CN, Burgess S. Factorial Mendelian randomization: using genetic variants to assess interactions. *International Journal of Epidemiology* 2019; **49**(4): 1147-58.

187. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther* 2008; **88**(11): 1322-35.

188. Thomas MC, Cooper ME, Zimmet P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. *Nat Rev Nephrol* 2016; **12**(2): 73-81.

189. Wyld MLR, Morton RL, Aouad L, Magliano D, Polkinghorne KR, Chadban S. The impact of comorbid chronic kidney disease and diabetes on health-related quality-of-life: a 12-year community cohort study. *Nephrol Dial Transplant* 2021; **36**(6): 1048-56.

190. Hawkes S, Allotey P, Elhadj AS, Clark J, Horton R. The Lancet Commission on Gender and Global Health. *The Lancet* 2020; **396**(10250): 521-2.

191. Vogel B, Acevedo M, Appelman Y, et al. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. *Lancet* 2021; **397**(10292): 2385-438.

192. Chen L, Peters JE, Prins B, et al. Systematic Mendelian randomization using the human plasma proteome to discover potential therapeutic targets for stroke. *Nature Communications* 2022; **13**(1): 6143.

193. Schmidt AF, Finan C, Gordillo-Marañón M, et al. Genetic drug target validation using Mendelian randomisation. *Nature Communications* 2020; **11**(1): 3255.

194. Di Angelantonio E, Thompson SG, Kaptoge S, et al. Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. *Lancet* 2017; **390**(10110): 2360-71.

195. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000; **342**(3): 145-53.

196. Marre M, Lievre M, Chatellier G, et al. Effects of low dose ramipril on cardiovascular and renal outcomes in patients with type 2 diabetes and raised excretion of urinary albumin: randomised, double blind, placebo controlled trial (the DIABHYCAR study). *BMJ* 2004; **328**(7438): 495.

197. Czernichow S, Zanchetti A, Turnbull F, et al. The effects of blood pressure reduction and of different blood pressure-lowering regimens on major cardiovascular events according to baseline blood pressure: meta-analysis of randomized trials. *J Hypertens* 2011; **29**(1): 4-16.

198. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; **360**(9349): 1903-13.

199. Kreutz R, Brunström M, Thomopoulos C, Carlberg B, Mancia G. Do recent meta-analyses truly prove that treatment with blood pressure-lowering drugs is beneficial at any blood pressure value, no matter how low? A critical review. *J Hypertens* 2022; **40**(5): 839-46.

200. Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in Medicine — Reporting of Subgroup Analyses in Clinical Trials. *New England Journal of Medicine* 2007; **357**(21): 2189-94.

Appendix

Table 1 Appendix. Genetic variants selected as an instrumental variable for systolic blood pressure.

(the information in this Table is extracted and modified from the main genome-wide association study by Evangelou et al.)[122]

SNP *	Chromosome	Position (GRCh37)	Allele1	Allele2	Freq1†	Effect †	Standard error †	P-value†	Source *
rs3737801	1	27960832	c	g	0.9142	0.4246	0.0954	8.67E-06	Novel:one-stage design
rs11210029	1	41865293	a	g	0.625	-0.1608	0.0476	0.000728	Novel:one-stage design
rs11579440	1	49052423	t	c	0.8468	0.2794	0.0653	1.86E-05	Novel:one-stage design
rs10923038	1	88651771	a	c	0.6166	0.1279	0.0481	0.00781	Novel:one-stage design
rs76719272	1	156129796	t	c	0.1309	-0.2747	0.0727	0.00016	Novel:two-stage design
rs1043069	1	180859368	t	g	0.6225	0.2696	0.0478	1.72E-08	Novel:two-stage design
rs4651224	1	184585182	t	c	0.4518	0.144	0.047	0.002185	Novel:two-stage design
rs12042924	1	197297417	t	c	0.5202	-0.1372	0.0465	0.003202	Novel:two-stage design
rs33996239	1	203109801	t	c	0.0577	-0.427	0.1058	5.43E-05	Novel:two-stage design
rs7555285	1	209970355	c	g	0.7951	0.173	0.0565	0.002212	Novel:two-stage design
rs260508	1	2187085	t	g	0.6167	0.1696	0.0477	0.000377	Novel:two-stage design
rs2807337	1	22577371	t	c	0.3721	0.1938	0.0478	5.02E-05	Novel:two-stage design
rs4926499	1	249155909	c	g	0.8262	0.2922	0.0752	0.000102	Novel:two-stage design
rs79598313	1	27284913	t	c	0.0275	0.5126	0.1518	0.00073	Novel:two-stage design
rs839755	1	43856410	a	c	0.6224	-0.1877	0.047	6.55E-05	Novel:two-stage design
rs7514579	1	94051350	a	c	0.7765	0.3027	0.0559	6.16E-08	Novel:two-stage design
rs17396055	1	94730954	a	g	0.3317	-0.1525	0.049	0.001874	Novel:two-stage design
rs880315	1	10796866	t	c	0.652	-0.5218	0.0499	1.33E-25	Previously reported
rs4846049	1	11850365	t	g	0.3264	-0.4146	0.0489	2.44E-17	Previously reported
rs17367504	1	11862778	a	g	0.8444	0.7774	0.0639	4.81E-34	Previously reported
rs5068	1	11905974	a	g	0.9387	1.0914	0.0989	2.53E-28	Previously reported
rs3820068	1	15798197	a	g	0.7977	0.3361	0.0596	1.69E-08	Previously reported
rs7515635	1	42408070	t	c	0.4684	0.2382	0.0463	2.70E-07	Previously reported

