Doctoral theses at NTNU, 2023:394

Nikhil Arora

Sleep traits, their interplay and the risks of acute myocardial infarction and atrial fibrillation

Prospective cohort and Mendelian randomization studies using UK Biobank and the HUNT Study

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

NTNU
Norwegian University of
Science and Technology

Nikhil Arora

Sleep traits, their interplay and the risks of acute myocardial infarction and atrial fibrillation

Prospective cohort and Mendelian randomization studies using UK Biobank and the HUNT Study

Thesis for the Degree of Philosophiae Doctor

Trondheim, December 2023

Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the Degree of Philosophiae Doctor

Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

ISBN 978-82-326-7488-6 (printed ver.)
ISBN 978-82-326-7487-9 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (online ver.)

Doctoral theses at NTNU, 2023:394

Printed by NTNU Grafisk senter

Norwegian summary

Søvnvariabler alene og i samspill, og risiko for akutt hjerteinfarkt og atrieflimmer: Prospektiv kohort- og Mendelsk randomiserings-studium med bruk av UK Biobank og HUNT-studien

Søvnproblemer er et stort folkehelseproblem, og det er estimert at 30% av den generelle befolkningen opplever ett eller flere langvarige symptomer på søvnløshet (f.eks. innsovningsvansker, gjentatte oppvåkninger, eller tidlig morgenoppvåkning). God søvn innebærer mer enn bare fravær av søvnforstyrrelser, og man kan undersøke søvnhelsen gjennom ulike søvnkarakteristikker som blant annet søvnvarighet, søvneffektivitet, døgnrytmepreferanse (kronotype), symptomer på søvnløshet, og søvnkvalitet. De siste årene har det blitt et økt fokus på hvilken betydning søvn har i forhold til sentrale kroppslige funksjoner som autonom dysregulering og hormonforstyrrelser, funksjoner som kan være tilknyttet kardiometabolske risikofaktorer og hjerte- og karsykdommer.

Hjerte- og karsykdommer, inkludert akutt hjerteinfarkt (AMI), er en viktig bidragsyter til helsetapsjusterte leveår og tidlig død globalt. Atrieflimmer (AF) – bidrar til morbiditet og dødelighet, og det er forventet en fordobling i prevalensen innen 2050. Det er verdt å merke seg at en stor del av hjerte- og karsykdommene ikke kan forklares av etablerte risikofaktorer som ugunstige lipidprofiler, høyt blodtrykk, diabetes, og røyking. Flere studier har vist en sammenheng mellom ulike søvnkarakteristikker (f.eks. symptomer på søvnløshet, søvnvarighet og kronotype) og en økt risiko for hjerte- og karsykdommer. Til tross for at disse søvnkarakteristikkene er sterkt relatert til hverandre, finnes det få studier som har undersøkt om det er en kombinert effekt av flere søvnkarakteristikker på risiko for AMI og AF.

Vi har benyttet tradisjonelle prospektive observasjonelle studiedesign og Mendelsk randomisering (MR) med data fra UK Biobank og Helseundersøkelsen i Trøndelag (HUNT2) for å undersøke hvorvidt det er et samspill mellom ulike søvnkarakteristikker på risikoen for AMI og AF. Vi estimerte additiv interaksjon mellom søvnkarakteristikkene ved bruk av RERI (relative excess risk due to interaction).

De tradisjonelle observasjonelle analysene viste at personer som hadde kombinasjoner av enkelte søvnkarakteristikker (dvs. symptomer på søvnløshet med kort søvnvarighet, symptomer på søvnløshet med lang søvnvarighet, symptomer på søvnløshet med kveldskronotype, kort søvnvarighet med kveldskronotype, og lang søvnvarighet med kveldskronotype) hadde høyere risiko for AMI enn dem som bare hadde en av de gitte søvnkarakteristikkene. Vi fant en statistisk signifikant interaksjon mellom symptomer på søvnløshet og lang søvnvarighet. Vi fant ingen tydelig interaksjon når vi undersøkte

risikoen for AF, men vi fant at personer med en kombinasjon av symptomer på søvnløshet og langvarig søvnvarighet, og personer med symptomer på søvnløshet som også hadde en kveldskronotype hadde høyere risiko enn dem som bare hadde en av disse søvnkarakteristikkene.

Analysene basert på faktoriell MR viste ingen interaksjon mellom genetisk predisposisjon for to søvnkarakteristikker på risikoen for AMI eller AF. Personer med genetisk predisposisjon for kombinasjonene symptomer på søvnløshet med kortvarig søvn, symptomer på søvnløshet med morgonkronotype og kortvarig søvn med morgonkronotype, hadde riktignok en større risiko for AMI og AF enn dem som kun hadde genetisk predisposisjon for en søvnkarakteristikk.

Ettersom det ikke var fullstendig samsvar mellom de tradisjonelle analysene og MR-analysene, er det vanskelig å trekke sikre overordnete konklusjoner. Til tross for dette gir resultatene våre et viktig bidrag til det samlede evidensgrunnlaget for uheldige helseeffekter av enkelte søvnkarakteristikker, samt samspillet mellom dem. Personer som sover et tilstrekkelig antall timer (7-8 timer) uten symptomer på søvnløshet ser ut til å ha en redusert risiko for både AMI og AF. Dette er kunnskap som kan være relevant for forebyggende arbeid. Videre forskning er nødvendig for å undersøke mekanismene som ligger til grunn for disse sammenhengene.

Kandidat: Nikhil Arora
Institutt: Institutt for samfunnsmedisin og sykepleie, NTNU
Hovedveileder: Linn Beate Strand
Biveiledere: Rebecca Claire Richmond, Bjørn Olav Åsvold, Eivind Schjelderup Skarpsno, og Laxmi Bhatta
Finansieringskilde: Nasjonalforeningen for folkehelsen

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden ph.d. i medisin og helsevitenskap.

Disputas finner sted i ØHA11 på Øya helsehus, fredag 8. desember 2023, kl.12.15.

Nasjonalforeningen for folkehelsen

Summary

Poor sleep is a major public health concern, with an estimated 30% of the general population reportedly experiencing one or more insomnia symptoms at any given point in time. Good sleep entails more than the mere absence of sleep disorders, and sleep health can be assessed through measurable constructs of sleep, including sleep duration, sleep efficiency, sleep timing (e.g., chronotype), alertness/sleepiness, and sleep satisfaction/ quality. In recent years, there has been a growing emphasis on the significance of sleep health in relation to critical bodily functions such as autonomic dysregulation and hormonal disturbances, which are potentially linked to cardiometabolic risk factors and cardiovascular diseases (CVDs).

CVDs, including acute myocardial infarction (AMI), represent a significant contribution to the burden of disability-adjusted life years and premature mortality on a global scale. Atrial fibrillation (AF) — being the most common arrhythmia — exerts a substantial impact on morbidity and mortality, and its prevalence is projected to double by the year 2050. Notably, a considerable proportion of CVDs cannot be explained by established risk factors, such as unfavorable lipid profiles, high blood pressure, diabetes, and cigarette smoking. Numerous studies have established links between various individual sleep traits (i.e., insomnia symptoms, sleep duration, and chronotype) and an elevated risk of CVD development. However, sleep traits are often interconnected, and only a limited number of studies have assessed their joint effects on AMI/AF risk.

We have leveraged data obtained from UK Biobank and the second survey of the Trøndelag Health Study (HUNT2) to investigate the joint influence of sleep traits on the risks of incident AMI and AF using prospective observational and Mendelian randomization (MR) study designs. Furthermore, we assessed relative excess risk due to interaction (RERI) for the joint association of sleep traits on an additive scale.

Through our observational analyses, we detected the joint associations of certain sleep traits on the risks of AMI and AF. Specifically, individuals who exhibited combinations of certain sleep traits (i.e., insomnia symptoms with short sleep duration, insomnia symptoms with long sleep duration, insomnia symptoms with evening chronotype, short sleep duration with evening chronotype, and long sleep duration with evening chronotype) had higher risk of incident AMI than those who exhibited only one sleep trait. Notably, interaction was observed for insomnia symptoms with long sleep duration. In the context of AF, we observed that individuals with the combinations of insomnia symptoms with long sleep duration, as well as insomnia symptoms with evening chronotype had higher risk than those who exhibited only one sleep trait; however, no substantial interaction was found.

In our factorial MR analyses, we detected the impacts of genetic predisposition to two sleep traits together on the risks of both AMI and AF. Although individuals with genetic predisposition to insomnia symptoms with short sleep duration, insomnia symptoms with morning chronotype, and short sleep duration with morning chronotype had higher risks of incident AMI and AF than those genetically predisposed to only one sleep trait, no interaction was found.

Since we could not triangulate the findings across the observational and MR study designs in this thesis, it is difficult to synthesize overall conclusions. Despite this, our results add to the body of evidence regarding the adverse health effects of certain sleep traits and their combinations. For instance, healthy sleep of adequate duration (7–8 h) that is free from insomnia symptoms can reduce the risks of incident AMI and AF, which could be relevant for preventive initiatives. Further research on this topic is warranted, particularly to address the underlying mechanisms of these observed associations.

Candidate: Nikhil Arora
Department: Department of Public Health and Nursing, NTNU
Main supervisor: Linn Beate Strand
Co-supervisors: Rebecca Claire Richmond, Bjørn Olav Åsvold, Eivind Schjelderup Skarpsno, and Laxmi Bhatta
Funding source: Norwegian Health Association

List of papers

This thesis is based on the following papers:

I. Arora N, Richmond RC, Brumpton BM, Åsvold BO, Dalen H, Skarpsno ES, Strand LB. **Self-reported insomnia symptoms, sleep duration, chronotype and the risk of acute myocardial infarction (AMI): a prospective study in the UK Biobank and the HUNT Study.** *Eur J Epidemiol*. 2023 Jun;38(6):643-656. doi: 10.1007/s10654-023-00981-x.

II. Arora N, Bhatta L, Skarpsno ES, Dalen H, Åsvold BO, Brumpton BM, Richmond RC, Strand LB. **Investigating the causal interplay between sleep traits and risk of acute myocardial infarction: a Mendelian randomization study.** *BMC Medicine.* 2023;21:385. doi: 10.1186/s12916-023-03078-0.

III. Arora N, Brumpton BM, Åsvold BO, Loennechen JP, Malmo V, Bhatta L, Skarpsno ES, Richmond RC, Strand LB. **A Mendelian randomization study investigating the role of sleep traits and their joint effects on the incidence of atrial fibrillation.** Manuscript.

Acknowledgements

This doctoral work was carried out between September 2020 and August 2023 at the Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim. During my research period, I had a research stay at the Population Health Science Institute, Bristol Medical School, University of Bristol, United Kingdom, from February to May 2023. This work was funded by the Norwegian Health Association (Norwegian: Nasjonalforeningen for folkehelsen) and my research stay was funded by the Liaison Committee for Education, Research and Innovation in Central Norway (Norwegian: Helse Midt-Norge).

I would like to express my sincere gratitude to *Associate Professor Linn Beate Strand*, my main supervisor, for her invaluable guidance and unwavering support throughout my doctoral journey. Her expertise, mentorship and encouragement have been instrumental in shaping my research. Additionally, her insightful feedback and useful critiques have significantly enriched the quality of my work. I am deeply indebted to Linn for her time, patience, commitment and support.

I also wish to express my gratitude to my co-supervisor, *Dr Rebecca Claire Richmond* at the University of Bristol. She made me feel welcome during my research stay in Bristol. Despite her other roles at the University, she dedicated her undivided attention to my project, and I am truly grateful for that. Under her supervision, I learned the skills necessary for conducting the Mendelian randomization analyses used in this thesis.

I am extremely grateful to *Associate Professor Ben Michael Brumpton* for his constant support and contribution to this thesis. His expert knowledge of genetic analyses helped me to make well-informed decisions. Likewise, I would like to thank my co-supervisor, *Professor Bjørn Olav Åsvold*, for his professional suggestions and advice. I also wish to thank my co-supervisor, *Dr Eivind Schjelderup Skarpsno*, for our important discussions on the epidemiological challenges that I faced during my research and for always encouraging me to think outside of the box. Moreover, I am thankful to *Dr Laxmi Bhatta* for taking over the role of co-supervision while Rebecca was on leave and for our important discussions on Mendelian randomization methods.

I would like to thank my co-authors, *Professor Håvard Dalen*, *Professor Jan Pål Loennechen*, and *Dr Vegard Malmo*, for extracting cardiovascular disease data from the patient records as well as contributing with valuable feedback.

This work is based on data from UK Biobank and the second survey of the Trøndelag Health Study (HUNT2). I am very appreciative of each individual who volunteered to take part in these population studies. I wish to thank the *HUNT Research Centre* for providing the data, and the HUNT Cloud team for providing me with the necessary setup to conduct my analyses.

I would also like to acknowledge *Professor Kristian Hveem* and my colleagues at the *HUNT Center for Molecular and Clinical Epidemiology* (formerly the *K.G. Jebsen Center for Genetic Epidemiology*) for fostering an exceptional learning environment. Beyond that, I am truly grateful for the delightful and insightful life discussions we shared over lunch throughout the past few years. Their camaraderie and support have contributed to my personal and professional growth, making my time at the center truly enriching and memorable.

I would also like to give a special mention to *Nayan Deepak Parlikar* and *Marta Riise Moksnes* for being not only incredible colleagues but also wonderful friends. Thank you for the unfiltered advice during the most crucial times and for reminding me about the important things in life.

Last, but not least, I would like to thank my parents and my sister for always supporting me and for inspiring me to be inquisitive and chase my aspirations.

To anyone else I may have inadvertently missed, I am grateful for your support in any capacity during the completion of this thesis.

Thank you!

Nikhil Arora

Abbreviations

AF	Atrial fibrillation
AMI	Acute myocardial infarction
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
GRS	Genetic risk score
GWAS	Genome-wide association study
HADS	Hospital Anxiety and Depression Scale
HDL	High-density lipoprotein
HF	Heart failure
HR	Hazard ratio
HRC	Haplotype Reference Consortium
HUNT	Trøndelag Health Study
HUNT2	Trøndelag Health Study 1995–97, Survey 2
ICD	International Statistical Classification of Diseases
ISI	Insomnia Severity Index
LDL	Low-density lipoprotein
MCTQ	Munich Chronotype Questionnaire
MEQ	Morningness-Eveningness Questionnaire
MR	Mendelian randomization
NHS	National Health Service
NREM	Non-rapid eye movement
OSA	Obstructive sleep apnea
PA	Physical activity
PSQI	Pittsburgh Sleep Quality Index
RCT	Randomized controlled trial
REM	Rapid eye movement
RERI	Relative excess risk due to interaction

RR	Relative risk
SCN	Suprachiasmatic nucleus
SE	Standard error
SHHS	Sleep Heart Health Study
SNP	Single nucleotide polymorphism
TDI	Townsend Deprivation Index
TSPS	Two-stage predictor substitution
uwGRS	Unweighted genetic risk score
wGRS	Weighted genetic risk score
WHI	Women's Health Initiative
WHIIRS	Women's Health Initiative Insomnia Rating Scale

Table of contents

Norwegian summary ..... **i**

Summary ..... **iii**

List of papers ..... **v**

Acknowledgements ..... **vii**

Abbreviations ..... **ix**

1 Introduction **1**

1.1 Cardiovascular diseases 1

1.1.1 Acute myocardial infarction 2

1.1.2 Atrial fibrillation 3

1.1.3 Risk factors for acute myocardial infarction and atrial fibrillation 4

1.1.3.1 Non-modifiable risk factors 4

1.1.3.2 Modifiable risk factors 5

1.2 Sleep 6

1.2.1 Sleep cycle and stages 7

1.2.2 Sleep traits and their interplay 7

1.2.3 Sleep traits of interest 8

1.3 Sleep traits and cardiovascular health 10

1.3.1 Sleep traits and acute myocardial infarction 12

1.3.2 Sleep traits and atrial fibrillation 13

1.3.3 Possible mechanisms linking sleep traits to acute myocardial infarction and atrial fibrillation 13

1.4 The puzzle of causation — a backdrop on causal inference 14

1.4.1 Mendelian randomization 16

1.4.2 Factorial Mendelian randomization 17

1.4.3 Triangulation in causal inference 19

2 Aims **21**

2.1 Overall aim 21

2.2 Specific aims 21

3 Materials and methods **23**

3.1 Study populations 23

3.1.1 UK Biobank 23

3.1.2 The Trøndelag Health Study 23

3.2 Study variables 24

3.2.1 Sleep traits 24

3.2.2 Genotyping and genetic instruments 25

3.2.3 Outcome ascertainment 27

3.2.4 Covariates 28

3.3 Study cohorts and design 32
3.3.1 Paper I 32
3.3.2 Paper II 33
3.3.3 Paper III 34
3.4 Statistical analyses 35
3.4.1 Observational analyses 35
3.4.2 Mendelian randomization analyses 36
3.4.3 Additional and sensitivity analyses 38
3.5 Ethics 40

4 Results 41
4.1 Paper I: Main findings 41
4.2 Paper II: Main findings 46
4.3 Paper III: Main findings 50

5 Discussion 59
5.1 Methodological considerations 60
5.1.1 Random error and statistical precision 60
5.1.2 Systematic error and internal validity 62
5.1.2.1 Selection bias 62
5.1.2.2 Information bias 63
5.1.2.3 Confounding 65
5.1.2.4 Reverse causation 67
5.1.2.5 Pleiotropy 67
5.1.2.6 Weak instrument bias 68
5.1.2.7 Winner's curse 68
5.1.3 Generalizability (external validity) 69
5.2 Appraisal of the findings 70
5.2.1 Sleep traits and the risk of acute myocardial infarction (Papers I and II) 70
5.2.2 Sleep traits and the risk of atrial fibrillation (Paper III) 73

6 Conclusions and future perspectives 75

References 77

Papers I – III and appendices 97

1 Introduction

On a global scale, cardiovascular diseases (CVDs) pose a substantial public health challenge, accounting for roughly one-third of the total annual mortality [1]. Some well-known factors that increase the risk of developing CVDs include unfavorable cholesterol levels, high blood pressure, diabetes, and cigarette smoking [2]. Notably, a considerable proportion of CVDs cannot be explained by these known risk factors. Given the high burden of CVDs, it is important to identify novel risk factors.

Roughly one-third of the general population regularly suffers from one or more insomnia symptoms (i.e., having difficulties initiating sleep, maintaining sleep or having poor sleep quality) [3] and the prevalence of insomnia is increasing [4, 5]. This emphasizes the public health importance of any effect of sleep on the occurrence of CVDs. Understanding the impact of sleep is crucial for gaining further knowledge of the causes of CVDs and implementing effective preventive measures to alleviate the burden of CVDs and their associated consequences.

Although numerous studies have investigated the associations between various individual sleep traits (i.e., insomnia symptoms, sleep duration, and chronotype) and the risk of CVDs, several questions remain unanswered. Since sleep traits are correlated, how sleep traits interact to influence risk of CVDs has not been well explored. This thesis extends on previous findings and aims to provide new knowledge about the joint effects of sleep traits on the risk of acute myocardial infarction (AMI) and atrial fibrillation (AF) using observational and Mendelian randomization (MR) study designs.

This chapter provides an overview of AMI and AF, which are relevant to the research conducted in this thesis, followed by a discussion of the established risk factors associated with AMI and AF. The subsequent section describes the sleep cycle and its stages, as well as the sleep traits of interest in this thesis, followed by the existing knowledge and possible mechanisms underlying the association of sleep traits and cardiovascular health. Finally, this chapter discusses the concept of causal inference in epidemiology and highlights recent advancements in the field of genomics and the application of MR.

1.1 Cardiovascular diseases

CVDs encompass a range of pathological conditions affecting the cardiovascular system, involving the heart and/or blood vessels. Globally, an estimated 18.6 million people succumb to CVDs each year, with approximately 85% of these deaths being attributed to AMI and stroke [1]. In Norway, one-fifth (21%) of the total population currently bears the

burden of a diagnosed CVD or manifests a substantial vulnerability to CVDs — a proportion that is projected to increase due to the ageing population [6].

1.1.1 Acute myocardial infarction

AMI, commonly referred to as *heart attack*, is a leading CVD that impacts an estimated 7.29 million individuals globally [2]. Notably, it is the world's foremost cause of mortality. AMI is the primary manifestation of coronary artery disease (CAD) or coronary heart disease (CHD), wherein blood flow to a portion of the heart muscle (myocardium) is significantly reduced or completely blocked, resulting in myocardial cell death (necrosis) [7]. Despite a decline in the number of new AMI cases in Norway between 2001 and 2014, approximately 11 000 individuals are diagnosed with AMI annually [6].

Atherosclerosis, a slowly progressing disease of the large arteries, serves as the underlying pathophysiologic mechanism contributing to the occurrence of AMI [8]. It begins with injury to the inner lining of the blood vessel (tunica intima) due to hyperlipidemia, high blood pressure, free radicals, or other mechanical stressors, which attract circulating low-density lipoprotein (LDL) cholesterol. The accumulated LDL cholesterol within the intima is taken up by monocytes attracted to the site of injury, which differentiates into macrophages, a type of white blood cell of the immune system. The macrophages overwhelmed with excessive cholesterol transform into *foam cells* and constitute the formation of fatty streaks in the intima (i.e., the first signs of atherosclerotic lesions). This stage is often observed in young people and is generally reversible through lifestyle modifications, including dietary adjustments and physical activity. The fatty streaks evolve into atherosclerotic plaques, narrowing the lumen. Meanwhile, the T-lymphocytes in the intima secrete cytokines and growth factors, in response smooth muscle cells (from tunica media) start to migrate and proliferate in the intima. Eventually, the growing lesion begins to encroach on the lumen. The proliferating smooth muscle cells secrete proteins, including collagen, which form a fibrous cap over the accumulating plaque. This cap helps to stabilize the plaque. Over time, the plaque continues to grow, further narrowing the lumen and impeding blood flow. The plaque can become calcified and hardened, making it more stable but less flexible. However, atherosclerotic plaques are prone to rupture, which can trigger an acute thrombosis (clot formation) by activating platelets and the clotting cascade, resulting in AMI [8].

Patients with AMI present with symptoms of myocardial ischemia, which include chest pain (radiating to the neck, jaw, left shoulder or arm), breathlessness, and fatigue [7]. Additional symptoms such as perspiration, nausea, abdominal pain, and syncope may also be present. Sometimes, AMI can be asymptomatic and go undetected. AMI is usually suspected from a combination of the patient's history, symptoms, electrocardiogram (ECG)

alterations, and changes in biochemical markers. However, the criteria for diagnosing AMI include the detection of rise and/or fall in cardiac biomarkers (preferably troponin (cTn)) with at least one value surpassing the 99th percentile of the upper reference level, together with clinical evidence of acute myocardial ischemia manifested through either one of its symptoms, ECG changes, imaging (echocardiography) evidence of new loss of viable myocardium/regional wall motion abnormality, or thrombus detection by angiography/autopsy. Some common ECG changes during or after an AMI episode include ST segment elevation, ST segment depression, pathologic Q-waves or T-wave inversion [7].

AMI is a life-threatening emergency that could lead to hemodynamic deterioration and sudden death. Over the past decades, there has been a decline in the incidence and mortality rates of AMI, which can be attributed to enhanced preventive medical protocols and the concurrent improvement of risk factor management [9–11]. AMI causes irreversible damage to the myocardium, causing scar formation. The heart is often remodeled following AMI, which is characterized by the dilation and segmental hypertrophy of remaining viable tissue, which compromises the systolic and diastolic function. The prognosis of those who survive an episode depends on the extent of the residual myocardial ischemia, the degree of myocardial damage and its associated complications [7, 12].

1.1.2 Atrial fibrillation

The resting heart rate for adults typically ranges from 60 to 100 beats per minute (bpm), where a healthy heart exhibits a characteristic rhythm that can be detected on an ECG. Irregularities in the rate or rhythm are known as arrhythmias [13]. AF is the most frequent sustained cardiac arrhythmia, affecting 46.3 million individuals globally [14, 15]. Accompanying the ageing of the global population, improved survival with chronic ailments and enhanced detection, AF is emerging as a global epidemic [14, 16]. In Norway, the estimated prevalence of AF was reported as 3.4% at the end of 2014 [17].

The rate and rhythm of the heart are regulated by the autonomic nervous system [18]. During each cardiac cycle, pacemaker cells located in the sinoatrial or sinus node in the right atrium initiate an electrical impulse. This impulse propagates through the atria, causing the right and left atria to contract, thereby facilitating the movement of blood towards the ventricles. Subsequently, another group of pacemaker cells located in the atrioventricular node between the atria and ventricles acts as a conduit for transmitting the impulse from the atria to the ventricles. This enables the ventricular walls to contract, thereby pumping blood out of the heart. This entire process operates in a synchronized manner and is known as the *normal sinus rhythm*, as seen on an ECG [18]. Arrythmias

typically occur as a result of a pathology affecting the conduction system of the heart, primarily due to structural alterations in the heart [12]. This causes the normal sinus rhythm to become desynchronized, leading to a chaotic rhythm characterized by automatic impulse triggers and abnormal conduction. AF is initiated by rapid firings of impulse from ectopic sites in the pulmonary vein or damaged atrial tissue, and it is sustained due to re-entrant conduction within the atria or continuous ectopic firings [19]. As a result, the atria lose their ability to contract and/or facilitate the efficient movement of blood into the ventricles, thus progressively weakening the heart and increasing the likelihood of clot formation [19].

AF can manifest with varying degrees of symptoms, or even be asymptomatic [20]. An estimated one-third of individuals with AF do not display any symptoms [21]. The main symptoms associated with AF are palpitations, breathlessness, and fatigue [20]. AF commonly presents in three forms: paroxysmal (episodes that self-terminate within 7 days), persistent (prolonged episodes that can be terminated by medical or electrical cardioversion) or permanent [12]. However, in most cases, paroxysmal AF transitions into permanent AF as the underlying disease process advances. The sometimes transient presentation of AF episodes and symptoms can pose diagnostic challenges. The diagnosis of AF requires confirmation by ECG. AF is characterized by irregular R-R intervals with narrow QRS complexes and the absence of distinct P waves on the ECG [22].

Although arrhythmia itself is not life-threatening, AF accompanying comorbidities and complications like heart failure (HF) and stroke increase the risk of cardiovascular and all-cause mortality among individuals with AF [15, 20, 23]. HF affects approximately 20–30% of individuals with AF. Moreover, AF is associated with a 2- to 5-fold increase in the risk of stroke and is accountable for 20–30% of all ischemic strokes [20]. Other comorbidities include AMI, chronic kidney disease, venous thromboembolism, dementia, and cancer [15, 20].

1.1.3 Risk factors for acute myocardial infarction and atrial fibrillation

The established risk factors for AMI and AF can be categorized into modifiable and non-modifiable factors.

1.1.3.1 Non-modifiable risk factors

Age. Advancing age is a significant risk factor, with the incidence of AMI rising steeply after the age of 45 in men and 55 in women [14]. Similarly, the prevalence and incidence of AF increase with age, particularly after the age of 60 [15, 24].

Sex. Men have a higher risk of AMI and AF when compared to premenopausal women, with women experiencing a steeper increase in these risks following menopause [25–27].

Genetics. Individuals with a family history are at an increased risk of AMI, indicating a genetic predisposition [28]. Additionally, certain genetic variants associated with cardiac development, electrophysiology, contractility, structure, and immune response pathways are linked to an increased risk of AF [29].

1.1.3.2 Modifiable risk factors

Lifestyle risk factors. Lifestyle risk factors for AMI and AF include tobacco smoking, excessive alcohol intake, physical inactivity, obesity, and poor diet [30, 31]. Smoking tobacco products significantly increases the risk of developing AMI by damaging the arterial walls and promoting early atherosclerosis [32]. Excessive alcohol intake, particularly binge drinking, has been linked to an increased risk of AMI [33]. Smoking and binge drinking have also been shown to impede electrical impulse conduction in cardiac tissue, thereby predisposing to atrial arrhythmias [34–36]. Physical inactivity and obesity enhance the risk by promoting metabolic abnormalities, including insulin resistance and systemic inflammation, thus increasing the risk of AMI [37, 38]. While a meta-analysis found no evidence linking regular physical activity with AF in the general population [39], it has been found that athletes have a higher likelihood of developing AF than non-athletes [40]. Obesity has also been strongly associated with an increased risk of AF [41]. Moreover, an unhealthy diet high in saturated and trans fats is associated with an increased risk of AMI [42].

Cardiometabolic risk factors. Metabolic syndrome encompasses conditions such as hypertension, dyslipidemia, and diabetes mellitus, all of which are important risk factors for developing AMI and AF [30, 31, 43]. Hypertension exerts excess strain on the heart, leading to increased myocardial oxygen demand and subsequent damage to the coronary arteries, thus increasing the risk of AMI [44]. As with AMI, hypertension increases the risk of AF by causing the structural and electrical remodeling of the atria [45]. Dyslipidemia, characterized by high levels of LDL cholesterol and low levels of high-density lipoprotein (HDL) cholesterol, promotes the formation of atherosclerotic plaques that can rupture and cause AMI [8, 46, 47]. When accompanied by CHD, dyslipidemia can promote arrhythmias by causing re-entry formation, focal ectopic activity and neural remodeling, thereby increasing the likelihood of AF [48]. In turn, AF can induce atherosclerosis, a mismatch of blood supply and oxygen demand, as well as thrombosis formation, which further worsens or exacerbates CHD. Consequently, AF and CHD form a vicious cycle, promoting the occurrence and progression of each other [48]. Diabetes mellitus, especially when poorly controlled, contributes to AMI risk by causing endothelial dysfunction and accelerated atherosclerosis [49]. High glucose levels also contribute to AF risk through various mechanisms, including inflammation, oxidative stress, and atrial remodeling [50].

Other risk factors. Individuals with lower education, lower income and that are single have a higher risk of AMI [51, 52]. Psychosocial factors such as depression, anxiety, social isolation, and chronic stress have been found to significantly contribute to the pathogenesis of AMI [53]. Obstructive sleep apnea (OSA), a disorder characterized by repeated pauses in breathing during sleep, has been strongly associated with an increased risk of AF, likely due to intermittent hypoxia, sleep fragmentation, intrathoracic pressure swings, hypercapnia, and increased sympathetic activity as a consequence [54]. Additionally, valvular heart diseases can also contribute to atrial remodeling and development of arrhythmias [55].

1.2 Sleep

A healthy individual devotes on average one-third of their lifespan to sleep [56]. However, *why we sleep* remains a mystery. Sleep is considered a fundamental physiological process that serves a crucial role in maintaining overall health and well-being. In its natural state, sleep is characterized by reduced consciousness and diminished responsiveness to external stimuli. During sleep, the body enters a heightened anabolic state, accelerating intricate processes of growth, tissue repair, and rejuvenation of the various systems of the body for its proper functioning [56, 57]. Some theories have been proposed regarding the functions of sleep [58]. Somatic theories relate sleep to the rejuvenation of bodily functions affected by wakefulness. Neural theories primarily relate sleep to the brain and can be further categorized as metabolic and cognitive theories. Metabolic theories suggest that sleep detoxifies the substances that accumulate from increased oxidative metabolism during wakefulness, whereas cognitive theories suggest that sleep helps in the consolidation of memory and learning [58]. Although scientists have begun to understand how sleep affects the way body functions, much remains to be discovered.

Sleep is regulated by two biological processes: sleep-wake homeostasis and circadian rhythm [59, 60]. Sleep-wake homeostasis acts as a balance between the body's need for sleep (*sleep drive*) and wakefulness (*alert drive*). The sleep drive accumulates over the time awake (due to the build-up of adenosine), thereby promoting the onset of sleep. It is also the reason for prolonged or deeper sleep following a period of insufficient sleep. Conversely, the alert drive grows after a period of sleep, prompting wakefulness [59]. Circadian rhythm is the internal biological clock controlled by the suprachiasmatic nucleus (SCN) located in the hypothalamus in the brain. The SCN responds to light and dark signals and sets off a chain reaction regulating hormone production and suppression, thereby affecting sleep. At sunrise, cortisol is released, enhancing alertness and facilitating awakening. As dusk sets in, melatonin levels begin to rise and remain elevated throughout the night, promoting sleep [60].

1.2.1 Sleep cycle and stages

On a typical night, sleep occurs in multiple cycles, each lasting between 70 and 120 minutes [61]. A healthy adult usually goes through three to five sleep cycles each night [62]. Each cycle consists of four sleep stages, including three that form non-rapid eye movement (NREM) sleep, followed by one for rapid eye movement (REM) sleep [61, 62]. Each sleep stage is characterized by distinct brain activity, body responses and specific sleep features.

Stage 1 NREM sleep (or N1), also known as drowsiness or light sleep, marks the transition from wakefulness to sleep [61, 62]. During this brief period lasting 1–7 minutes, despite the body not being fully relaxed, body and brain activities start to decelerate, which is accompanied by occasional twitches.

Stage 2 NREM sleep (or N2) lasts for 10–25 minutes, during which time the body enters a more subdued state characterized by a reduction in heart rate and breathing, muscle relaxation, drop in body temperature, and the absence of eye movements [61, 62].

Stage 3 NREM sleep (or N3), also known as the period of deep sleep or slow-wave sleep, persists for 20–40 minutes and is when muscle tone, heart rate, and breathing decrease to their lowest levels during sleep, and the body relaxes even further [61, 62].

Stage 4 REM sleep lasts 10–60 minutes and is distinguished by increased brain activity and vivid dreaming [61, 62]. The body experiences temporary paralysis of the muscles, except for the respiratory muscles (causing fast breathing) and the muscles of the eyes (resulting in rapid eye movements). REM sleep is linked to cognitive processing, emotional regulation, and memory consolidation. REM sleep periods occur every 90 to 120 minutes and tend to become longer as the night progresses [61, 62].

1.2.2 Sleep traits and their interplay

The discipline of sleep medicine has primarily focused on the identification and study of sleep disorders, and, more recently, sleep deficiency [63]. However, it has become evident that good sleep encompasses more than the mere absence of sleep disorders. The term ‘sleep health’ is infrequently used and lacks a consensus on its definition. Buysse proposed a definition that elucidates its multifaceted nature. According to Buysse, *‘sleep health is a multidimensional pattern of sleep-wakefulness, adapted to individual, social, and environmental demands, that promotes physical and mental well-being. Good sleep health is characterized by subjective satisfaction, appropriate timing, adequate duration, high efficiency, and sustained alertness during waking hours’* [63]. This definition is based on

measurable constructs of sleep that are most clearly associated with physical, mental, and neurobehavioral well-being, and presented as five dimensions of sleep health, as follows:

a) Sleep duration, which refers to the total amount of sleep obtained within a 24-hour period.
b) Sleep continuity or efficiency, denoting the ease of falling asleep and returning to sleep.
c) Sleep timing, which pertains to the placement of sleep episodes within a 24-hour period (e.g., chronotype).
d) Alertness/sleepiness, which involves the ability to maintain attentive wakefulness.
e) Sleep satisfaction/quality, representing the subjective assessment of the overall quality of sleep as 'good' or 'poor'.

This conceptualization of sleep health acknowledges that optimal sleep health may manifest differently in various situations and among different individuals. Although this comprehensive framework provides clear benchmarks for evaluating and promoting healthy sleep patterns, a consensus on how these dimensions should be measured remains lacking [63].

Sleep is a multifaceted biological phenomenon, and its assessment involves the measurement of various sleep dimensions [63]. These dimensions are indicators of sleep traits or behaviors exhibited by an individual. Sleep traits are often interconnected, meaning that changes in one sleep trait can lead to compensatory adjustments in other sleep traits. For instance, difficulty falling asleep may result in shorter sleep duration and excessive daytime sleepiness, while individuals with the late chronotype (characterized by staying up late) tend to have reduced sleep duration. Moreover, Vgontzas *et al*. suggested the co-occurrence of insomnia with objective short sleep duration as the most biologically severe sleep disorder phenotype [64]. This suggests the possibility of sleep traits influencing each other in a concerted manner, thus underscoring the evaluation of combinations of sleep traits and various sleep patterns as extremely crucial.

1.2.3 Sleep traits of interest

On a population level, comprehensively measuring all sleep traits can be challenging and resource intensive. In the subsequent sub-sections, I discuss the sleep traits of interest in this thesis.

Insomnia

Insomnia can be characterized as a subjective feeling of nocturnal symptoms such as difficulty initiating sleep, difficulty maintaining sleep, or early-morning awakenings. These symptoms persist despite adequate opportunity for sleep and result in daytime impairment.