rs10922502	1	89360158	a	g	0.6407	-0.2283	0.0483	2.32E-06	Previously reported
rs55732192	2	162278233	t	g	0.0962	-0.2807	0.0798	0.000433	Novel:one-stage design
rs6712203	2	165557318	t	c	0.3779	-0.1943	0.0477	4.57E-05	Novel:one-stage design
rs11694601	2	174949358	a	g	0.5927	-0.1422	0.047	0.00249	Novel:one-stage design
rs1837164	2	178716601	a	t	0.3753	0.186	0.0472	8.27E-05	Novel:one-stage design
rs296797	2	201102905	t	c	0.4142	0.2067	0.0467	9.68E-06	Novel:one-stage design
rs1047891	2	211540507	a	c	0.3243	-0.1647	0.0511	0.00127	Novel:one-stage design
rs10189186	2	53025757	a	g	0.5357	0.1752	0.0459	0.000135	Novel:one-stage design
rs28377357	2	112769721	a	g	0.3	-0.1588	0.0502	0.001572	Novel:two-stage design
rs6723509	2	122000745	t	c	0.8617	0.2553	0.0677	0.000162	Novel:two-stage design
rs72844590	2	138421227	t	g	0.1441	0.0856	0.0692	0.2158	Novel:two-stage design
rs79523138	2	161368213	a	g	0.8849	-0.3083	0.0749	3.83E-05	Novel:two-stage design
rs6739913	2	185033065	a	g	0.7095	-0.1523	0.0506	0.002638	Novel:two-stage design
rs28558491	2	187816321	t	c	0.7362	-0.1935	0.0531	0.000265	Novel:two-stage design
rs67720684	2	18975439	a	c	0.2295	0.0834	0.0546	0.1269	Novel:two-stage design
rs12694277	2	213188795	t	c	0.2914	-0.219	0.051	1.77E-05	Novel:two-stage design
rs1044822	2	230629138	t	c	0.142	-0.2655	0.0657	5.38E-05	Novel:two-stage design
rs139354822	2	242344695	t	c	0.9675	0.4794	0.1554	0.002042	Novel:two-stage design
rs35590893	2	43716933	a	g	0.2716	-0.1215	0.0515	0.01822	Novel:two-stage design
rs6545155	2	50429861	t	c	0.7852	0.2182	0.0559	9.56E-05	Novel:two-stage design
rs2920899	2	55279681	t	g	0.7851	0.1653	0.0573	0.003886	Novel:two-stage design
rs72816333	2	60096560	a	t	0.8277	0.254	0.0606	2.79E-05	Novel:two-stage design
rs2300481	2	66782467	t	c	0.3886	0.2043	0.0472	1.50E-05	Novel:two-stage design
rs1446468	2	164963486	t	c	0.4512	-0.487	0.0468	2.26E-25	Previously reported
rs6712094	2	165043460	a	g	0.7296	0.42	0.0525	1.17E-15	Previously reported
rs6749447	2	169041386	t	g	0.7323	-0.067	0.0523	0.2008	Previously reported
rs6434404	2	191494411	a	g	0.3247	0.2228	0.0498	7.53E-06	Previously reported
rs1344653	2	19730845	a	g	0.4961	-0.1568	0.0456	0.00058	Previously reported
rs55780018	2	208526140	t	c	0.548	-0.3278	0.0488	1.85E-11	Previously reported
rs2972146	2	227100698	t	g	0.6362	0.2486	0.0476	1.76E-07	Previously reported