The diagnosis of insomnia disorder follows the criteria outlined in both the International Classification of Sleep Disorders, Third Edition (ICSD-3) [65], and the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [66], which share similar guidelines for establishing the presence of insomnia disorder. The criteria specify that nocturnal symptoms must cause clinically significant daytime impairment, occur at least three nights per week for at least three months, and must not be attributed to other sleep-related, medical, or mental health disorder(s), or substance abuse.

The prevalence of insomnia differs based on how it is defined. Insomnia has been regarded both as a symptom and as a disorder in its own right. Most often, insomnia is defined by an individual's self-reported difficulties with sleep. An estimated 30% of the general population reports having experienced one or more insomnia symptoms [3]. Insomnia is the most common sleep disorder, with approximately 10% of the global population meeting the criteria for insomnia disorder [67, 68], with the prevalence steadily increasing [4]. In Norway, the prevalence of insomnia among the adult population has increased from 11.9% in the late 2000s to 15.5% in the late 2010s [5, 69]. A recent investigation based on a cohort from northern Norway (the Tromsø 7 Study; 2015–16) found that the prevalence of insomnia among adults aged 40 years and older has increased further to 20%, which was especially pronounced among women [70]. The prevalence was found to be even higher (30.8%) among young adults aged 18–35 years in Norway [71].

Insomnia complaints are highly subjective since they rely on the patient's own experience and perception of the sleep disturbance and are often misreported or not reported in medical records [72]. At a population level, insomnia symptoms are usually assessed subjectively by framing simple questions on various insomnia symptoms. Some validated questionnaires, such as the Insomnia Severity Index (ISI) and the Pittsburgh Sleep Quality Index (PSQI), are designed to enhance the evaluation of sleep disorders, including insomnia [73, 74]. The ISI captures a comprehensive picture of insomnia severity, whereas the PSQI evaluates sleep quality. Another questionnaire designed to measure insomnia is the Bergen Insomnia Scale [75]. However, detailed questionnaires often are unavailable in large observational studies.

Sleep duration

The amount of sleep required to maintain health and well-being varies among individuals, with some requiring less sleep (*short sleepers*) while others require more sleep (*long sleepers*) [76]. Research conducted over recent decades has aimed to determine the ideal amount of sleep required each night for a healthy adult. Conflicting perspectives have emerged, with some researchers claiming that 5–6 hours of sleep per night is necessary and any additional sleep is surplus [77], whereas others suggested that 9–10 hours of sleep per night is optimal [78]. Nevertheless, there is a strong consensus among sleep experts that the

optimal sleep duration for most adults falls within the range of 7–9 hours [79, 80]. The suggested sleep duration recommendations are well tailored to offer guidance from a population-level perspective, while personalized advice for individuals should consider their circumstances and needs.

At the population level, sleep duration can be measured in two ways: subjective measurements through questionnaires and objective measurements using actigraphy. However, there remains some uncertainty about whether subjective measurements reflect time in bed or actual sleep time. Although actigraphy measurements are less frequently measured in large-scale populations, it was observed that actigraphy tends to overestimate sleep duration [81].

Chronotype

A chronotype pertains to an individual's inclination to sleep at a certain time of the day. It distinguishes between morning persons (also known as *early birds*) who prefer to get up and go to bed early, and evening persons (commonly referred to as *night owls*) who prefer to get up and go to bed late. An individual's chronotype is closely linked to their circadian rhythm [82]. Although it is very difficult or impossible to deliberately alter an individual's inherent chronotype, it may shift over the life course concomitant with shifts in the circadian rhythm [83, 84].

At the population level, chronotype can be subjectively measured using a single question asking an individual about their sleep schedule preference. However, some validated measures, such as the Morningness-Eveningness Questionnaire (MEQ) and the Munich Chronotype Questionnaire (MCTQ), were developed and use diverse questions to reliably estimate chronotype [85, 86]. The MCTQ primarily focuses on the assessment of actual wake and sleep times, while the MEQ incorporates inquiries that encompass a broader range of activities, including meal and exercise times. An alternative approach is to use the timing of sleep as a proxy for chronotype, assuming a morning preference if going to bed and rising earlier, while assuming an evening preference if going to bed and getting up later.

1.3 Sleep traits and cardiovascular health

Regulation of the cardiovascular system is primarily governed by the autonomic nervous system. The transition from wakefulness to sleep brings about notable changes in the autonomic control of the cardiovascular system, which are evident as changes in the heart rate and blood pressure (BP). During sleep, the heart rate slows by an average of 10–20 bpm below the resting heart rate [87]. Notably, the heart rate is lower during NREM sleep when compared to REM sleep [88, 89]. In healthy adults, BP undergoes fluctuations over a

24-hour period. After the onset of sleep, there is a sudden drop in BP, which reaches its lowest point during the initial sleep cycle and gradually increases towards alertness levels during the remaining sleep time [90, 91]. This decline in BP at the onset of sleep is believed to be restorative for the cardiovascular system [92]. Furthermore, the stage of sleep also affects BP, with lower levels observed during NREM sleep stages and levels comparable to alertness levels being observed during REM sleep [93].

Numerous studies conducted over the years have examined the associations between sleep traits and CVDs [94–106]. Notably, poor sleep is an important risk factor associated with CVDs. A meta-analysis of prospective cohort studies on insomnia symptoms (i.e., difficulty falling asleep, difficulty maintaining sleep or non-restorative sleep) and the risk of CVDs (including CHD, AMI, and stroke) found that individuals with insomnia symptoms had a 45% increase in the risk of developing or dying from CVDs (relative risk (RR) 1.45; 95% confidence interval (CI) 1.29, 1.62) [94]. A larger and more recent meta-analysis found that the insomnia symptoms of difficulty falling asleep and non-restorative sleep were associated with increased CVD mortality [95]. Moreover, MR studies (i.e., a study design that uses genetic variants as instrument for a modifiable risk factor to investigate the causal influence on an outcome) have found that genetically predicted insomnia was associated with an increased risk of a range of CVDs [96–98].

In a meta-analysis, it was found that compared to 7 h sleep duration per day, there was an 11% increase in the risk of CHD for every hour decrease in sleep duration (RR 1.11; 95% CI 1.05, 1.16) and a 7% increase in the risk of CHD for every hour increase in sleep duration (RR 1.07; 95% CI 1.00, 1.15) [99]. Moreover, some meta-analyses of prospective studies also found that both short and long sleep durations were associated with an increased risk of morbidity or mortality from CVDs when compared to normal sleep duration [100–103]. Additionally, an MR study has revealed that a genetically predicted increase of 1 h in sleep duration had a protective effect on CVDs, while genetically predicted short sleep duration was linked to an increased risk of CVDs [104]. These results suggest the presence of a U- or J-shaped association of sleep duration on the risk of CVDs [107].

Only a few studies have investigated the association of chronotype and the risk of CVDs, with evening chronotypes being associated with an increased risk of CVD risk factors [108, 109]. On the contrary, a prospective study on the association of sleep onset timing and the incidence of CVDs found that sleep onset earlier than 10:00 PM and later than 11:00 PM were associated with increased risk of CVDs when compared to sleep onset between 10:01 PM and 11:00 PM [105]. Since MR investigations of chronotype are scarce and lack compelling evidence [106], it remains unclear whether chronotype is causally associated with an increased risk of CVDs.

Sleep traits are often correlated and can together assert their influence on disease risk. Solely focusing on one sleep element may provide a partial recognition of clinically relevant sleep phenotypes while overlooking their potential health implications. While individual sleep traits have been extensively researched, evidence of the joint association of sleep traits on the risk of cardiovascular outcomes remains limited. A few observational studies have investigated the joint effects of sleep traits and have found evidence that a combination of sleep traits may further increase the risk of CVDs [110–114], including CAD/CHD [110, 111, 115]. For instance, insomnia with short sleep duration — considered the most biologically severe sleep disorder phenotype [64] — is associated with increased cardiometabolic risk [116–119]. To date, MR investigations exploring the joint effects of sleep traits remain lacking.

1.3.1 Sleep traits and acute myocardial infarction

A recent meta-analysis of observational studies showed that individuals with insomnia had a 69% increase in the risk of AMI (RR 1.69; 95% CI 1.41, 2.02) when compared to individuals without insomnia [120]. Moreover, the symptoms of initiating and maintaining sleep were associated with a 13% increased risk of AMI (RR 1.13; 95% CI 1.04, 1.23) [120]. In HUNT2, individual insomnia symptom(s) and a cumulative number of insomnia symptoms were previously reported to be associated with an increased risk of AMI [121]. Also, MR studies found that genetically predicted insomnia was associated with an increased risk of CAD and AMI [96–98, 106].

Two recent meta-analyses of prospective cohort studies have shown that both short and long sleep durations increase the risk of CHD [101, 102]. This was also found for AMI in an observational study performed previously based on data from UK Biobank (hazard ratio (HR) $_{\text{short sleep}}$ 1.20; 95% CI 1.07, 1.33 and HR $_{\text{long sleep}}$ 1.34; 95% CI 1.13, 1.58) [122]. Additionally, MR studies found that a genetically predicted increase of 1 h in sleep duration had protective effects on CAD and AMI, and genetically predicted short sleep duration was associated with an increased risk of CAD and AMI [104, 122].

An observational study from the Sleep Heart Health Study (SHHS) found that individuals with sleep onset timing later than midnight had a 63% increased risk of AMI (HR 1.63; 95% CI 1.09, 2.43) when compared to those with sleep onset between 10:01 PM and 11:00 PM [123]. Any compelling evidence of an association of chronotype and the risk of AMI is lacking from both observational and MR study designs.

A cross-sectional investigation by Kalmbach *et al.* involving 3 911 subjects from the Evolution of Pathways to Insomnia Cohort (EPIC) study found that subjects who had self-reported insomnia disorder with short sleep duration exhibited an increased likelihood of AMI (odds ratio (OR) 3.23; 95% CI 1.45, 7.21) when compared to those who never had

insomnia disorder with 6 h or more of sleep duration [124]. Investigations exploring the joint effects of sleep traits on the risk of AMI using more robust observational and MR study designs remain largely lacking.

1.3.2 Sleep traits and atrial fibrillation

Two recent observational studies have identified insomnia symptoms as risk factors for AF [125, 126]. Some recent MR studies also found that genetically predicted insomnia increased the risk of AF [96–98].

A recent meta-analysis of cohort studies found that both short and long sleep duration increases the risk of AF (HR 1.21; 95% CI 1.02, 1.44 and HR 1.18; 95% CI 1.03, 1.35, respectively) [127]. Also, MR studies have found that a genetically predicted increase of 1 h in sleep duration had a protective effect on AF, while genetically predicted short sleep duration was associated with an increased risk of AF [104, 128].

An observational study based on UK Biobank data found a weak negative association (HR 0.97; 95% CI 0.93, 1.00) between morning chronotype and the risk of AF [126]. Notably, genetically-determined chronotype has not been explored in relation to AF using MR.

A recent observational study on sleep patterns and the risk of incident arrhythmias found that poor sleep scores represented by the combination of unfavorable sleep behaviors (i.e., presence of insomnia symptoms, abnormal sleep duration, evening chronotype, snoring or daytime sleepiness) increased the risk of AF [126]. However, evidence of the joint effects of sleep traits on the risk of AF remains lacking.

1.3.3 Possible mechanisms linking sleep traits to acute myocardial infarction and atrial fibrillation

The mechanisms thought to be underlying the increased risk of AMI due to insomnia symptoms or short sleep duration are multifaceted [129]. Insomnia and short sleep duration independently increase the risk of autonomic dysfunction by increasing sympathetic activity (stress response), consequently leading to elevated metabolic rate, increased heart rate, and reduced heart rate variability [130–132]. Furthermore, experimental studies have demonstrated that sleep restrictions can cause hormonal imbalance, triggering the activation of proinflammatory pathways [133], increased appetite [134, 135], and increased insulin resistance [136]. These disturbances in autonomic function and hormonal regulation subsequently contribute to hypertension [137, 138], diabetes [136], dyslipidemia, and obesity [134, 135]. Together, these accelerate endothelial dysfunction and atherosclerosis, thereby leading to cardiac dysfunction [139].

The definitive mechanisms mediating the detrimental effects of sleep disturbances on AF risk remain elusive. Nonetheless, it is well known that insomnia and short sleep duration trigger a range of physiological processes, including dysfunction of the autonomic nervous system [130–132], activation of proinflammatory and oxidative stress pathways [140], dysregulation of the hypothalamic-pituitary axis (HPA) [141], and activation of the renin-angiotensin system [142]. These responses may contribute to atrial remodeling and fibrosis [143–145], resulting in the loss of atrial muscle mass and subsequent promotion of proarrhythmic conditions conducive to AF incidence. OSA is a common cause of poor sleep and has been recognized as a risk factor for AF [54]. OSA alters the intrathoracic pressure, leading to cyclic augmentation of atrial wall stress. This stress can further exacerbate autonomic dysfunction and inflammation pathways [146], which are potentially pathophysiological in the development of AF.

While knowledge about the biological mechanisms involving long sleep duration is limited, the association of long sleep duration on the risk of AMI and AF may be explained by poor sleep quality, depression or other underlying comorbidities [147]. People reporting long sleep duration are more likely to experience poor sleep quality due to fragmented sleep with repeated awakenings [147]. In turn, this poor sleep quality increases sympathetic activity and activates an inflammatory response [148, 149]. These physiological changes are associated with the development of arterial stiffness and the onset of atherosclerosis [139, 149], while also potentially contributing to atrial remodeling [150].

The underlying mechanisms by which chronotype may influence AMI and AF are not fully understood. However, studies have found that individuals with evening chronotype are more likely to be susceptible to cardiometabolic risk behaviors and risk factors [108, 109].

The interconnection and the compensatory adjustment in sleep traits due to changes in a vicinal sleep trait demonstrate the intricacy of the sleep process and the importance of considering multiple sleep traits when investigating sleep traits. Although no study has explored the mechanism by which the combination of sleep traits may influence the risk of AMI/AF, it is plausible that these sleep traits might individually via different and complementary pathways could operate synergistically to increase the risk of AMI/AF.

1.4 The puzzle of causation — a backdrop on causal inference

Epidemiology is defined as *'the study of the occurrence and distribution of health-related events, states, and processes in specified populations, including the study of determinants influencing such processes and the application of this knowledge to control relevant health problems'* [151]. Over the years, large epidemiological population-based studies have yielded important insights into the distribution and etiology of disease [152–154]. Being

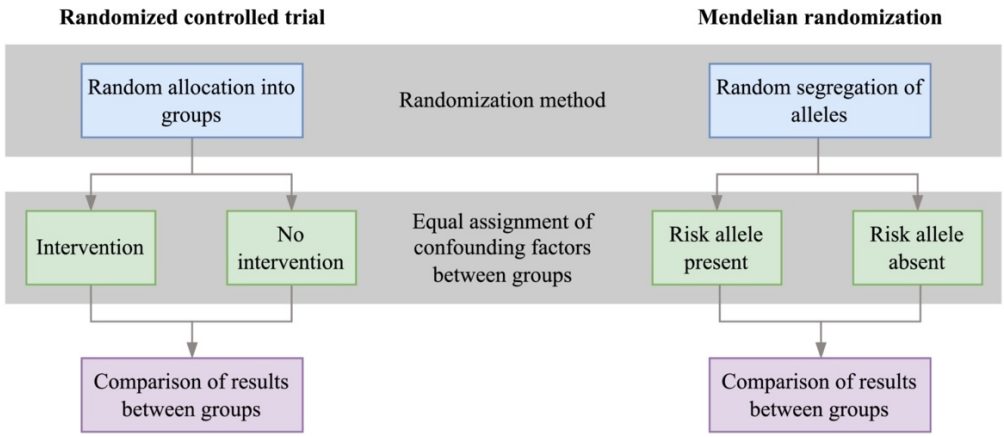
able to establish causation is central to epidemiology. Given that the practice of this discipline aims to identify the causes of diseases, epidemiologists have traditionally turned to Bradford and Hill's criteria for causality to distinguish non-causal from causal associations in their research [155]. Subsequently, they developed theoretical frameworks outlining the requisite designs and methods used to conduct analyses that could draw valid and robust conclusions [156–158]. Observational study designs — including cross-sectional, case-control, and prospective cohort studies — are susceptible to various sources of bias, including confounding, reverse causation, and measurement error (details in the Discussion chapter). This results in misleading/spurious associations, despite the best efforts to enhance the design and analysis of these studies [159]. Randomized controlled trials (RCTs) are widely regarded as the gold standard for establishing causal relationships between an exposure or risk factor and an outcome because the randomization ensures confounding is independent of the exposure/risk factor status [160]. However, RCTs are very costly and not always ethical, practical or timely [161].

In recent decades, advancements in the field of genomics — with breakthroughs in the development of deoxyribonucleic acid (DNA) sequencing techniques, together with parallel progress in other fields such as epidemiology, biotechnology, computer science, and statistics — have paved the way for the field of *genetic epidemiology*. As a result, it has become feasible to conduct hypothesis-free statistical association tests between phenotypes of interest and millions of genetic variants spanning the entire genome in large populations, which are commonly known as genome-wide association studies (GWASs) [162]. GWASs identify genetic variants (i.e., single nucleotide polymorphisms (SNPs)) that influence traits, segregating either between cases and controls or along the distribution of continuous traits. GWASs have provided detailed insights into the genetic architecture of a large number of polygenic complex traits [163]. The discovery of multiple signals in GWASs has made it possible to further incorporate these genetic variants into genome-wide polygenic scores (or genetic risk scores (GRSs), polygenic risk scores or weighted allele scores) [164, 165]. GRS aggregates information across the entire genome to identify the genetic contribution to a trait, particularly when there may not be one single gene responsible for the acquisition of the trait (i.e., a monogenic trait). In the context of a polygenic complex trait, each genetic variant explains very little variation in the trait, but cumulative risk across many genetic variants may account for a substantial proportion of variation in the trait. The availability of a large resource with GWAS summary statistics for a broad range of phenotypes has promoted the use of other approaches aimed at enhancing causal inferences, such as Mendelian randomization (MR).

1.4.1 Mendelian randomization

MR is an application of instrumental variable (IV) analysis that uses genetic variants as an instrument for a modifiable risk factor to investigate the causal influence on an outcome [166, 167]. It leverages the principle of random assortment of alleles from parents to offspring during meiosis (Mendel's law of independent assortment), as well as the principle of germline DNA not being modified by lifestyle factors later in life, thus making it less susceptible to the biases observed in conventional observational study designs. The MR design closely imitates that of an RCT, rendering it akin to *nature's own randomized trial* [168, 169]. In contrast to a typical RCT, where study participants are randomly assigned to a treatment or non-treatment (or placebo) group, MR compares groups of individuals who have been *naturally randomized* to (on average) higher or lower genetic risk for an exposure of interest (Figure 1). The genetic risk can be quantified using a genetic instrument, which can be a single or multiple genetic variant(s) or a GRS that serves as a proxy for the exposure under investigation.

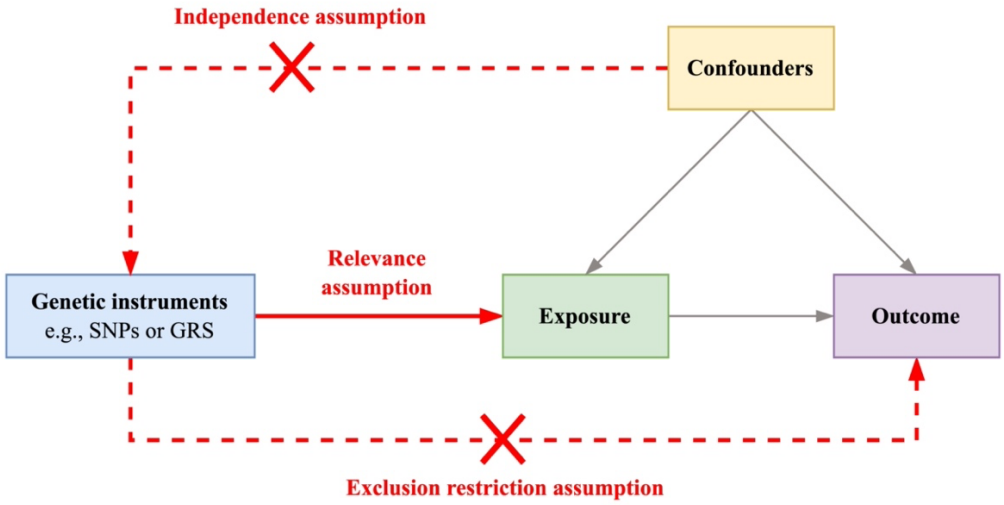
Figure 1: Mendelian randomization compared to a randomized controlled trial.



There are three core assumptions of MR (as illustrated by the directed acyclic graph presented in Figure 2) [170, 171]. These assumptions state the following:

1. The genetic instrument must be robustly associated with the exposure (relevance assumption).
2. The genetic instrument should not be associated with any confounders of the exposure-outcome association (independence assumption).
3. The genetic instrument should only affect the outcome via the exposure of interest, i.e., no independent pathway except through the exposure (exclusion restriction assumption).

Figure 2: Directed acyclic graph presenting Mendelian randomization assumptions.



MR can be applied in two settings: a) One-sample MR can be performed where individual-level data, i.e., information on genetic variants, exposure, and outcome are available for all study participants [172]. The genetic variants are identified from a GWAS of the exposure of interest [173]. b) Two-sample MR (also known as summary MR) can be conducted where estimates of the genetic associations for the exposure and outcome are available from two separate GWASs [174]. For a single genetic variant, a Wald ratio can be estimated by dividing the SNP-outcome association (sample 1) by the SNP-exposure association (sample 2). In the case of multiple genetic variants as instruments, the Wald ratio estimates are combined using an inverse-variance weighted fixed-effects meta-analysis [175].

In recent years, MR methodologies have expanded with the introduction of novel techniques aimed at extending the applicability of MR to investigate more complex causal associations [176]. One such advancement is factorial MR, which enables the investigation of the joint effects of two risk factors on a single outcome [177, 178].

1.4.2 Factorial Mendelian randomization

A factorial MR design closely imitates a factorial randomized trial (Figure 3) [177, 179]. A 2x2 factorial randomized trial explores the effect of two binary treatments (labelled as A and B) on a binary outcome, where the study participants are randomly assigned to one of four groups: those who receive treatment A only; those who receive treatment B only; those who receive both treatments A and B; those who receive neither treatment A nor B [180]. Similarly, in a 2x2 factorial MR design, the dichotomization of GRS for two risk factors

Figure 3: Factorial Mendelian randomization compared to a factorial randomized clinical trial.

A) Factorial randomized clinical trial

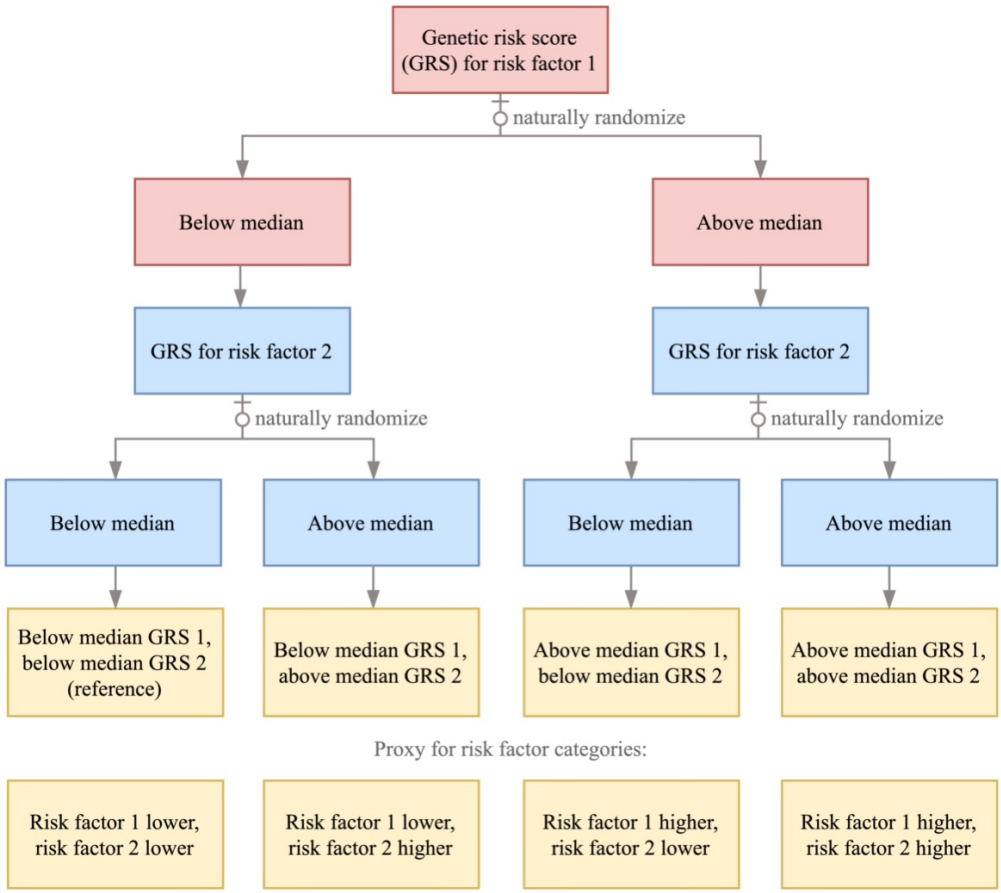
		Randomization of A	
		Control	Treatment A
Randomization of B	Control	Incidence under usual care	Incidence under treatment A
	Treatment B	Incidence under treatment B	Incidence under both treatments A and B

B) Factorial Mendelian randomization

		Genetic risk score 1	
		Below median	Above median
Genetic risk score 2	Below median	Risk factor 1 lower, risk factor 2 lower	Risk factor 1 higher, risk factor 2 lower
	Above median	Risk factor 1 lower, risk factor 2 higher	Risk factor 1 higher, risk factor 2 higher

across their median will allow the *natural randomization* of study participants into one of four groups: higher genetic risk for risk factor 1 only; higher genetic risk for risk factor 2 only; higher genetic risks for both risk factors 1 and 2; lower genetic risks for both risk factors 1 and 2 (Figure 4). This allows for an assessment of the joint causal influence of two risk factors on a binary outcome [177].

Figure 4: Design of a 2x2 factorial Mendelian randomization study.



Understanding how causes of disease cumulatively increase disease risk can have important public health implications. The above-additive effects due to multiple risk factors can lead to a greater burden of disease in the population. Factorial MR offers a valuable approach to assess the combined causal effects of the simultaneous occurrence of two or more risk factors for disease. By using factorial MR, we can gain insights into the collective influence of multiple risk factors and their implications for disease development.

1.4.3 Triangulation in causal inference

Triangulation involves addressing a causal question through the integration of results derived from different methodological approaches that have distinct and unrelated key sources of potential bias [181]. It is widely acknowledged that no solitary method enables us to draw robust conclusions on causation due to the limitations and sources of bias inherent in each method. Nonetheless, the use of different methods can help us gain stronger support for causation. For instance, if different methods yield consistent results, then more compelling conclusions can be made [181].

This thesis aimed to strengthen the establishment of causal relationships by employing triangulation, which involved combining evidence from two distinct methodological approaches — observational studies utilizing multivariable regression analysis, as well as MR analysis.

2 Aims

2.1 Overall aim

The overarching aim of this thesis was to investigate sleep traits (i.e., insomnia symptoms, sleep duration, and chronotype) and their interplay as risk factors for the development of CVDs. More specifically, we aimed to investigate the individual and joint causal influence of sleep traits on the risk of incident AMI and AF.

2.2 Specific aims

I. To prospectively investigate the individual and joint associations of sleep traits on the risk of incident AMI (Paper I).

II. To examine the individual and joint causal effects of sleep traits on the risk of incident AMI using MR (Paper II).

III. To prospectively investigate the individual and joint associations of sleep traits on the risk of incident AF (Paper III).

IV. To examine the individual and joint causal effects of sleep traits on the risk of incident AF using MR (Paper III).

3 Materials and methods

3.1 Study populations

In this thesis, all papers are based on data from UK Biobank and the second survey of the Trøndelag Health Study (HUNT2; 1995–97).

3.1.1 UK Biobank

UK Biobank is a large population-based prospective study of middle-aged adults (aged 40 to 69 years) based in the United Kingdom [182, 183]. All individuals registered with the UK National Health Service (NHS) living within a 25-mile radius of one of 22 study centers located throughout England, Scotland, and Wales were invited to participate during the period March 2006 – July 2010.

In total, ~9.2 million individuals were invited and 502 460 (5.5%) participated [184, 185]. Upon recruitment, participants completed a touchscreen questionnaire along with a brief computer-assisted interview. The questionnaire elicited information on various factors such as socio-demographics and lifestyle. Physical measurements including height and weight were also recorded, and bodily fluid samples (e.g., blood, saliva, and urine) were collected from participants. In addition to data collected directly from participants, all participants consented to have their electronic health records (from general practitioners, hospitals, and health registries) linked to UK Biobank for research purposes. More information on UK Biobank can be found at https://www.ukbiobank.ac.uk/.

3.1.2 The Trøndelag Health Study

The Trøndelag Health Study (HUNT), formerly known as the Nord-Trøndelag Health Study, is the largest population-based health study in Norway, primarily conducted in the northern region of Trøndelag County [153, 186]. Prior to 2018, the northern region of Trøndelag constituted one of the 19 counties in Norway, which was later united with its southern counterpart (Sør-Trøndelag) to establish Trøndelag County [187]. The northern region of Trøndelag County is predominantly rural and its population is fairly representative of Norway regarding socio-demographic characteristics, as well as mortality and morbidity [188].

The HUNT Study is an ongoing collaboration between the HUNT Research Centre (Faculty of Medicine and Health Science, Norwegian University of Science and Technology (NTNU)), Trøndelag County Council, the Central Norway Regional Health Authority, and the Norwegian Institute of Public Health. The HUNT Study currently consists of four surveys carried out at different time points spanning a 35-year period: HUNT1 (1984–86), HUNT2 (1995–97), HUNT3 (2006–08), and HUNT4 (2017–19). For

the adult part of each survey, inhabitants in the area aged 20 years or older were invited to participate. Over the years, the study has expanded from collecting health-related data through basic questionnaires and clinical examinations in HUNT1 to questionnaires involving a wide range of self-reported and clinical information, interviews, clinical examinations, and the collection of blood samples (HUNT2 and onwards), as well as urine, saliva, and fecal samples in HUNT4. Recently, the adult population of the former Sør-Trøndelag region was also invited to participate in the questionnaire. In total, the HUNT Research Centre has compiled a database of approximately 230 000 participants [186]. More information on the HUNT Study can be found at http://www.ntnu.edu/hunt.

HUNT2

Due to the non-availability of data pertaining to all sleep trait variables in HUNT1 and HUNT3, as well as the short follow-up period in HUNT4, we only used data from HUNT2 in this thesis. In total, 93 898 individuals were invited during the 1995–97 period, and 65 228 (69.5%) participated. An invitation letter was sent by mail along with a self-administered questionnaire. The completed questionnaire was returned by participants at the health examination site, where clinical examinations were conducted, and blood samples were drawn by trained personnel. Subsequently, a second questionnaire with more detailed questions about their health and lifestyle was handed out at the examination site along with a prepaid envelope. The questionnaire was completed at home and returned by mail. Detailed information regarding the HUNT2 study has been published elsewhere [188].

3.2 Study variables

3.2.1 Sleep traits

Insomnia symptoms

Insomnia symptoms were defined as an individual having two night-time insomnia symptoms (i.e., difficulty falling asleep, difficulty maintaining sleep or waking up too early) without any related daytime impairment. Notably, this definition does not encompass all components included in the frameworks for diagnosing insomnia [68]. Thus, the term *insomnia symptoms* is used throughout this thesis.

In UK Biobank, insomnia symptoms were assessed by the following question: ‘Do you have trouble falling asleep at night or do you wake up in the middle of the night?’, with the response options of ‘Never/rarely’, ‘Sometimes’, ‘Usually’ or ‘Prefer not to answer’. Participants were classified as having insomnia symptoms if they answered ‘Usually’, and classified as not having insomnia symptoms if they answered ‘Never/rarely’ or ‘Sometimes’. Other responses were coded as missing.

In HUNT2, insomnia symptoms were assessed by the following two questions: (1) 'Have you had difficulty falling asleep in the last month?'; (2) 'During the last month, have you woken too early and not been able to get back to sleep?'. These questions had the response options 'Never', 'Sometimes', 'Often' or 'Almost every night'. Participants who responded 'Often' or 'Almost every night' to at least one of these questions were classified as having insomnia symptoms. For participants who answered only one of these insomnia symptom questions, we did the following: (1) if they answered 'Often' or 'Almost every night' to one of the questions but did not answer the other, they were classified as having insomnia symptoms; (2) if they answered 'Never' or 'Sometimes' to one of the questions but did not answer the other, they were excluded to avoid potential misclassification. The remaining participants were classified as not having insomnia symptoms.

Sleep duration

Sleep duration was assessed by the questions 'About how many hours of sleep do you get in every 24 hours? (please include naps)' and 'How many hours do you usually spend lying down (i.e., sleeping and/or napping) during a 24-hour period?' for UK Biobank and HUNT2, respectively. The answers could only contain integer values. Any influence of poor health on implausible short or long sleep duration was avoided by excluding extreme responses of less than 3 hours or more than 18 hours. Participants were classified into normal (7–8 h), short (≤6 h) or long (≥9 h) sleep durations. In Papers II and III, in addition to the use of continuous measurement on 24-hour sleep duration (h), binary variables for short (≤6 vs. 7–8 h) and long (≥9 vs. 7–8 h) sleep durations were constructed.

Chronotype

In UK Biobank, chronotype (morning or evening chronotype) was assessed by the question 'Do you consider yourself to be?', with the following response options of 'Definitely a "morning" person', 'More a "morning" than an "evening" person', 'More an "evening" than a "morning" person', 'Definitely an "evening" person', 'Do not know' or 'Prefer not to answer'. Participants were classified as having a morning chronotype if they reported 'Definitely a "morning" person' or 'More a "morning" than an "evening" person', and as having an evening chronotype if they reported 'More an "evening" than a "morning" person' or 'Definitely an "evening" person'. Other responses were coded as missing. Chronotype was not assessed in the HUNT Study.

3.2.2 Genotyping and genetic instruments

The stored blood samples were used to extract DNA samples from 488 377 UK Biobank participants. Since a detailed account of genotyping, pre-imputation quality control and imputation procedures has been provided elsewhere [184], a brief overview is provided here. The samples were assayed using two very similar genotyping arrays, the UK BiLEVE

Axiom™ Array by Affymetrix1 (N = 49 950) and the closely-related UK Biobank Axiom™ Array (N = 438 427). The variants were imputed to the Haplotype Reference Consortium (HRC) and UK10K + 1000 Genomes reference panels. Additionally, an in-house quality control measure was applied, which excluded samples with third-degree or close relatives, sex mismatch, those identified as outliers of heterozygosity and those of non-European ancestry [189].

The HUNT Study has DNA extracted from blood samples obtained from approximately 88 000 participants across HUNT2, HUNT3, and HUNT4 [190]. Since this thesis used genetic data from participants genotyped in HUNT2 and HUNT3, the information about genotyping is limited to these studies. The samples were assayed with one of three different Illumina HumanCoreExome genotyping chips (HumanCoreExome 12 v.1.0, HumanCoreExome 12 v.1.1 or UM HUNT Biobank v.1.0), where genotypes from different chips were quality controlled separately and reduced to a common set of variants. Sample quality control measures were similar to those applied to the UK Biobank. Imputation was performed in two rounds using the HRC (involving joint imputation with HUNT - Whole Genome Sequencing (HUNT-WGS) samples) and the Trans-Omics for Precision Medicine (TOPMed) reference panels, respectively. A detailed account of the genotyping, quality control measures applied and imputation have been described elsewhere [190].

Table 1: Genome-wide significant genetic instruments of sleep traits obtained from discovery genome-wide association studies.