rs55701159	2	25139596	t	g	0.887	0.2999	0.0742	5.27E-05	Previously reported
rs1275988	2	26914364	t	c	0.6055	-0.5157	0.0466	1.83E-28	Previously reported
rs9678851	2	27887034	a	c	0.559	-0.1135	0.0474	0.01662	Previously reported
rs7562	2	28635740	t	c	0.5297	0.1555	0.047	0.00093	Previously reported
rs13420463	2	37517566	a	g	0.7775	0.2751	0.0555	7.19E-07	Previously reported
rs262986	3	183435713	a	g	0.4712	-0.2288	0.0468	1.01E-06	Novel:one-stage design
rs1882289	3	114461208	a	g	0.8814	-0.2919	0.0708	3.78E-05	Novel:two-stage design
rs9875380	3	132780356	t	c	0.4619	-0.2472	0.0457	6.21E-08	Novel:two-stage design
rs863930	3	135949737	t	g	0.4671	-0.191	0.046	3.28E-05	Novel:two-stage design
rs78151625	3	158316726	t	c	0.831	-0.222	0.0618	0.000329	Novel:two-stage design
rs189267552	3	20073193	a	t	0.0141	-0.7415	0.2166	0.000618	Novel:two-stage design
rs12638085	3	30405936	a	t	0.3525	0.2514	0.0486	2.30E-07	Novel:two-stage design
rs6788984	3	41107173	a	g	0.858	0.3015	0.066	4.92E-06	Novel:two-stage design
rs6774721	3	49381898	a	g	0.1465	-0.2171	0.0689	0.001627	Novel:two-stage design
rs9857362	3	74710462	a	c	0.5249	0.1736	0.0473	0.000241	Novel:two-stage design
rs347591	3	11290122	t	g	0.6625	0.2842	0.0489	6.31E-09	Previously reported
rs11128722	3	14958126	a	g	0.5628	-0.2518	0.047	8.53E-08	Previously reported
rs143112823	3	154707967	a	g	0.076	-0.4019	0.0949	2.29E-05	Previously reported
rs3097937	4	124794644	a	t	0.8075	0.2388	0.0587	4.77E-05	Novel:one-stage design
rs6823767	4	151295085	t	c	0.7227	-0.1566	0.0528	0.003027	Novel:one-stage design
rs7439567	4	138464842	t	c	0.4157	0.245	0.0474	2.39E-07	Novel:two-stage design
rs17035181	4	157678511	t	g	0.8549	0.2169	0.0653	0.000898	Novel:two-stage design
rs2610990	4	18008232	a	g	0.2693	-0.2325	0.0523	8.86E-06	Novel:two-stage design
rs231708	4	2694773	c	g	0.6983	-0.2643	0.0499	1.19E-07	Novel:two-stage design
rs12511987	4	46595623	t	g	0.8224	-0.2456	0.0614	6.26E-05	Novel:two-stage design
rs1347345	4	95938386	a	g	0.6206	-0.1645	0.0478	0.000583	Novel:two-stage design
rs13112725	4	106911742	c	g	0.7682	0.397	0.0557	1.01E-12	Previously reported
rs2291435	4	38387395	t	c	0.5248	-0.2419	0.0463	1.74E-07	Previously reported
rs2014912	4	86715670	t	c	0.1515	0.5122	0.0644	1.80E-15	Previously reported
rs1650911	5	141740620	c	g	0.7619	0.2465	0.0584	2.42E-05	Novel:one-stage design

rs12153395	5	179411477	a	g	0.1133	-0.2602	0.0764	0.000661	Novel:one-stage design
rs4957026	5	361148	a	g	0.3503	0.2214	0.0497	8.29E-06	Novel:one-stage design
rs6875372	5	64079015	a	t	0.5154	0.2228	0.0459	1.18E-06	Novel:one-stage design
rs1871190	5	97953719	t	g	0.3472	0.1658	0.0495	0.000805	Novel:one-stage design
rs62373688	5	127352807	a	t	0.1259	0.3593	0.0714	4.76E-07	Novel:two-stage design
rs10069690	5	1279790	t	c	0.2583	0.3827	0.0627	1.03E-09	Novel:two-stage design
rs702395	5	140086677	t	c	0.4369	0.2367	0.0468	4.31E-07	Novel:two-stage design
rs74774746	5	33411769	c	g	0.2639	-0.1177	0.0541	0.02953	Novel:two-stage design
rs13179413	5	55868097	t	c	0.2775	0.1383	0.0544	0.01098	Novel:two-stage design
rs3121685	5	65662133	t	c	0.4815	-0.2015	0.046	1.17E-05	Novel:two-stage design
rs246973	5	68007803	t	c	0.2833	0.1984	0.0509	9.60E-05	Novel:two-stage design
rs709668	5	96174186	a	g	0.1965	-0.2755	0.0576	1.72E-06	Novel:two-stage design
rs10077885	5	114390121	a	c	0.498	-0.2465	0.0484	3.54E-07	Previously reported
rs1008058	5	122435627	a	g	0.1183	0.3142	0.0766	4.12E-05	Previously reported
rs13359291	5	122476457	a	g	0.1654	0.4005	0.062	1.06E-10	Previously reported
rs6595838	5	127868199	a	g	0.2891	0.2361	0.0507	3.14E-06	Previously reported
rs11953630	5	157845402	t	c	0.3694	-0.4463	0.0501	5.15E-19	Previously reported
rs1421811	5	32714270	c	g	0.6116	0.4743	0.0477	2.46E-23	Previously reported
rs1173771	5	32815028	a	g	0.3976	-0.5227	0.0468	6.04E-29	Previously reported
rs10059921	5	87514515	t	g	0.0846	-0.3732	0.0919	4.89E-05	Previously reported
rs7765526	6	147713764	a	g	0.4682	0.2317	0.047	8.11E-07	Novel:one-stage design
rs9449350	6	82281417	t	c	0.673	-0.2333	0.0488	1.72E-06	Novel:one-stage design
rs9401090	6	119113317	t	c	0.7538	0.2512	0.054	3.32E-06	Novel:two-stage design
rs10782230	6	126228512	a	g	0.4907	0.2787	0.0459	1.27E-09	Novel:two-stage design
rs9885632	6	131311909	t	c	0.7338	0.245	0.052	2.42E-06	Novel:two-stage design
rs7763294	6	140383733	t	g	0.3169	-0.2059	0.0493	2.95E-05	Novel:two-stage design
rs2745599	6	1613686	a	g	0.5476	0.2128	0.0513	3.30E-05	Novel:two-stage design
rs9368222	6	20686996	a	c	0.2767	0.1639	0.0511	0.001338	Novel:two-stage design
rs6911827	6	22130601	t	c	0.4623	0.152	0.0473	0.001295	Previously reported
rs2270860	6	43270151	t	c	0.3092	0.2966	0.05	3.09E-09	Previously reported