Sleep traits	**Discovery GWASs**	**N**	**Cohorts used by the discovery GWASs**		**No. of SNPs identified**
			UK Biobank	**23andMe**	
Insomnia symptoms	Jansen *et al*., 2019 [191]	1 331 010	109 402 cases and 277 131 controls	288 557 cases and 655 920 controls	248
24-hour sleep duration (h)	Dashti *et al*., 2019 [192]	446 118	446 118 samples	Not included	78
Short sleep (≤6 vs. 7–8 h)	Dashti *et al*., 2019 [192]	411 934	106 192 cases and 305 742 controls	Not included	27
Long sleep (≥9 vs. 7–8 h)	Dashti *et al*., 2019 [192]	339 926	34 184 cases and 305 742 controls	Not included	8
Chronotype (morning preference)*	Jones *et al.*, 2019 [193]	651 295	252 287 cases and 150 908 controls	120 478 cases and 127 622 controls	351

GWAS, genome-wide association study; N, sample size; SNPs, single nucleotide polymorphisms.
* In the discovery GWAS of chronotype, the chronotype increasing allele is morning preference.

A total of 248 SNPs were identified as robustly associated with insomnia symptoms at P $<5x10^{-8}$ based on the meta-analysis of UK Biobank (n = 386 533) and 23andMe (n = 944 477) cohorts in a GWAS conducted by Jansen *et al.* [191]. A large GWAS performed by Dashti *et al.* based on UK Biobank (n = 446 118) identified 78 SNPs as being robustly associated with 24-hour sleep duration [192]. They additionally identified 27 SNPs specific to short sleep duration and 8 SNPs specific to long sleep duration. A genome-wide

association meta-analysis by Jones *et al.* based on UK Biobank (n = 403 195) and 23andMe (n = 248 100) identified 351 SNPs robustly associated with chronotype (morning vs. evening preference) [193]. A list with detailed information on the discovery GWASs used to obtain the genetic instruments is summarized in Table 1.

3.2.3 Outcome ascertainment

The International Statistical Classification of Diseases (ICD) is a standardized system for the classification and coding of health information that serves as a universal language for the reporting of diagnoses, symptoms, and procedures [194]. It is used by healthcare professionals, policymakers, and researchers across regions and countries to ensure the accuracy and comparability of health data.

In UK Biobank, participants were followed via linkage to the Hospital Episode Statistics (HES) for England, the Scottish Morbidity Record (SMR), and the Patient Episode Database for Wales (PEDW), where health-related outcomes had been recorded using ICD-9 and ICD-10 codes. Mortality information was obtained from NHS Digital for participants in England and Wales, and from the NHS Central Register (part of the National Records of Scotland) for participants in Scotland, where the cause of death had been recorded by ICD-10 codes.

In HUNT2, participants were followed via linkage to the medical records provided by the Nord-Trøndelag Hospital Trust for three hospitals (St. Olavs Hospital, Levanger Hospital, and Namsos Hospital), where health-related outcomes had been defined by ICD-9 and ICD-10 codes. Mortality information was obtained via linkage to the National Cause of Death Registry, where the cause of death had been defined by ICD-10 codes.

AMI ascertainment (Papers I and II)

Hospitalizations or deaths due to AMI were identified using ICD-9 code 410 and ICD-10 codes I21 and I22. Incident cases were defined as the first occurrence of either hospitalization or death attributed to AMI during the follow-up period. Participants with any prior AMI episode(s) before their date of participation in the study cohorts regarded as prevalent cases were excluded. Each participant was followed until either first diagnosis or death due to AMI, death due to other causes, loss to follow-up, or the end of the follow-up period (March 23, 2021 for UK Biobank and December 31, 2020 for HUNT2), whichever came first.

AF ascertainment (Paper III)

Hospitalizations or deaths due to AF were identified using ICD-9 code 427.3 and ICD-10 code I48. Incident cases were defined as the first occurrence of either hospitalization or

death due to AF during follow-up from the baseline. All participants with any episode(s) of AF before their date of participation in the study cohorts regarded as prevalent cases were excluded. Each participant was followed up until either first diagnosis or death due to AF, death due to other causes, loss to follow-up, or the end of the follow-up period (March 23, 2021 for UK Biobank and December 31, 2020 for HUNT2), whichever came first.

3.2.4 Covariates

Information on the characteristics of study participants, including their socio-demographic variables such as gender, age, marital status, ethnicity (for UK Biobank), education attainment, and employment status, as well as their lifestyle factors (e.g., smoking status, alcohol intake, physical activity, and use of sleep medication(s)), were collected using a self-administered questionnaire. Participants attended examination stations where clinical examinations were performed, and blood samples were drawn by trained staff.

Questionnaire

Marital status. In UK Biobank, participants were categorized as 'Married' if they cohabit with their husband, wife, or partner, and as 'Unmarried' if they do not. In cases where information regarding marital status was unavailable, the number of individuals residing in a household was used to categorize those living alone as 'Unmarried'. In HUNT2, participants were categorized as 'Unmarried', 'Married' or 'Separated/Divorced/Widowed'.

Alcohol intake. In UK Biobank and HUNT2, participants were asked about their frequency of alcohol intake and were categorized as 'Never/rarely' for non-drinkers or those who consume alcohol only on special occasions, 'Monthly' for those who drink 1–3 times per month, 'Weekly' for those who drink 1–4 times per week, or 'Daily/almost daily' for those who consume alcohol more frequently. In HUNT2, for the observations where information on alcohol intake frequency was not available, an additional covariate for participants that had never consumed alcohol was used to categorize those as 'Never/rarely'. Consequently, data on alcohol intake were categorized as falling into one of four categories: 'Never/rarely', 'Monthly', 'Weekly' or 'Daily/almost daily'.

Smoking status. The information on smoking status was categorized as 'Never', 'Previous' or 'Current' smoker for UK Biobank and HUNT2. Former smokers were defined as those who quit smoking, while current smokers were defined as those currently smoking cigarettes, cigars, or pipes either occasionally or daily.

Physical activity. In UK Biobank, information on physical activity (PA) was collected using adapted questions from the validated short International Physical Activity Questionnaire (IPAQ) [195, 196], which assessed total PA, including walking, moderate PA, and vigorous PA performed over the last week. Participants were categorized into one

of the three mutually exclusive PA categories of 'High' (≥1 h of moderate PA or ≥½ h of vigorous PA above the basal level of activity on most days), 'Moderate' (≥½ h of moderate PA above the basal level of activity on most days) or 'Low/inactive' (anything else) based on standard scoring criteria [197], where approximately 5000 steps per day was considered basal activity. In HUNT2, PA was classified based on self-reported leisure time light and hard PA during the past year. Light PA was characterized as activities that did not cause shortness of breath or sweating, while hard PA was characterized as activities that resulted in shortness of breath or sweating. Participants were instructed to consider their commute to work as part of their leisure time. Participants were categorized into one of three mutually exclusive PA categories of 'High' (defined by ≥1 h of hard PA regardless of light PA or ≥3 h of light PA with <1 h of hard PA), 'Moderate' (defined by ≥3 h of light PA with no hard PA or <3 h of light PA with <1 h of hard PA); 'Low/inactive' (for anything else). This categorization strategy was previously used by Brumpton *et al.* [198]. The questions on PA from HUNT2 were reported to have acceptable reliability and validity for hard PA, but poor for light PA [199].

Education. In UK Biobank and HUNT2, participants were asked about their education attainment and were categorized as '10 years or less' (for primary and lower secondary school education), '11–13 years' (for upper secondary school education) or '14 years or more' (for university/college education).

Socioeconomic status. The Townsend Deprivation Index (TDI) was used as a measure to account for varying socioeconomic disparities and urban-rural mix within the UK Biobank participants. The index was derived from census data on housing, employment, car availability, and social class based on the postal codes of participants, with higher values indicating a higher level of deprivation. The TDI has been validated for use in a UK-based population [200]. The HUNT2 population is fairly representative of Norway regarding socioeconomic characteristics [188]. Thus, any potential socioeconomic differences would be largely captured by education attainment.

Ethnicity. In UK Biobank, participants were categorized based on their ethnicity as 'White', 'Mixed', 'Asian/Asian British', 'Black/Black British', 'Chinese' or 'Other'. Although information on ethnicity was not available in HUNT2, the Nord-Trøndelag region is predominantly composed of Caucasians (exceeding 97%), thereby comprising a homogenous population pool [188].

Shift work. UK Biobank collected separate responses from participants regarding working shifts or working night shifts, which were subsequently combined to generate a proxy variable. The final value was determined based on the highest response category, and this proxy variable was later dichotomized. Responses of 'Usually' or 'Always' were

categorized as ‘Yes’, while all other responses as ‘No’. In HUNT2, participants were also asked about working shifts, at night or on-call, and their responses were dichotomized as ‘Yes’ or ‘No’. Furthermore, information on current employment/work status from both UK Biobank and HUNT2 was used to categorize those without paid employment and those who were self-employed as ‘No’ for observations where information on working shifts, at night or on-call was unavailable.

Use of sleep medication. In UK Biobank, the use of sleep medication(s) was ascertained by the self-reported use of medications from the list of sleep medications, as used by Daghlas *et al*. [122], along with five other commonly used anxiolytics or sleep medications (list included in Table 2). Responses were then categorized as either ‘Yes’ or ‘No’ for the use of sleep medication(s). In HUNT2, participants were asked about their use of any anxiolytics or sleep medications in the last month and were categorized as ‘Yes’ if they reported daily or weekly intake, and ‘No’ otherwise.

Table 2: List of medications used to define the sleep medication covariate in UK Biobank.

Sleep medication	**Treatment/medication code (UK Biobank field ID: 20003)**
Oxazepam	1140863442
Meprobamate	1140863378
Medazepam	1140863372
Bromazepam	1140863318
Lorazepam	1140863302
Clobazam	1140863268
Chlormezanone	1140863262, 1140868274
Temazepam	1140863202
Nitrazepam	1140863182, 1140863104
Lormetazepam	1140863176
Diazepam	1140863152, 1141157496
Zopiclone	1140863144
Triclofos sodium	1140863140
Methyprylon	1140856040
Prazepam	1140855944
Triazolam	1140855914
Ketazolam	1140855860
Dichloralphenazone	1140855824
Clomethiazole	1140909798
Zaleplon	1141171404
Butobarbital	1141180444
Clonazepam	1140872150
Flurazepam	1140863110
Loprazolam	1140863120
Alprazolam	1140863308
Butobarbitone	1140882090

Chronic illness. In UK Biobank and HUNT2, participants were asked about suffering from any long-standing illness, disability, or injury of a physical or psychological nature that impairs their functioning in everyday life. Their responses were categorized as 'Yes' or 'No'.

Clinical measures

Body mass index. In UK Biobank, weight was determined using a Tanita BC-418MA body composition analyzer to the nearest 0.1 kg, while height was measured using a Seca 202 height measure. In HUNT2, weight was measured to the nearest 0.5 kg and height was measured to the nearest 1 cm. Participants were instructed to wear light clothing and no shoes during the measurements. Body mass index (BMI) was then calculated by dividing the weight (in kg) by the square of the height (in meters).

Blood pressure. The methods and protocols for obtaining BP measurements differed between UK Biobank and HUNT2. In UK Biobank, systolic and diastolic BP readings were obtained using an automated method (with an Omron HEM-705 IT electronic BP monitor) and/or manual method (with a sphygmomanometer). Two sets of measurements were taken 1 min apart, and their average was used in the analysis. In cases where automated readings were unavailable, manual readings were used. In HUNT2, systolic and diastolic BP readings were obtained using an automated method (with a Dinamap 845XT (Critikon) sphygmomanometer based on oscillometry). Three sets of measurements were taken 1 min apart, and the average of the second and third measurements were used in the analysis.

Laboratory measures

In accordance with the standard operating procedures for UK Biobank, a random (non-fasting) blood sample was collected from each participant and stored in refrigerators at temperatures ranging from 2 to 8°C. Fasting time was noted as the duration between the last food or drink intake and the blood sample collection. The samples were transported to a central laboratory on a daily basis for storage and analysis. Serum samples were centrifuged at 2000 RCF for 10 min, and the concentrations of glucose, total cholesterol, HDL-cholesterol, and triglycerides were analyzed using a Beckman Coulter AU5800 automated analyzer. Glucose was measured using hexokinase analysis, while total cholesterol, HDL-cholesterol, and triglycerides were measured by CHO-POD analysis, enzyme immunoinhibition analysis, and GPO-POD analysis, respectively [201].

In HUNT2, a random (non-fasting) blood sample was collected from each participant. The serum was separated from the blood by centrifugation within 2 h of collection at the screening site and was stored in a refrigerator at 4°C. The time between the last meal and venipuncture was recorded. The samples were later transported to the central laboratory at Levanger Hospital, where they were analyzed using a Hitachi 911 Autoanalyzer (Hitachi,

Mito, Japan). The samples were transported to the laboratory either on the same day or within 2 to 3 days (e.g., on weekends). The serum concentrations of glucose, total cholesterol, HDL-cholesterol, and triglycerides were analyzed using reagents from Boehringer Mannheim (Mannheim, Germany). The day-to-day coefficients of variation were 1.3–2.0%, 1.3–1.9%, 2.4%, and 0.7–1.3%, respectively. Glucose was measured using an enzymatic hexokinase method, total cholesterol and HDL-cholesterol were measured using an enzymatic colorimetric cholesterol esterase method, and triglycerides were measured using an enzymatic colorimetric method [188].

Depression and anxiety

Anxiety and depression episodes in the UK Biobank participants were identified from hospital records using ICD-10 codes F40 and F41 for anxiety; and F32, F33, F34, F38, and F39 for depression. This information was then used to create two binary proxy variables, one each for anxiety and depression, which were categorized as 'Yes' or 'No'.

The HUNT2 participants were evaluated for symptoms of anxiety and depression using the Hospital Anxiety and Depression Scale (HADS). This questionnaire consisted of 14 Likert-scaled items (7 each for anxiety and depression) having a 4-point scale ranging from 0 (not at all) to 3 (very often). The responses were summed to generate anxiety and depression scores ranging from 0 to 21, with higher scores indicating a greater likelihood of anxiety and depression. A score of ≥8 each for depression and anxiety represents cases [202, 203]. The HADS does not include items related to sleep difficulties or somatic symptoms. This assessment tool is useful in both primary care and hospital settings for measuring anxiety and depression symptom severity [204], and its psychometric properties have previously been validated as part of the HUNT Study [205].

3.3 Study cohorts and design

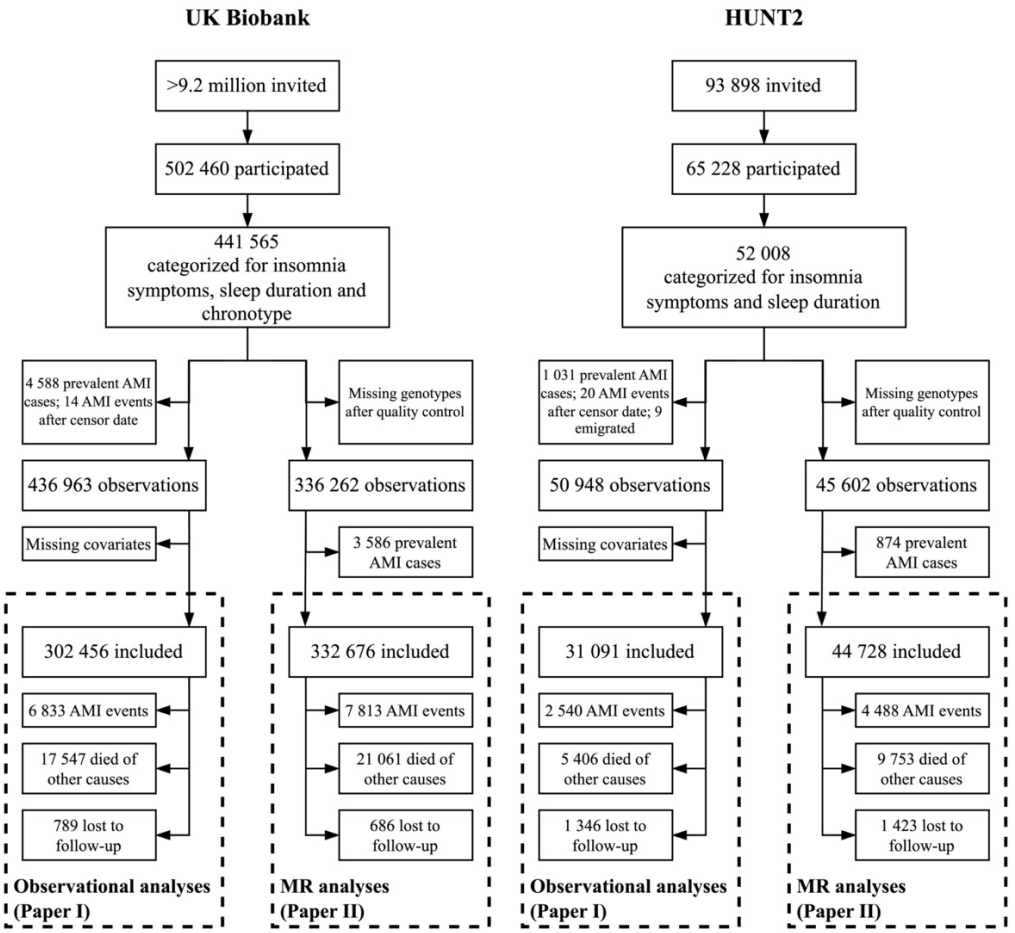
We used prospective cohort and MR study designs to address the study aims, where MR was used as our main approach for causal inference. This thesis draws on the principle of triangulation, which suggests that the findings will be strengthened if the results from the different approaches with unrelated sources of bias all point to the same conclusion. Furthermore, these approaches were applied to the UK Biobank and HUNT2 separately.

3.3.1 Paper I

This is a prospective cohort study investigating the association of sleep traits and the risk of incident AMI. Overall, there were 441 565 participants in UK Biobank who had information available for sleep traits of interest. After excluding participants who had any prior episode(s) of AMI, AMI episode(s) after the censor date and missing information on covariate(s), a total of 302 456 participants were included in the analyses. There were

52 008 participants in HUNT2 who had information available for sleep traits of interest. After excluding participants who had any prior episode(s) of AMI, AMI episode(s) after the censor date, and those who had emigrated and missing information on covariate(s), a total of 31 091 participants were included in the analyses. A flow chart depicting the selection of study participants is presented in Figure 5.

Figure 5: Selection of study participants – Paper I and Paper II.