rs10948071	6	43280713	t	c	0.5993	-0.2074	0.0465	8.13E-06	Previously reported
rs1563788	6	43308363	t	c	0.2937	0.3062	0.0501	9.79E-10	Previously reported
rs78648104	6	50683009	t	c	0.8985	-0.3571	0.083	1.69E-05	Previously reported
rs35410524	6	96885405	t	c	0.1917	0.2999	0.0588	3.38E-07	Previously reported
rs1870735	7	155744303	c	g	0.4548	0.2137	0.0486	1.08E-05	Novel:one-stage design
rs12979	7	24738164	c	g	0.8745	0.2241	0.0693	0.001227	Novel:one-stage design
rs34072724	7	130432469	a	g	0.4828	-0.1967	0.0465	2.37E-05	Novel:two-stage design
rs12703989	7	140238048	a	g	0.494	0.1026	0.0474	0.03035	Novel:two-stage design
rs11771693	7	150050111	a	g	0.6743	0.169	0.0502	0.000757	Novel:two-stage design
rs10274928	7	28142088	a	g	0.4932	0.1644	0.0475	0.000538	Novel:two-stage design
rs10233127	7	30933453	a	t	0.1087	0.2638	0.0805	0.001051	Novel:two-stage design
rs6593297	7	56122058	a	t	0.3178	0.0982	0.0523	0.06052	Novel:two-stage design
rs6963105	7	75097488	a	g	0.4432	-0.2035	0.0531	0.000127	Novel:two-stage design
rs848445	7	77572461	t	c	0.2821	-0.2067	0.0528	9.04E-05	Novel:two-stage design
rs17477177	7	106411858	t	c	0.7906	-0.5642	0.0564	1.60E-23	Previously reported
rs4728142	7	128573967	a	g	0.4383	-0.2155	0.0467	3.91E-06	Previously reported
rs13238550	7	131059056	a	g	0.3909	0.1695	0.0472	0.000329	Previously reported
rs10224002	7	151415041	a	g	0.7186	-0.2375	0.0525	5.99E-06	Previously reported
rs6969780	7	27159136	c	g	0.0961	0.3697	0.0793	3.12E-06	Previously reported
rs142449193	8	102750597	t	c	0.0491	-0.4354	0.112	0.000102	Novel:one-stage design
rs4875958	8	1721090	a	g	0.7099	0.2209	0.0515	1.83E-05	Novel:one-stage design
rs2979470	8	30288272	t	c	0.4873	0.2114	0.046	4.25E-06	Novel:one-stage design
rs2354862	8	64501744	a	c	0.6441	0.2139	0.0485	1.03E-05	Novel:one-stage design
rs13253358	8	68920135	t	c	0.297	0.1945	0.0504	0.000113	Novel:one-stage design
rs61040371	8	8503700	t	c	0.6221	0.191	0.0475	5.68E-05	Novel:one-stage design
rs62526122	8	92769569	a	g	0.2707	0.1739	0.0557	0.001806	Novel:one-stage design
rs1986971	8	10268736	a	g	0.7048	0.2632	0.051	2.49E-07	Novel:two-stage design
rs4598218	8	129483956	t	c	0.614	0.1523	0.048	0.001523	Novel:two-stage design
rs4129585	8	143312933	a	c	0.4438	0.1977	0.0467	2.30E-05	Novel:two-stage design
rs1906672	8	38130025	a	g	0.2275	0.2644	0.055	1.51E-06	Novel:two-stage design