3.3.2 Paper II

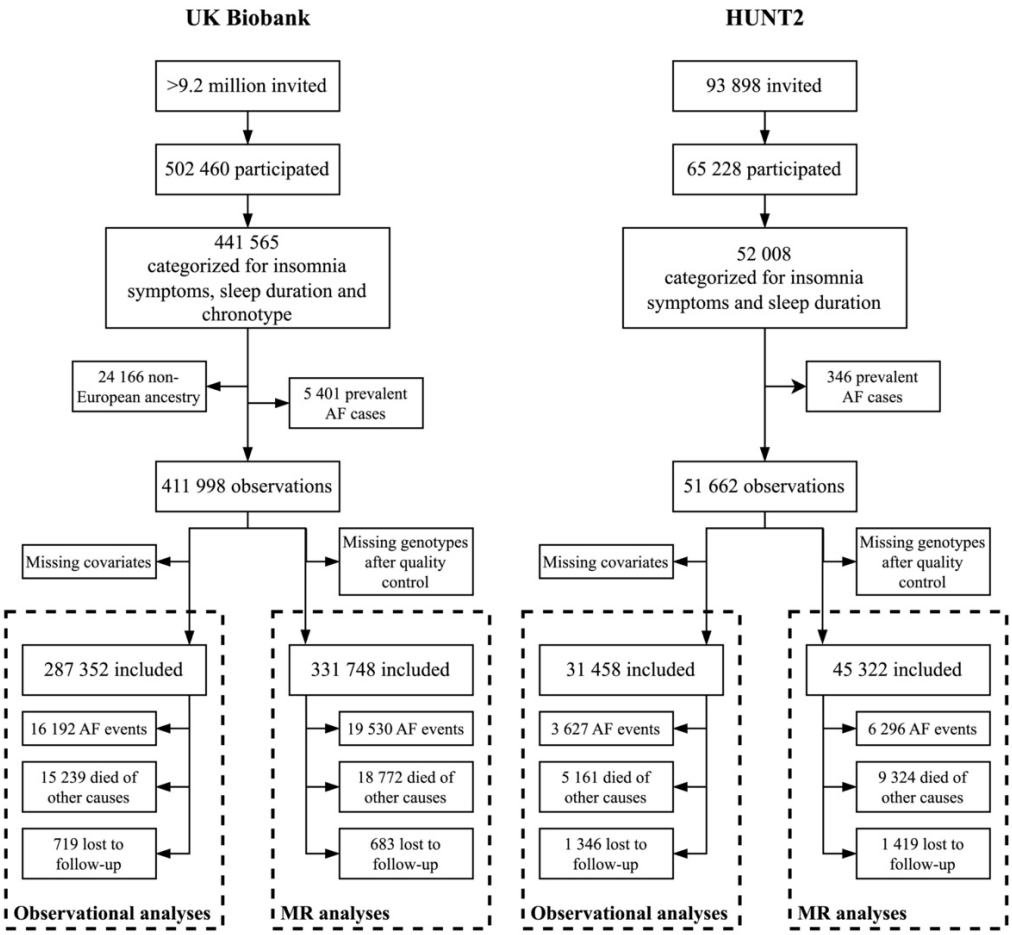
This is an MR study examining the causal effects of sleep traits on the risk of incident AMI. There were 441 565 participants who were categorized for sleep traits of interest in UK Biobank. After excluding participants who had missing genotypes following quality control and those with a prior episode(s) of AMI, a total 332 676 participants were included in the analyses. Overall, there were 52 008 participants who were categorized for sleep

traits of interest in HUNT2. After excluding participants who had missing genotypes after quality control and those with a prior episode(s) of AMI, a total of 44 728 participants were included in the analyses. The selection process of study participants is shown in Figure 5.

3.3.3 Paper III

This is a prospective cohort and MR study investigating the causal influence of sleep traits on the risk of incident AF. All participants included were of European ancestry, which enabled the comparison of observational and MR estimates within the same underlying population. There were 441 565 participants who were categorized for sleep traits of interest in UK Biobank. After excluding participants with non-European ancestry and those with a prior diagnosis of AF, a total of 287 352 participants were included in the observational analyses following the exclusion of participants with missing covariate(s) information, and a total of 331 748 participants were included in the MR analyses following the exclusion of participants with missing genotypes on quality control. Overall, there were 52 008 participants who were categorized for sleep traits of interest in HUNT2. After excluding participants with a previous diagnosis of AF, a total of 31 458 participants were included in the observational analyses following the exclusion of participants with missing covariate(s) information, and a total of 45 322 participants were included in the MR analyses following the exclusion of participants with missing genotypes on quality control. The selection of study participants is summarized in Figure 6.

Figure 6: Selection of study participants – Paper III.



3.4 Statistical analyses

All statistical analyses were conducted using R versions 4.1.1 for macOS (Paper I) and 3.6.3 for Linux (Papers II and III) (R Foundation for Statistical Computing, Vienna, Austria), where the UK Biobank and HUNT2 cohorts were analyzed separately.

3.4.1 Observational analyses

We investigated the prospective associations of self-reported sleep traits and the subsequent risk of incident AMI (Paper I) and AF (Paper III) to estimate (1) the associations of individual sleep traits on the risk of incident AMI/AF, and (2) the joint associations of any two sleep traits (i.e., insomnia symptoms and short sleep duration; insomnia symptoms and long sleep duration; insomnia symptoms and chronotype; short sleep duration and chronotype; long sleep duration and chronotype) on the risk of incident AMI/AF.

To investigate the association of each individual sleep traits on the risk of incident AMI/AF, we used Cox proportional hazards models. We calculated HRs with 95% CIs using different models adjusting for potential confounding factors. The crude model (Model 1) was adjusted for age at recruitment and gender only. The main model (Model 2) was adjusted for age at recruitment, gender, marital status, alcohol intake frequency, smoking status, BMI, physical activity, education, TDI (for UK Biobank only), shift work, and employment status. Additionally, systolic BP, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression and anxiety were adjusted in an additional model (Model 3).

To assess the joint association of sleep traits, we generated subgroups for each combination of these sleep traits. HRs with 95% CIs for each subgroup were then calculated using the Cox proportional hazards model adjusted for potential confounding factors using different models, as previously described. For instance, for the joint association of insomnia symptoms and short sleep duration, subgroups include no insomnia symptoms with normal sleep duration (reference group), insomnia symptoms with normal sleep duration (HR_A), no insomnia symptoms with short sleep duration (HR_B), and insomnia symptoms with short sleep duration (HR_{AB}). The joint associations of two sleep traits together on the subsequent risk of incident AMI/AF were then assessed for relative excess risk due to interaction (RERI) with 95% CIs [206, 207]. The RERI was calculated on the additive scale using the formula: $RERI = HR_{AB} – HR_A – HR_B + 1$, when none of the HRs were less than 1 (i.e., preventive) [208]. The additive scale is used to examine biological interaction between multiple risk factors that collectively assert their influence on disease risk [209, 210]. In brief, RERI >0 and the lower limit of 95% CI >0 suggest a synergistic effect of two sleep traits together on incident AMI/AF, i.e., their joint effect on incident AMI/AF is even greater than the sum of their individual effects.

3.4.2 Mendelian randomization analyses

We used one-sample MR and factorial MR to examine the individual and joint causal effects of sleep traits, respectively, on the risk of incident AMI (Paper II) and AF (Paper III).

Genetic risk scores

To overcome the weak effects of most SNPs on their corresponding sleep traits, GRSs were created as instruments for each sleep trait [173]. Weighted GRS (wGRS) was computed by summing the participants' sleep trait-increasing alleles (morning preference alleles for chronotype), weighted by the variant effect sizes from the external GWAS. We used wGRS for the main analysis in HUNT2 only, whereas in UK Biobank, we used unweighted GRS (uwGRS) computed by simply summing the sleep trait-increasing alleles. Since all

included discovery GWASs used the UK Biobank cohort, the use of internal weights to calculate wGRS is not recommended [173]. Instrument strength was assessed by the regression of each sleep trait on their respective GRS, reporting the variance explained (R^2) and F-statistics.

One-sample MR analysis

We performed a one-sample MR analysis to examine the causal effects of individual sleep traits on the risk of incident AMI/AF. A two-stage predictor substitution (TSPS) regression estimator method was used to calculate average causal HRs with 95% CIs. The first stage involved the regression of each sleep trait (linear regression for 24-hour sleep duration and logistic regression for other sleep traits) on their GRS. The second stage consisted of performing a Cox regression of AMI/AF status on the fitted values from the first-stage regression, with adjustment for age at recruitment, gender, assessment center (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2) and genotyping chip in both stages. As recommended for MR analysis with a binary outcome [211], the first-stage regression was limited to participants who did not experience the outcome of interest. To obtain corrected standard errors, a bootstrapping method was applied with 2000 iterations in UK Biobank and 5000 iterations in HUNT2 (only for our analyses on AMI in Paper II) [211]. The causal estimates for binary exposures (insomnia symptoms, short sleep duration, long sleep duration, and chronotype) were scaled to represent the risk increase in incident AMI/AF per doubling in the odds of these exposures by multiplying the obtained β values by 0.693, as previously explained [212]. The causal estimate for 24-hour sleep duration indicates the risk increase in incident AMI/AF per additional hour of sleep.

Factorial MR analysis

Furthermore, we performed a 2x2 factorial MR analysis to examine the joint causal effects of sleep traits on the risk of incident AMI/AF. Participants were dichotomized across their median GRS (uwGRS for UK Biobank and wGRS for HUNT2) for each sleep trait, with a group equal to or below the median representing low genetic risk for the sleep trait, and a group above the median representing high genetic risk for the sleep trait. Thus, for any combination of two sleep traits, participants were categorized into four subgroups according to their genetic predisposition. We then used Cox regression to estimate the causal effect across these participant subgroups with adjustment for age at recruitment, gender, assessment center (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2), and genotyping chip. For instance, when combining insomnia symptoms and short sleep duration, participants were categorized into the following groups: *'Both GRS ≤ median'* (reference group; representing low genetic risks for both insomnia symptoms and short sleep duration), *'Insomnia GRS > median'* (HR_A;

representing high genetic risk for insomnia symptoms only), *'Short sleep GRS > median*' (HR_B; representing high genetic risk for short sleep duration only), and *'Both GRS > median'* (HR_{AB}; representing high genetic risks for both insomnia symptoms and short sleep duration). RERI was then assessed for the joint causal effect on the additive scale using the formula: $RERI = HR_{AB} – HR_A – HR_B + 1$, when none of the HRs were less than 1 (i.e., preventive) [208].

3.4.3 Additional and sensitivity analyses

We tested the proportionality of hazards using log-log curves (in Paper I) and the Schoenfeld residuals test (in Papers I, II and III). The covariates in the models that showed evidence against proportionality ($p < 0.10$) were stratified.

In Paper I, we conducted several stratified analyses to assess whether the individual or joint associations of sleep traits with incident AMI could be modified by other factors. Thus, we investigated the potential effect modification by age (above and below 65 years), gender, shift work (Yes/No), depression (Yes/No in UK Biobank; HADS – Depression score above or below 8 in HUNT2), and anxiety (Yes/No in UK Biobank; HADS – Anxiety score above or below 8 in HUNT2). We also performed formal tests for interaction on these variables.

Several sensitivity analyses were performed to assess the robustness of our findings, as described below:
In Paper I:

a) We repeated the original analyses after excluding the first 2 years of follow-up to mitigate the potential influence of reverse causation as an explanation for the observed associations.
b) We additionally adjusted for any self-reported chronic disorder(s) at baseline in our models since sleep disturbances may co-exist with certain illnesses and chronic pain [213].
c) We repeated the original analyses restricting the UK Biobank to only the White British subset.
d) We repeated the original analyses in HUNT2, restricting the end of the follow-up period to December 31, 2008 (i.e., approximating a similar duration of follow-up as in UK Biobank) to facilitate better comparisons.

In Papers II and III:

a) We repeated the one-sample MR and 2x2 factorial MR analyses using uwGRS in HUNT2.
b) We investigated the associations between GRS and potential confounders of the exposure-outcome relationship to assess the second assumption of MR. Furthermore, we repeated the one-sample MR analyses adjusted for any potential

confounders found to be strongly associated with the sleep trait GRS after correcting for multiple comparisons (i.e., Bonferroni correction).

c) We obtained estimates of the SNP-exposure and SNP-outcome associations from the same individuals and applied two-sample MR methods, such as MR-Egger, weighted median, and weighted mode-based methods, to investigate potential directional pleiotropy (elaborated in the Discussion chapter). These methods can be applied in a one-sample setting [214, 215]. Each of these methods makes different assumptions about the genetic instruments used, where the MR-Egger regression method gives a valid causal estimate under the instrument strength independent of direct effect (InSIDE) assumption and its intercept allows the size of any unbalanced pleiotropic effect to be determined [216], the weighted median method assumes that at least 50% of genetic variants are valid [217], and the weighted mode-based estimation method assumes that a plurality of genetic variants are valid [218]. Thus, consistent estimates across these methods strengthen causal evidence. To further investigate pleiotropy due to insomnia symptoms' instruments, 57 SNPs found to be robustly associated with insomnia by Lane *et al*. [219] in a different GWAS on UK Biobank (n = 345 022 cases and 108 357 controls) representing crucial variants with effect sizes for any insomnia symptoms ('Sometimes'/'Usually' as cases vs. 'Never/rarely' as controls) were used in a post hoc one-sample MR Cox regression analysis using different methods.

d) We repeated the one-sample MR analysis using genetic variants that replicated at a genome-wide significance level in a large independent dataset for insomnia symptoms (23andMe, n = 944 477) [191] and chronotype (23andMe, n = 240 098) [193] to evaluate the impact due to winner's curse.

e) We repeated the factorial MR analysis using two continuous GRSs (for any combinations of two sleep traits) as quantitative traits and their product term, to avoid potential bias due to arbitrary dichotomization and to maximize power [177]. RERI was then assessed for the joint causal effect on the additive scale using the formula: $\text{RERI} = \exp(\beta_1 + \beta_2 + \beta_{(\text{product term})}) - \exp(\beta_1) - \exp(\beta_2) + 1$ [220].

The stratified and sensitivity analyses performed in the observational study on AMI (in Paper I) were not conducted for the observational analyses on AF (in Paper III), where MR was considered the main approach for drawing causal inferences from the investigation of sleep traits on the risk of incident AF. In Paper III, the observational analyses were only performed to compare the observational findings with the MR findings. We assessed the robustness of our MR findings from Paper III using several sensitivity analyses, as previously mentioned.

3.5 Ethics

All study participants have given their informed consent. UK Biobank received ethics approval from the NHS National Research Ethics Service on June 17, 2011 (reference number 11/NW/0382), which was extended on May 10, 2016 (reference number 16/NW/0274). This analysis of UK Biobank was conducted under application number 40135. The HUNT Study was approved by the Data Inspectorate of Norway and recommended by the Regional Committee for Ethics in Medical Research (REK; reference number 152/95/AH/JGE). Ethical approval for conducting this study was also obtained from the Regional Committee for Ethics in Medical Research in Northern Norway (REK nord; reference number 2020/47206).

4 Results

4.1 Paper I: Main findings

We prospectively investigated the individual and joint associations of sleep traits on the subsequent risk of incident AMI in two large population-based cohorts — UK Biobank and HUNT2. Among 302 456 UK Biobank participants without prior episode(s) of AMI, a total of 6 833 were diagnosed with AMI during a mean of 11.7 years of follow-up. Among 31 091 HUNT2 participants without prior episode(s) of AMI, a total of 2 540 were diagnosed with AMI during a mean of 21.0 years of follow-up.

Individual sleep traits and the risk of incident AMI *(Observational analysis)*

Based on our main model adjusted for potential confounders (Model 2), the participants who reported insomnia symptoms had HRs of 1.11 (95% CI 1.05, 1.16) and 1.09 (95% CI 0.98, 1.21) for incident AMI in UK Biobank and HUNT2, respectively, when compared to those without insomnia symptoms (Table 3). When compared to participants who reported normal sleep duration (7–8 h), the HRs for incident AMI in UK Biobank were 1.09 (95% CI 1.04, 1.16) and 1.14 (95% CI 1.05, 1.24) for those who reported short (≤6 h) and long (≥9 h) sleep duration, respectively. The corresponding HRs in HUNT2 were similar for those who reported short sleep duration (HR 1.05; 95% CI 0.89, 1.24), but not for those who reported long sleep duration (HR 0.97; 95% CI 0.88, 1.06). Compared to morning chronotypes, the HR for incident AMI was 1.08 (95% CI 1.03, 1.13) for evening chronotypes in UK Biobank.

The estimated associations remained fairly unchanged in Model 3 (Table 3).

Combination of sleep traits and the risk of incident AMI *(Observational analysis)*

Compared to participants who reported normal sleep duration without insomnia symptoms, the adjusted multivariable HR for incident AMI in UK Biobank was 1.07 (95% CI 0.99, 1.15) for those who reported normal sleep duration with insomnia symptoms, whereas the HR increased to 1.16 (95% CI 1.07, 1.25) for those who reported short sleep duration with insomnia symptoms and 1.40 (95% CI 1.21, 1.63) for those who reported long sleep duration with insomnia symptoms (Table 4). The corresponding HRs in HUNT2 were similar for those who reported normal sleep duration with insomnia symptoms (HR 1.09; 95% CI 0.95, 1.25) and those who reported short sleep duration with insomnia symptoms (HR 1.17; 95% CI 0.87, 1.58), but not for those who reported long sleep duration with insomnia symptoms (HR 1.02; 95% CI 0.85, 1.23). In UK Biobank, we found statistical evidence for biological interaction beyond additivity for long sleep duration with insomnia symptoms (RERI 0.25; 95% CI 0.01, 0.48), but no such evidence for short sleep duration

Table 3: Hazard ratios (95% confidence intervals) for incident acute myocardial infarction (AMI) according to self-reported insomnia symptoms, sleep duration, and chronotype in UK Biobank and HUNT2.