rs6996733	8	60535824	t	c	0.8439	0.1904	0.0647	0.003269	Novel:two-stage design
rs72688070	8	81393697	t	c	0.1714	-0.1536	0.0621	0.01338	Novel:two-stage design
rs62491354	8	9730663	a	g	0.1401	0.3376	0.0663	3.59E-07	Novel:two-stage design
rs35783704	8	105966258	a	g	0.1092	-0.5219	0.0773	1.50E-11	Previously reported
rs2898290	8	11433909	t	c	0.4835	0.3419	0.0466	2.12E-13	Previously reported
rs4841569	8	11452177	a	g	0.4123	-0.3758	0.0511	1.94E-13	Previously reported
rs6557876	8	25900675	t	c	0.2511	-0.3667	0.0533	5.98E-12	Previously reported
rs520015	9	211762	c	g	0.5144	0.2043	0.0456	7.60E-06	Novel:one-stage design
rs9886665	9	22942770	t	c	0.2721	0.1887	0.0519	0.000277	Novel:one-stage design
rs60191654	9	753648	a	g	0.8143	-0.2311	0.0584	7.50E-05	Novel:one-stage design
rs7023828	9	128498594	t	c	0.423	-0.2466	0.0464	1.10E-07	Novel:two-stage design
rs1891730	9	130309028	t	c	0.6198	-0.1749	0.0479	0.000257	Novel:two-stage design
rs184457	9	131940019	a	g	0.2995	-0.1157	0.0498	0.02015	Novel:two-stage design
rs28558845	9	4334791	c	g	0.1568	-0.2472	0.0652	0.00015	Novel:two-stage design
rs1332813	9	9350706	t	c	0.3515	0.1771	0.0472	0.000175	Novel:two-stage design
rs7045409	9	95201540	a	t	0.3681	-0.1498	0.0473	0.001553	Novel:two-stage design
rs111245230	9	113169775	t	c	0.9662	-0.6917	0.1299	9.99E-08	Previously reported
rs11592107	10	122968964	a	g	0.3087	0.2721	0.0495	3.93E-08	Novel:one-stage design
rs72834453	10	124235226	t	g	0.8742	-0.2378	0.0712	0.000839	Novel:one-stage design
rs3802517	10	28233469	a	t	0.4668	0.188	0.0456	3.80E-05	Novel:one-stage design
rs11187142	10	94468685	t	c	0.1047	0.298	0.0763	9.34E-05	Novel:one-stage design
rs11197813	10	118523933	a	g	0.7025	-0.1612	0.0505	0.0014	Novel:two-stage design
rs7912283	10	133773019	a	g	0.642	-0.2008	0.0505	7.02E-05	Novel:two-stage design
rs1133400	10	134459388	a	g	0.7954	-0.307	0.0601	3.24E-07	Novel:two-stage design
rs34130368	10	48411796	t	g	0.1197	-0.1772	0.0816	0.02998	Novel:two-stage design
rs56352451	10	5804865	t	c	0.1337	0.3049	0.0672	5.69E-06	Novel:two-stage design
rs12572586	10	74751579	t	c	0.9383	-0.4496	0.1012	8.86E-06	Novel:two-stage design
rs112184198	10	102604514	a	g	0.1058	-0.5331	0.0761	2.40E-12	Previously reported
rs1004467	10	104594507	a	g	0.9028	0.8884	0.0785	1.08E-29	Previously reported
rs11191548	10	104846178	t	c	0.9129	1.0233	0.0818	6.19E-36	Previously reported

rs4746172	10	75855842	t	c	0.7348	-0.1017	0.0528	0.0542	Previously reported
rs932764	10	95895940	a	g	0.5561	-0.3654	0.0467	4.84E-15	Previously reported
rs10766533	11	19224677	a	t	0.7004	0.2572	0.0515	5.85E-07	Novel:one-stage design
rs11031051	11	30355707	a	c	0.683	-0.1902	0.0493	0.000116	Novel:two-stage design
rs190194639	11	34068037	t	c	0.0823	0.3274	0.0862	0.000146	Novel:two-stage design
rs1585453	11	46884713	a	t	0.8866	-0.2449	0.0775	0.00157	Novel:two-stage design
rs4385883	11	51539339	a	t	0.7047	0.2189	0.0566	0.000112	Novel:two-stage design
rs4980515	11	63744609	t	c	0.504	0.227	0.0464	1.01E-06	Novel:two-stage design
rs67976715	11	68023742	c	g	0.2282	0.2708	0.0555	1.04E-06	Novel:two-stage design
rs10743086	11	8774923	a	g	0.2086	-0.2193	0.0567	0.000111	Novel:two-stage design
rs7129220	11	10350538	a	g	0.1233	0.3919	0.0724	6.28E-08	Previously reported
rs1401454	11	16250183	t	c	0.3998	0.3365	0.0469	7.10E-13	Previously reported
rs757081	11	17351683	c	g	0.6644	-0.2958	0.0487	1.29E-09	Previously reported
rs5219	11	17409572	t	c	0.3755	0.32	0.0471	1.12E-11	Previously reported
rs661348	11	1905292	t	c	0.5632	-0.3417	0.0502	9.56E-12	Previously reported
rs217727	11	2016908	a	g	0.192	0.3626	0.061	2.85E-09	Previously reported
rs11537751	11	47587452	t	c	0.0521	0.3936	0.1076	0.000256	Previously reported
rs11229457	11	58207203	t	c	0.2144	-0.2886	0.0563	2.97E-07	Previously reported
rs3741378	11	65408937	t	c	0.1328	-0.4169	0.0696	2.15E-09	Previously reported
rs7927515	11	76125330	a	c	0.3455	0.1705	0.0488	0.000479	Previously reported
rs117206641	12	133086888	t	c	0.1145	0.3348	0.0783	1.88E-05	Novel:one-stage design
rs28621435	12	13860990	a	g	0.1187	-0.3138	0.0729	1.69E-05	Novel:one-stage design
rs4143175	12	67782397	t	c	0.239	0.3055	0.0533	9.90E-09	Novel:one-stage design
rs5742643	12	102837863	t	c	0.2505	-0.2603	0.0534	1.07E-06	Novel:two-stage design
rs11112548	12	105871914	a	t	0.9558	0.5768	0.1203	1.64E-06	Novel:two-stage design
rs11571376	12	1059556	c	g	0.7011	-0.1164	0.0506	0.0215	Novel:two-stage design
rs2024385	12	12888438	a	t	0.4186	-0.243	0.0467	1.99E-07	Novel:two-stage design
rs7976167	12	24210599	t	c	0.6893	0.1409	0.0489	0.003922	Novel:two-stage design
rs10437954	12	58003922	a	g	0.9064	-0.4326	0.0832	2.01E-07	Novel:two-stage design
rs7963801	12	79685226	t	c	0.4129	-0.2145	0.0482	8.45E-06	Novel:two-stage design