	Insomnia symptoms		Sleep duration			Chronotype	
	No	**Yes**	**Short**	**Normal**	**Long**	**Morning**	**Evening**
UK Biobank (n = 302 456)							
AMI events/ Person-years	4 784/ 2 583 503	2 049/ 964 868	1 794/ 844 021	4 365/ 2 445 890	674/ 258 460	4 206/ 2 229 937	2 627/ 1 318 434
Model 1	Reference	1.19 (1.13, 1.25)	1.20 (1.14, 1.27)	Reference	1.29 (1.19, 1.40)	Reference	1.14 (1.08, 1.19)
Model 2	Reference	1.11 (1.05, 1.16)	1.09 (1.04, 1.16)	Reference	1.14 (1.05, 1.24)	Reference	1.08 (1.03, 1.13)
Model 3	Reference	1.08 (1.03, 1.14)	1.09 (1.03, 1.15)	Reference	1.10 (1.01, 1.19)	Reference	1.06 (1.01, 1.12)
HUNT2 (n = 31 091)							
AMI events/ Person-years	2 120/ 577 219	420/ 76 391	151/ 41 306	1 668/ 470 344	721/ 141 960	-	-
Model 1	Reference	1.17 (1.06, 1.30)	1.15 (0.97, 1.35)	Reference	1.05 (0.96, 1.15)	-	-
Model 2	Reference	1.09 (0.98, 1.21)	1.05 (0.89, 1.24)	Reference	0.97 (0.88, 1.06)	-	-
Model 3	Reference	1.08 (0.96, 1.21)	1.09 (0.93, 1.29)	Reference	0.94 (0.85, 1.03)	-	-

Model 1, adjusted for age and gender.
Model 2, adjusted for the covariates in Model 1 along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index (for UK Biobank), ethnicity (for UK Biobank), shift work, and employment status.
Model 3, adjusted for the covariates in Model 2 along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table 4: Hazard ratios (95% confidence intervals) for incident acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and sleep duration in UK Biobank and HUNT2.

	No insomnia symptoms			**Insomnia symptoms**		
	Sleep duration			**Sleep duration**		
	Short	**Normal**	**Long**	**Short**	**Normal**	**Long**
UK Biobank (n = 302 456)						
AMI events/ Person-years	900/ 427 642	3 395/ 1 951 096	489/ 204 765	894/ 416 379	970/ 494 794	185/ 53 695
Model 1	1.16 (1.08, 1.25)	Reference	1.22 (1.11, 1.34)	1.32 (1.23, 1.42)	1.12 (1.05, 1.21)	1.72 (1.49, 2.00)
Model 2	1.07 (0.99, 1.15)	Reference	1.09 (0.99, 1.20)	1.16 (1.07, 1.25)	1.07 (0.99, 1.15)	1.40 (1.21, 1.63)
Model 3	1.08 (1.00, 1.16)	Reference	1.05 (0.96, 1.16)	1.13 (1.04, 1.21)	1.05 (0.98, 1.13)	1.32 (1.14, 1.54)
HUNT2 (n = 31 091)						
AMI events/ Person-years	106/ 32 967	1 420/ 420 985	594/ 123 267	45/ 8 339	248/ 49 360	127/ 18 692
Model 1	1.10 (0.90, 1.34)	Reference	1.06 (0.96, 1.17)	1.38 (1.03, 1.86)	1.17 (1.03, 1.34)	1.19 (0.99, 1.43)
Model 2	1.02 (0.84, 1.24)	Reference	0.97 (0.88, 1.07)	1.17 (0.87, 1.58)	1.09 (0.95, 1.25)	1.02 (0.85, 1.23)
Model 3	1.08 (0.89, 1.32)	Reference	0.94 (0.85, 1.04)	1.18 (0.87, 1.60)	1.08 (0.94, 1.25)	0.97 (0.80, 1.19)

Model 1, adjusted for age and gender.
Model 2, adjusted for the covariates in Model 1 along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index (for UK Biobank), ethnicity (for UK Biobank), shift work, and employment status.
Model 3, adjusted for the covariates in Model 2 along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

with insomnia symptoms (RERI 0.02; 95% CI -0.11, 0.15). In HUNT2, we did not find evidence of interaction beyond additivity for short sleep duration (RERI 0.06; 95% CI -0.36, 0.48) or long sleep duration (RERI -0.04; 95% CI -0.28, 0.20) with insomnia symptoms.

Compared to morning chronotypes without insomnia symptoms, the HRs for incident AMI in UK Biobank were 1.08 (95% CI 1.02, 1.15) for evening chronotype without insomnia symptoms and 1.11 (95% CI 1.04, 1.18) for morning chronotype with insomnia symptoms, whereas the HR increased to 1.19 (95% CI 1.10, 1.29) for evening chronotype with insomnia symptoms (Table 5). There was no evidence of interaction beyond additivity for evening chronotype with insomnia symptoms (RERI -0.01; 95% CI -0.12, 0.12).

Table 5: Hazard ratios (95% confidence intervals) for incident acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and chronotype in UK Biobank.

	No insomnia symptoms		**Insomnia symptoms**	
	Chronotype		**Chronotype**	
	Morning	**Evening**	**Morning**	**Evening**
UK Biobank (n = 302 456)				
AMI events/ Person-years	2 953/ 1 625 404	1 831/ 958 099	1 253/ 604 533	796/ 360 335
Model 1	Reference	1.12 (1.06, 1.19)	1.17 (1.10, 1.25)	1.36 (1.26, 1.47)
Model 2	Reference	1.08 (1.02, 1.15)	1.11 (1.04, 1.18)	1.19 (1.10, 1.29)
Model 3	Reference	1.07 (1.01, 1.14)	1.09 (1.02, 1.17)	1.14 (1.06, 1.24)

Model 1, adjusted for age and gender.
Model 2, adjusted for the covariates in Model 1 along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index, ethnicity, shift work, and employment status.
Model 3, adjusted for the covariates in Model 2 along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

When compared to participants who reported normal sleep duration with morning chronotype, the HR for incident AMI in UK Biobank was 1.08 (95% CI 1.02, 1.15) for those who reported normal sleep duration with evening chronotype, whereas the HR increased to 1.18 (95% CI 1.08, 1.29) for those who reported short sleep duration with evening chronotype and 1.21 (95% CI 1.07, 1.37) for those who reported long sleep duration with evening chronotype (Table 6). There was no evidence of interaction beyond additivity for short sleep duration (RERI -0.01; 95% CI -0.14, 0.12) or long sleep duration (RERI -0.02; 95% CI -0.21, 0.18) with evening chronotype.

The estimated associations remained fairly unchanged in Model 3 (Table 4, Table 5, and Table 6).

Table 6: Hazard ratios (95% confidence intervals) for incident acute myocardial infarction (AMI) according to the joint association of self-reported chronotype and sleep duration in UK Biobank.

	Morning chronotype			**Evening chronotype**		
	Sleep duration			**Sleep duration**		
	Short	**Normal**	**Long**	**Short**	**Normal**	**Long**
UK Biobank (n = 302 456)						
AMI events/ Person-years	1 141/ 539 398	2 689/ 1 540 774	376/ 149 766	653/ 304 623	1 676/ 905 117	298/ 108 694
Model 1	1.21 (1.12, 1.29)	Reference	1.28 (1.15, 1.42)	1.37 (1.26, 1.49)	1.13 (1.06, 1.20)	1.46 (1.29, 1.64)
Model 2	1.10 (1.03, 1.18)	Reference	1.14 (1.03, 1.27)	1.18 (1.08, 1.29)	1.08 (1.02, 1.15)	1.21 (1.07, 1.37)
Model 3	1.09 (1.02, 1.17)	Reference	1.12 (1.00, 1.24)	1.16 (1.06, 1.27)	1.07 (1.01, 1.14)	1.15 (1.01, 1.29)

Model 1, adjusted for age and gender.
Model 2, adjusted for the covariates in Model 1 along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index, ethnicity, shift work, and employment status.
Model 3, adjusted for the covariates in Model 2 along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Additional and sensitivity analyses

In our additional analyses, we found no strong statistical evidence of interaction by age for any individual sleep traits (Paper I: Supplementary Table S3). However, for the combination of insomnia symptoms and chronotype, we found that young or middle-aged adults (<65 years) who were evening chronotypes without insomnia symptoms or morning chronotypes with insomnia symptoms had an increased risk of incident AMI when compared to morning chronotypes without insomnia symptoms. We did not find the similar increased risk of incident AMI in these phenotypes among the older participants (≥65 years). Additionally, we found no statistical evidence of interaction by gender, shift work, depression, or anxiety (Paper I: Supplementary Tables S4–S7).

After excluding the first 2 years of follow-up, a total of 6 089 and 2 390 participants were diagnosed with AMI within UK Biobank and HUNT2, respectively, and the estimated associations remained fairly unchanged but were less precise (Paper I: Supplementary Tables S8–S11).

Adjustment for chronic disorder(s) in our models did not change the associations in comparison to the findings of our main analysis (Paper I: Supplementary Tables S12–S15).

We obtained similar results when restricting the UK Biobank data to the White British subset (Paper I: Supplementary Tables S16–S19).

A total of 1 144 participants were diagnosed with AMI in HUNT2 until December 31, 2008 (i.e., mean follow-up of 11.6 years). When restricted to a shorter follow-up period, the estimated associations remained fairly unchanged but were less precise in comparison to the original follow-up period in HUNT2 (Paper I: Supplementary Tables S20–S21).

4.2 Paper II: Main findings

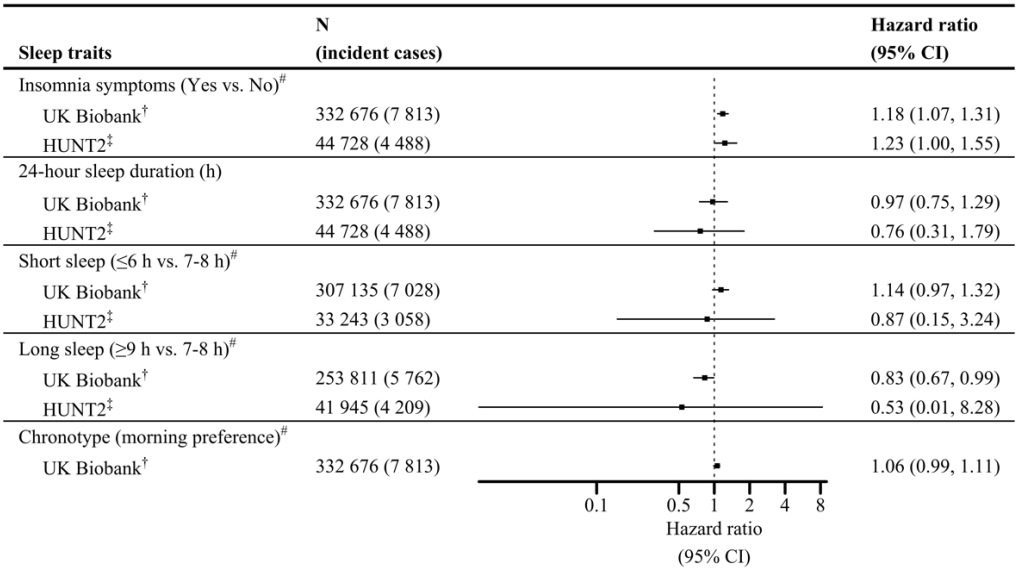
We used MR to examine the individual and joint causal effects of sleep traits on the subsequent risk of incident AMI in the UK Biobank and HUNT2. Among 332 676 UK Biobank participants without prior episode(s) of AMI, a total of 7 813 were diagnosed with AMI during a mean of 11.7 years of follow-up. Among 44 728 HUNT2 participants without prior episode(s) of AMI, a total of 4 488 were diagnosed with AMI during a mean 20.4 years of follow-up.

Individual sleep traits and the risk of incident AMI *(One-sample MR analysis)*

For UK Biobank, the variance explained (R^2) by the uwGRS in insomnia symptoms, 24-hour sleep duration (h), short sleep duration (≤6 vs. 7–8 h), long sleep duration (≥9 vs. 7–8 h), and morning chronotype were 0.41, 0.59, 0.18, 0.11, and 1.54%, respectively, and the

corresponding F-statistics were 1370.92, 1962.0, 558.68, 285.42, and 5202.20, respectively. For HUNT2, the variance explained (R^2) by the wGRS in insomnia symptoms, 24-hour sleep duration, short sleep duration, and long sleep duration were 0.16, 0.09, 0.01, and 0.01%, respectively, and the corresponding F-statistics were 71.17, 38.94, 4.97, and 4.07, respectively.

Figure 7: One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction in relation to individual sleep traits in UK Biobank and HUNT2.



CI, confidence interval.
† Derived using the unweighted genetic risk score for each sleep trait, with adjustment for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
‡ Derived using weighted genetic risk score for each sleep trait, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.
# Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait. Chronotype was missing in HUNT2.

There was evidence of an adverse causal effect on incident AMI risk per doubling in odds of insomnia symptoms in UK Biobank (HR 1.18; 95% CI 1.07, 1.31) and HUNT2 (HR 1.23; 95% CI 1.00, 1.55) (Figure 7). The estimates for 24-hour sleep duration suggested no causal effect on incident AMI per hour increase in sleep duration in UK Biobank (HR 0.97; 95% CI 0.75, 1.29) and HUNT2 (HR 0.76; 95% CI 0.31, 1.79). The sleep duration findings were further investigated using genetic variants specifically associated with short and long sleep durations. There was weak evidence of an adverse causal effect on incident AMI per doubling in odds of short sleep duration in UK Biobank (HR 1.14; 95% CI 0.97, 1.32) but not in HUNT2 (HR 0.87; 95% CI 0.15, 3.24). However, there was evidence of a protective causal effect on incident AMI per doubling in odds of long sleep duration in UK Biobank (HR 0.83; 95% CI 0.67, 0.99), which was underpowered in HUNT2 (HR 0.53; 95% CI 0.01, 8.28). Also, there was some evidence of an adverse causal effect on incident AMI per

doubling in odds of morning chronotype in UK Biobank (HR 1.06; 95% CI 0.99, 1.11) (Figure 7).

Combination of sleep traits and the risk of incident AMI *(2x2 factorial MR analysis)*

In UK Biobank, participants with high genetic risk for insomnia symptoms and high genetic risk for short sleep duration had a slightly higher risk of incident AMI (HR 1.03; 95% CI 0.96, 1.10 and HR 1.05; 95% CI 0.98, 1.12, respectively), whereas participants with high genetic risks for both traits had the highest risk (HR 1.10; 95% CI 1.03, 1.12) (Figure 8), but there was no evidence of interaction (RERI 0.03; 95% CI -0.07, 0.12). This pattern was however not consistent in HUNT2, with imprecise estimates and a lack of evidence of interaction (RERI -0.05; 95% CI -0.20, 0.09). The joint effects of insomnia symptoms and long sleep duration on the risk of incident AMI were inconclusive in both UK Biobank and HUNT2 (Figure 8).

Additionally, UK Biobank participants with high genetic risk for insomnia symptoms and high genetic risk for morning chronotype had a slightly higher risk of incident AMI (HR 1.03; 95% CI 0.97, 1.10 and HR 1.03; 95% CI 0.97, 1.10, respectively), whereas participants with high genetic risks for both sleep traits had the highest risk (HR 1.09; 95% CI 1.03, 1.17) (Figure 8). Notably, there was no evidence of interaction (RERI 0.03; 95% CI -0.06, 0.12). Similarly, the UK Biobank participants with high genetic risk for short sleep duration and high genetic risk for morning chronotype had a slightly higher risk of incident AMI (HR 1.04; 95% CI 0.98, 1.12 and HR 1.02; 95% CI 0.96, 1.10, respectively), whereas participants with high genetic risks for both had the highest risk (HR 1.11; 95% CI 1.04, 1.19), with no strong statistical evidence of interaction (RERI 0.05; 95% CI -0.05, 0.14). The joint effects of long sleep duration and morning chronotype were imprecise and inconclusive (Figure 8).

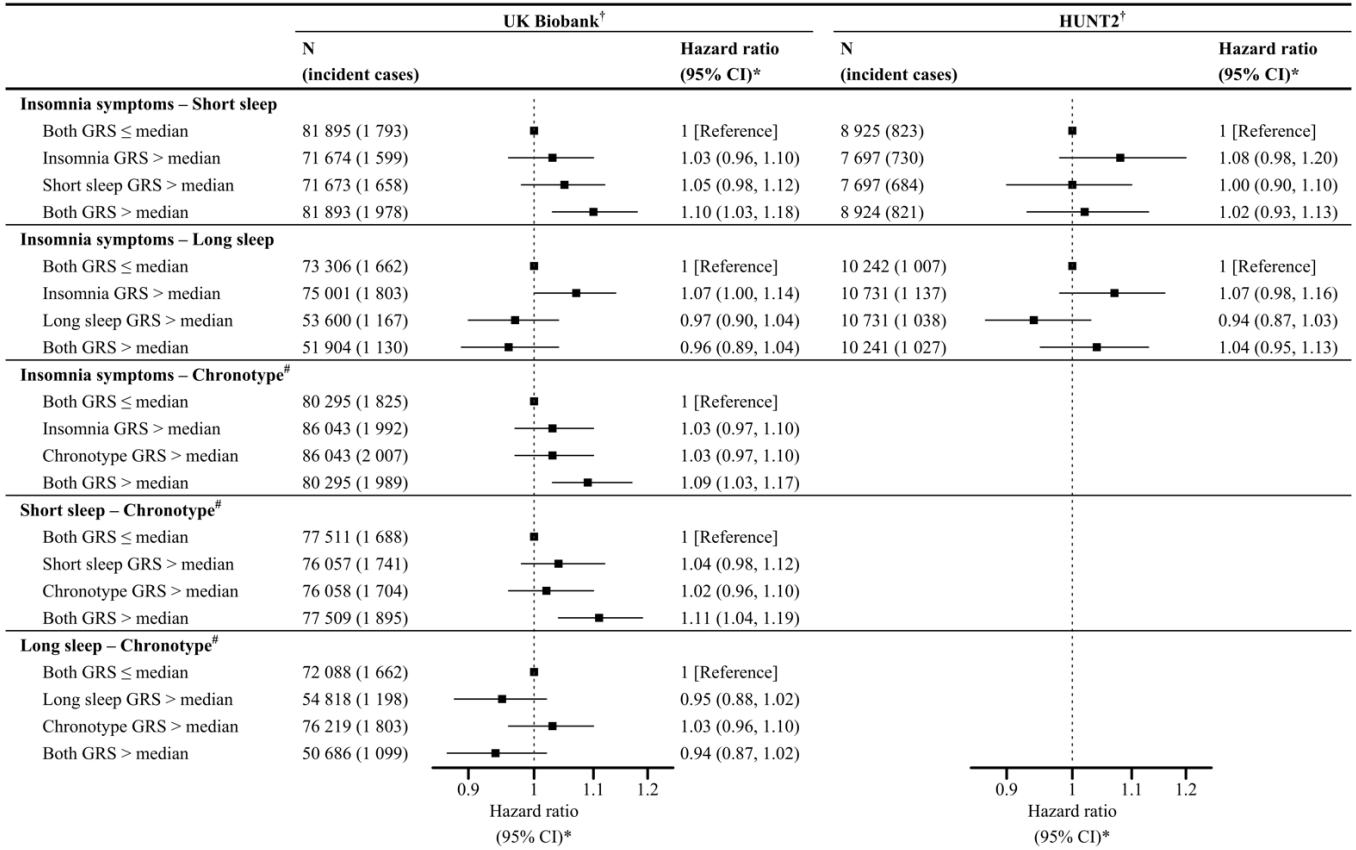
Sensitivity analyses

The one-sample MR and 2x2 factorial MR estimates in HUNT2 using the uwGRS for the sleep traits remained unchanged (Paper II: Supplementary Table S12 and Figure S2).