rs10858966	12	90567026	c	g	0.3035	0.2024	0.05	5.12E-05	Novel:two-stage design
rs2384550	12	115352731	a	g	0.3457	-0.2748	0.0473	6.29E-09	Previously reported
rs1126930	12	49399132	c	g	0.0343	0.5757	0.14	3.93E-05	Previously reported
rs73099903	12	53440779	t	c	0.0794	0.4218	0.0878	1.56E-06	Previously reported
rs7297416	12	54443090	a	c	0.6867	0.2816	0.05	1.84E-08	Previously reported
rs2681492	12	90013089	t	c	0.8344	0.7729	0.0615	3.26E-36	Previously reported
rs17249754	12	90060586	a	g	0.1637	-0.8015	0.0619	2.16E-38	Previously reported
rs2480171	13	21559858	t	c	0.1324	0.2057	0.0693	0.002978	Novel:one-stage design
rs1331012	13	27115424	t	g	0.269	0.1514	0.051	0.002962	Novel:one-stage design
rs4274337	13	41967193	a	g	0.177	-0.33	0.0612	6.93E-08	Novel:one-stage design
rs75961402	13	56398286	a	g	0.1516	0.2759	0.0635	1.40E-05	Novel:one-stage design
rs606950	13	22298923	a	g	0.6176	0.1755	0.047	0.000186	Novel:two-stage design
rs9532243	13	32191408	a	c	0.4797	0.2485	0.0452	3.89E-08	Novel:two-stage design
rs73187288	13	42738672	a	c	0.8935	-0.2492	0.0738	0.000731	Novel:two-stage design
rs912434	13	47189928	t	g	0.7628	0.2107	0.0531	7.30E-05	Novel:two-stage design
rs9526707	13	51489186	a	g	0.3166	-0.2364	0.0492	1.56E-06	Novel:two-stage design
rs78474310	13	73826901	a	g	0.9566	-0.4412	0.1138	0.000106	Novel:two-stage design
rs7988232	13	79808655	a	g	0.4146	0.1378	0.0463	0.002917	Novel:two-stage design
rs3011549	13	113634937	a	c	0.2888	0.226	0.0539	2.78E-05	Previously reported
rs63418562	13	30146201	t	c	0.7462	-0.3846	0.0529	3.74E-13	Previously reported
rs34983854	14	39858442	a	g	0.6064	-0.2259	0.0463	1.05E-06	Novel:one-stage design
rs8014182	14	103859962	t	c	0.1388	-0.3218	0.0655	8.80E-07	Novel:two-stage design
rs17115145	14	30122409	t	c	0.3909	0.1853	0.0462	6.08E-05	Novel:two-stage design
rs72683923	14	50735947	t	c	0.9767	0.7823	0.1705	4.45E-06	Novel:two-stage design
rs11623535	14	72462381	a	g	0.7393	0.1623	0.0513	0.001552	Novel:two-stage design
rs11159091	14	75074316	a	g	0.4654	0.1973	0.046	1.78E-05	Novel:two-stage design
rs9888615	14	53377540	t	c	0.2936	-0.2356	0.0499	2.32E-06	Previously reported
rs8016306	14	63928546	a	g	0.7931	0.1339	0.0554	0.01569	Previously reported
rs4965529	15	100145224	a	c	0.1657	-0.2802	0.0622	6.60E-06	Novel:two-stage design
rs11634028	15	76276150	a	t	0.205	0.2356	0.059	6.49E-05	Novel:two-stage design

rs3743157	15	85680532	a	c	0.1651	0.2069	0.0615	0.000766	Novel:two-stage design
rs11632436	15	86295286	c	g	0.5045	0.1907	0.0458	3.07E-05	Novel:two-stage design
rs35199222	15	81013037	a	g	0.4398	0.2436	0.0466	1.75E-07	Previously reported
rs2759308	15	81016227	a	g	0.4758	0.2592	0.046	1.79E-08	Previously reported
rs2379829	16	3538873	c	g	0.728	-0.2143	0.0521	3.84E-05	Novel:one-stage design
rs34941092	16	50550137	a	g	0.1491	-0.302	0.0651	3.53E-06	Novel:one-stage design
rs1012089	16	74171973	c	g	0.4758	-0.1354	0.0456	0.002974	Novel:one-stage design
rs3851018	16	86437811	c	g	0.5676	0.2224	0.0473	2.60E-06	Novel:one-stage design
rs6540125	16	87993889	t	g	0.3501	0.1864	0.0475	8.75E-05	Novel:one-stage design
rs35450617	16	6889675	t	g	0.6958	-0.1542	0.051	0.002489	Novel:two-stage design
rs7187540	16	85318302	a	c	0.3245	-0.193	0.0563	0.000606	Novel:two-stage design
rs9899540	17	30777924	a	t	0.4126	0.1809	0.0487	0.0002	Novel:one-stage design
rs112260610	17	64252393	t	c	0.1353	0.3389	0.0669	4.11E-07	Novel:one-stage design
rs4925159	17	18185510	a	g	0.4192	0.2134	0.0464	4.23E-06	Novel:two-stage design
rs1551355	17	30032420	t	c	0.2369	0.1842	0.0538	0.000621	Novel:two-stage design
rs34430710	17	56876627	a	t	0.6753	-0.2151	0.0487	9.87E-06	Novel:two-stage design
rs1036902	17	58950791	t	c	0.8404	-0.2107	0.0634	0.000888	Novel:two-stage design
rs112280096	17	79367409	a	c	0.37	-0.0932	0.0561	0.09643	Novel:two-stage design
rs12946454	17	43208121	a	t	0.739	-0.3193	0.0518	7.30E-10	Previously reported
rs7406910	17	46688256	t	c	0.0893	-0.4877	0.0812	1.93E-09	Previously reported
rs8068318	17	59483766	t	c	0.7271	0.4318	0.0536	8.20E-16	Previously reported
rs2240736	17	59485393	t	c	0.7328	0.4265	0.0525	4.49E-16	Previously reported
rs1154214	18	24546824	t	g	0.3963	-0.2163	0.046	2.57E-06	Novel:one-stage design
rs6567160	18	57829135	t	c	0.7644	0.1618	0.0541	0.002765	Novel:one-stage design
rs10460108	18	73034151	a	g	0.4819	0.2039	0.0452	6.40E-06	Novel:one-stage design
rs11876341	18	48799991	a	g	0.6949	-0.2167	0.0518	2.89E-05	Novel:two-stage design
rs10048404	18	54578482	t	c	0.3741	-0.2123	0.049	1.46E-05	Novel:two-stage design
rs12454712	18	60845884	t	c	0.6224	0.1891	0.0537	0.000429	Novel:two-stage design
rs34413141	18	777282	a	t	0.1796	-0.337	0.0599	1.83E-08	Novel:two-stage design
rs12958173	18	42141977	a	c	0.3	0.3518	0.0495	1.21E-12	Previously reported

rs7256564	19	33889593	a	g	0.3133	0.2039	0.0487	2.87E-05	Novel:one-stage design
rs73046792	19	49605705	a	g	0.1513	-0.2413	0.069	0.000474	Novel:one-stage design
rs2613765	19	5066330	a	g	0.4768	-0.1874	0.0455	3.85E-05	Novel:two-stage design
rs138877676	19	50935809	t	g	0.0211	-0.5482	0.2033	0.006999	Novel:two-stage design
rs17638167	19	11584818	t	c	0.047	-0.5228	0.1095	1.81E-06	Previously reported
rs8105753	19	31927547	a	c	0.6255	0.1895	0.0487	9.88E-05	Previously reported
rs4247374	19	7252756	t	c	0.1355	-0.5063	0.0753	1.76E-11	Previously reported
rs1764975	20	4101290	a	t	0.7894	0.2759	0.058	1.99E-06	Novel:one-stage design
rs6021247	20	50108980	a	g	0.5289	0.1623	0.0453	0.000338	Novel:two-stage design
rs6031435	20	42797358	a	g	0.5388	-0.2268	0.0456	6.72E-07	Previously reported
rs11701033	21	33788341	c	g	0.8169	-0.2465	0.0592	3.18E-05	Previously reported
rs9608690	22	28921347	a	g	0.0678	-0.308	0.0912	0.000733	Novel:one-stage design
rs28578714	22	50727921	t	c	0.6045	0.2346	0.0538	1.28E-05	Novel:two-stage design

* Candidate SNPs selected from Evangelou et al.[122]
† Regression coefficient and corresponding standard error derived from International Consortium for Blood Pressure Genome-Wide Association Studies (ICBP).[123]

Appendix 2. The Blood Pressure Lowering Treatment Trialists' Collaboration

Steering Committee: Kazem Rahimi (Chair), Koon Teo, Barry R Davis, John Chalmers, Carl J Pepine

Collaborating Trialists: A Adler (UKPDS [UK Prospective Diabetes Study]), L Agodoa (AASK [African-American Study of Kidney Disease and Hypertension]), A Algra (Dutch TIA Study [Dutch Transient Ischemic Attack Study]), F W Asselbergs (PREVEND-IT [Prevention of Renal and Vascular End-stage Disease Intervention Trial]), N Beckett (HYVET [Hypertension in the Very Elderly Trial]), E Berge (deceased) (VALUE trial [Valsartan Antihypertensive Long-term Use Evaluation trial]), H Black (CONVINCE [Controlled Onset Verapamil Investigation of Cardiovascular End Points]), E Boersma (EUROPA [European trial on reduction Of cardiac events with Perindopril among patients with stable coronary Artery disease]), F P J Brouwers (PREVEND-IT), M Brown (INSIGHT [International Nifedipine GITS Study: Intervention as a Goal in Hypertension]), J Brugts (EUROPA),C J Bulpitt (EWPHE [European Working Party on High Blood Pressure in the Elderly], HYVET), R P Byington (PREVENT [Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial]), J Chalmers (ADVANCE [Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation], PROGRESS [Perindopril protection against recurrent stroke]), W C Cushman (ACCORD [Action to Control Cardiovascular Risk in Diabetes], ALLHAT [Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial], SPRINT [Systolic Blood Pressure Intervention Trial]), J Cutler (ALLHAT), B R Davis (ALLHAT), R B Devereaux (LIFE [Losartan Intervention For Endpoint reduction in hypertension]), J P Dwyer (IDNT [Irbesartan Diabetic Nephropathy Trial]), R Estacio (ABCD [Appropriate Blood

Pressure Control in Diabetes]), R Fagard (Syst-Eur [SYSTolic Hypertension in EURope]), K Fox (EUROPA), T Fukui (CASE-J [Candesartan Antihypertensive Survival Evaluation in Japan]), A K Gupta (ASCOT-BPLA [AngloScandinavian Cardiac Outcomes Trial—Blood Pressure Lowering Arm]), R R Holman (UKPDS), Y Imai (HOMED-BP [Hypertension Objective Treatment Based on Measurement by Electrical Devices of Blood Pressure]), M Ishii (JMIC-B [Japan Multicenter Investigation for Cardiovascular Diseases-B]), S Julius (VALUE), Y Kanno (E-COST [Efficacy of Candesartan on Outcome in Saitama Trial]), S E Kjeldsen (VALUE, LIFE), J Kostis (SHEP [Systolic Hypertension in the Elderly Program]), K Kuramoto (NICS-EH [National Intervention Cooperative Study in Elderly Hypertensives]), J Lanke (STOP Hypertension-2 [Swedish Trial in Old Patients with Hypertension-2], NORDIL [Nordic Diltiazem]), E Lewis (IDNT), J B Lewis (IDNT), M Lievre (DIABHYCAR [Non-insulin-dependent diabetes, hypertension, microalbuminuria or proteinuria, cardiovascular events, and ramipril study]), L H Lindholm (CAPPP [Captopril Prevention Project], STOP Hypertension-2, NORDIL), S Lueders (MOSES [The Morbidity and Mortality After Stroke, Eprosartan Compared With Nitrendipine for Secondary Prevention]), S MacMahon (ADVANCE, PART-2 [Prevention of Atherosclerosis with Ramipril Trial]), G Mancia (INSIGHT), M Matsuzaki (COPE [The Combination Therapy of Hypertension to Prevent Cardiovascular Events]), M H Mehlum (VALUE), S Nissen (CAMELOT [Comparison of Amlodipine vs Enalapril to Limit Occurrences of Thrombosis]), H Ogawa (HIJ-CREATE [Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Heart Disease]), T Ogihara (CASE-J, COLM [Combinations of OLMesartan], COPE), T Ohkubo (HOMED-BP), C R Palmer (INSIGHT), A Patel (ADVANCE), C J Pepine (INVEST [International Verapamil SR-Trandolapril Study]), M A Pfeffer (PEACE [Prevention of

Events With Angiotensin- Converting Enzyme Inhibition]), B Pitt (PREVENT), N R Poulter (ASCOT), H Rakugi (CASE-J, VALISH [Valsartan in Elderly Isolated Systolic Hypertension Study]), G Reboldi (Cardio-Sis [CARDIOvascolari del Controllo della Pressione Arteriosa SIStolica]), C Reid (ANBP2 [The Second Australian National Blood Pressure Study]), G Remuzzi (BENEDICT [BErgamo NEphrologic DIabetes Complications Trial]), P Ruggenenti (BENEDICT), T Saruta (CASE-J), J Schrader (MOSES), R Schrier (deceased) (ABCD), P Sever (ASCOT-BPLA), P Sleight (deceased; CONVINCE, HOPE [Heart Outcomes Prevention Evaluation], ONTARGET [Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial], TRANSCEND [Telmisartan Randomised AssessmeNt Study in ACE iNtolerant subjects with cardiovascular Disease]), J A Staessen (Syst-Eur), H Suzuki (E-COST), L Thijs (Syst-Eur), K Ueshima (CASE-J, VALISH), S Umemoto (COPE), W H van Gilst (PREVEND-IT), P Verdecchia (Cardio-Sis), K Wachtell (LIFE), P Whelton (SPRINT), L Wing (ANBP2), M Woodward (ADVANCE, PROGRESS), Y Yui (JMIC-B), S Yusuf (HOPE, ONTARGET, TRANSCEND), A Zanchetti (deceased; ELSA [European Lacidipine Study on Atherosclerosis], VHAS [Verapamil in Hypertension and Atherosclerosis Study]), and Z Y Zhang (Syst-Eur).

Other members: C Anderson, C Baigent, B M Brenner, R Collins, D de Zeeuw, J Lubsen, E Malacco, B Neal, V Perkovic, A Rodgers, P Rothwell, G Salimi-Khorshidi, J Sundström, F Turnbull, G Viberti, and J Wang.