Several confounding factors were associated with sleep trait uwGRS in UK Biobank, and a few were associated with the sleep trait wGRS in HUNT2 after correcting for multiple comparisons (Paper II: Supplementary Tables S13 and S14). Furthermore, adjusting for these confounding factors in the one-sample MR analysis did show a slightly weaker adverse causal effect of insomnia symptoms in UK Biobank (HR 1.04; 95% CI 0.92, 1.17) and HUNT2 (HR 1.13; 95% CI 0.87, 1.47) (Paper II: Supplementary Table S15).

The causal estimates obtained using the MR-Egger, the weighted median, and weighted mode-based methods attenuated slightly and were less precise (Paper II: Supplementary

Figure 8: 2x2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of two sleep traits on the risk of incident acute myocardial infarction in UK Biobank and HUNT2.



CI, confidence interval; GRS, genetic risk score.
For each sleep trait combination, both GRS ≤ median represents low genetic risks for both sleep traits in combination, sleep trait 1 GRS > median represents high genetic risk for sleep trait 1 only, sleep trait 2 GRS > median represents high genetic risk for sleep trait 2 only, and both GRS > median represents high genetic risks for both sleep traits.
† Derived using the unweighted genetic risk score for each sleep trait in UK Biobank and using the weighted genetic risk score for each sleep trait in HUNT2.
* Adjusted for age, gender, assessment center (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2), and genotyping chip.
# Chronotype genetic risk score calculated using alleles for morning preference.

Tables S16 and S17, as well as Figures S3–S7). The MR-Egger regression for insomnia symptoms in UK Biobank showed evidence of directional pleiotropy (HR 0.77; 95% CI 0.62, 0.95; and intercept 0.007; 95% CI 0.003, 0.012). Furthermore, the post hoc one-sample MR analysis using insomnia symptom SNPs from Lane *et al*. [219] gave similar estimates (Paper II: Supplementary Table S18 and Figure S8).

The causal estimates were consistent when using GRSs comprising 116 insomnia SNPs (one missing from the HUNT imputed dataset) and 72 chronotype SNPs, which replicated at the genome-wide significance level ($P < 5x10^{-8}$) in the independent 23andMe dataset (Paper II: Supplementary Tables S19 and S20).

The estimates from factorial MR analysis using sleep trait GRS as quantitative traits (per standard deviation increase) and their product term inferred similar effects when compared to estimates from the 2x2 factorial MR (Paper II: Supplementary Figure S9). In UK Biobank, the GRSs for insomnia symptoms and short sleep duration were independently linked to an increased risk of incident AMI (HR 1.03; 95% CI 1.01, 1.06 and HR 1.02; 95% CI 0.99, 1.04, respectively), with no evidence of interaction (RERI 0.02; 95% CI -0.01, 0.04). Similarly, the GRSs for insomnia symptoms and morning chronotype were independently associated with an increased risk of incident AMI (HR 1.04; 95% CI 1.02, 1.06 and HR 1.03; 95% CI 1.00, 1.05, respectively), though there was no evidence of interaction (RERI 0.02; 95% CI -0.01, 0.04). Also, the GRSs for short sleep duration and morning chronotype were both independently linked to an increased risk of incident AMI (HR 1.02; 95% CI 1.00, 1.04 and HR 1.03; 95% CI 1.00, 1.05, respectively), with no evidence of interaction (RERI 0.01; 95% CI -0.02, 0.03).

4.3 Paper III: Main findings

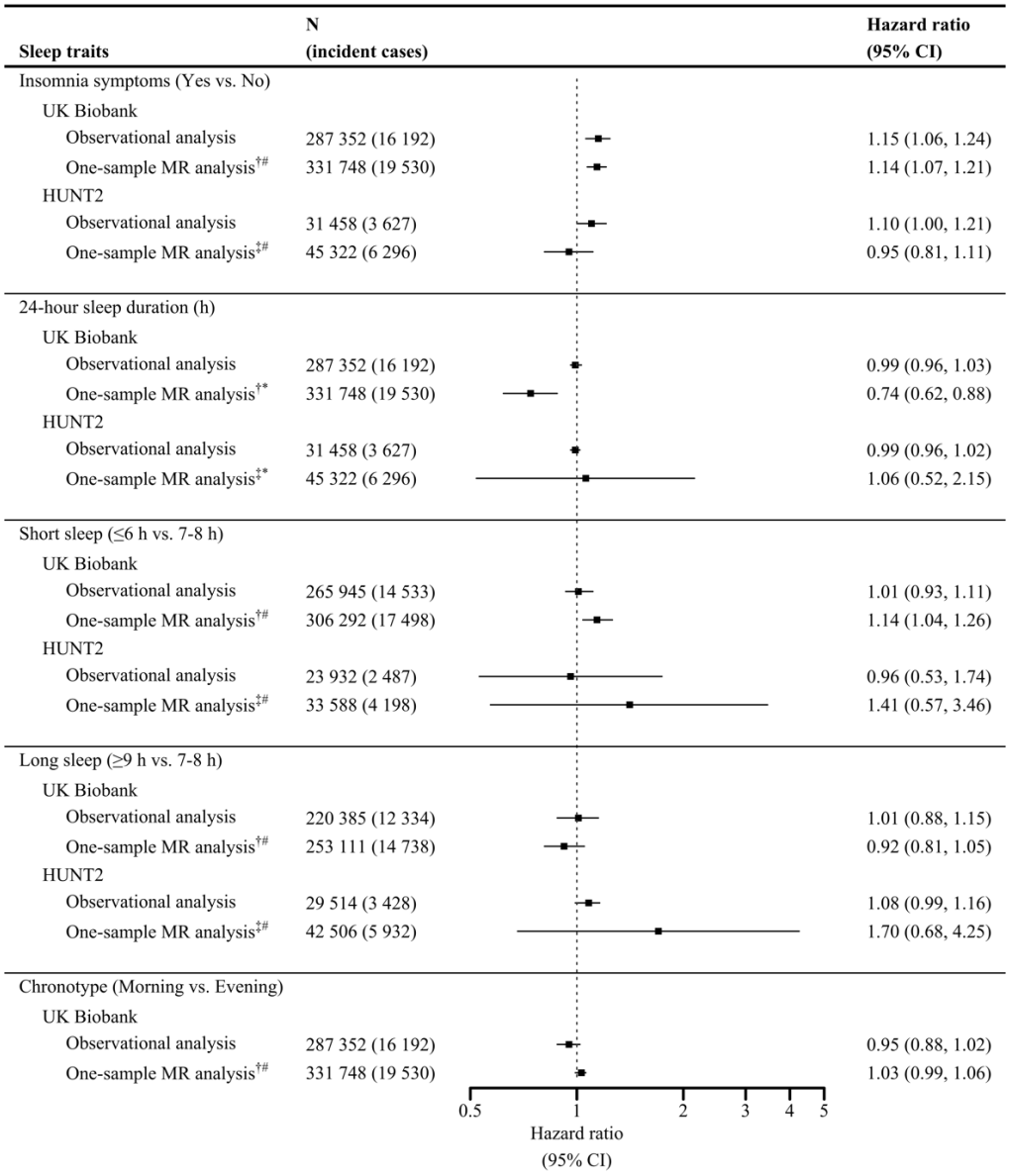
We used observational and MR analyses to investigate the individual and joint causal influence of sleep traits on the subsequent risk of incident AF in UK Biobank and HUNT2. For UK Biobank, among 287 352 participants in the observational analysis and 331 748 in the MR analysis without a prior diagnosis of AF, a total of 16 192 and 19 530, respectively, were diagnosed with AF during an average 11.6 years of follow-up. For HUNT2, among 31 458 participants in the observational analysis and 45 322 in the MR analysis without a prior diagnosis of AF, a total of 3 627 and 6 296, respectively, were diagnosed with AF during an average 20.7 years of follow-up.

Individual sleep traits and the risk of incident AF

Observational analysis

Based on our main model adjusted for potential confounders, insomnia symptoms were associated with an increased risk of incident AF in both UK Biobank and HUNT2, with

Figure 9: Observational and one-sample Mendelian randomization Cox regression analysis for incident atrial fibrillation in relation to individual sleep traits in UK Biobank and HUNT2.



CI, confidence interval; MR, Mendelian randomization.
Observational analysis represents the main model adjusted for age, gender, marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index (for UK Biobank only), shift work, and employment status.
Chronotype was missing in HUNT2.
† Derived using the unweighted genetic risk scores for each sleep trait, with adjustment for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
‡ Derived using the weighted genetic risk scores for each sleep trait, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.
# Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait.
* Hazard ratio (95% CI) scaled to per additional hour of sleep duration.

HRs of 1.15 (95% CI 1.06, 1.24) and 1.10 (95% CI 1.00, 1.21), respectively (Figure 9). No association was found for an additional hour increase in sleep duration on the risk of incident AF in UK Biobank and HUNT2, where the corresponding HRs were 0.99 (95% CI 0.96, 1.03) and 0.99 (95% CI 0.96, 1.02). Moreover, no associations were found for short and long sleep durations on the risk of incident AF in either cohort. The corresponding HRs in UK Biobank were 1.01 (95% CI 0.93, 1.11) and 1.01 (95% CI 0.88, 1.15), respectively; in HUNT2, these were 0.96 (95% CI 0.53, 1.74) and 1.08 (95% CI 0.99, 1.16), respectively. Additionally, no association was found for morning chronotype on the risk of incident AF in UK Biobank, where the HR was 0.95 (95% CI 0.88, 1.02) (Figure 9).

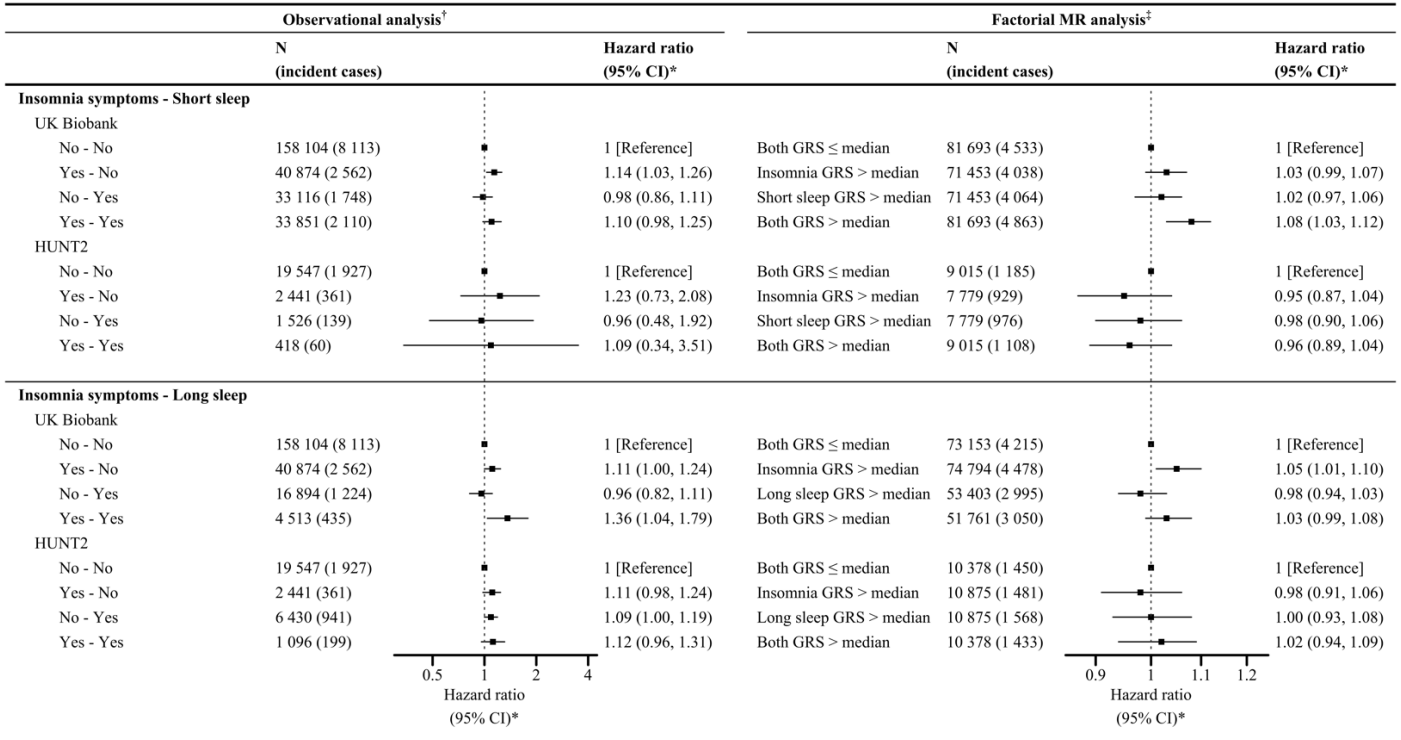
The estimated associations remained fairly unchanged in the additional model (Paper III: Supplementary Tables S3 and S4).

One-sample MR analysis

For UK Biobank, the variance explained (R^2) by the uwGRS in insomnia symptoms, 24-hour sleep duration (h), short sleep duration (≤6 vs. 7–8 h), long sleep duration (≥9 vs. 7–8 h), and morning chronotype were 0.41, 0.60, 0.18, 0.11, and 1.54%, respectively, and the corresponding F-statistics were 1369.47, 1993.0, 558.61, 270.36, and 5176.33, respectively. For HUNT2, the variance explained (R^2) by the wGRS in insomnia symptoms, 24-hour sleep duration, short sleep duration, and long sleep duration were 0.16, 0.09, 0.02, and 0.01%, respectively, and the corresponding F-statistics were 72.38, 42.63, 5.35, and 4.42, respectively.

Similar to the observational analysis, there was evidence suggesting that per doubling in odds of genetically-determined insomnia symptoms increased the incidence of AF in UK Biobank (HR 1.14; 95% CI 1.07, 1.21); however, this was not the case for HUNT2 (HR 0.95; 95% CI 0.81, 1.11) (Figure 9). Unlike the observational analysis, each hour increase in genetically-determined sleep duration decreased the incidence of AF in UK Biobank (HR 0.74; 95% CI 0.62, 0.88) but not in HUNT2 (HR 1.06; 95% CI 0.52, 2.15). Furthermore, there was evidence suggesting that per doubling in odds of genetically-determined short sleep duration increased the incidence of AF in UK Biobank (HR 1.14; 95% CI 1.04, 1.26); however, estimates were underpowered in HUNT2 (HR 1.41; 95% CI 0.57, 3.46). Similar to the observational analysis, genetically-determined long sleep duration was not associated with the incidence of AF in UK Biobank (HR 0.92; 95% CI 0.81, 1.05) and HUNT2 (HR 1.70; 95% CI 0.68, 4.25). Unlike the observational analysis, there was weak evidence suggesting that per doubling in odds of genetically-determined morning preference chronotype increased the incidence of AF in UK Biobank (HR 1.03; 95% CI 0.99, 1.06) (Figure 9).

Figure 10: Observational and 2x2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of insomnia symptoms and sleep duration on the risk of incident atrial fibrillation in UK Biobank and HUNT2.



CI, confidence interval; MR, Mendelian randomization; GRS, genetic risk score.

In the observational analysis for each sleep trait combination, 'No – No' represents the absence of both sleep traits in combination, 'Yes – No' represents the presence of the first sleep trait and absence of the second, 'No – Yes' represents the absence of first sleep trait and presence of second, and 'Yes – Yes' represents the presence of both sleep traits.

In the factorial MR analysis for each sleep trait combination, both GRS ≤ median represents low genetic risks for both sleep traits in combination, sleep trait 1 GRS > median represents high genetic risk for sleep trait 1 only, sleep trait 2 GRS > median represents high genetic risk for sleep trait 2 only, and both GRS > median represents high genetic risks for both sleep traits.

† Estimates from the main model adjusted for age, gender, marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index (for UK Biobank only), shift work, and employment status.

‡ Estimates adjusted for age, gender, assessment center (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2), and genotyping chip.

* Derived using the unweighted genetic risk score for each sleep trait in UK Biobank and using the weighted genetic risk score for each sleep trait in HUNT2.

Combination of sleep traits and the risk of incident AF

Observational analysis

Based on the main model, UK Biobank participants who reported short sleep duration without insomnia symptoms had little evidence of a decreased risk of incident AF (HR 0.98; 95% CI 0.86, 1.11), while those who reported short sleep duration with insomnia symptoms had weak evidence of an increased risk (HR 1.10; 95% CI 0.98, 1.25) and those who reported normal sleep duration with insomnia symptoms had strong evidence of an increased risk of incident AF (HR 1.14; 95% CI 1.03, 1.26) when compared to those who reported normal sleep duration without insomnia symptoms (Figure 10). There was a similar pattern in HUNT2; however, the corresponding HRs were imprecise.

The UK Biobank participants who reported long sleep duration without insomnia symptoms had little evidence of a decreased risk of incident AF (HR 0.96; 95% CI 0.82, 1.11), while those who reported long sleep duration with insomnia symptoms had strong evidence of an increased risk (HR 1.36; 95% CI 1.04, 1.79) and those who reported normal sleep duration with insomnia symptoms had slightly weaker evidence of an increased risk of incident AF (HR 1.11; 95% CI 1.00, 1.24) when compared to those who reported normal sleep duration without insomnia symptoms (Figure 10). However, the joint association of insomnia symptoms and long sleep duration on the risk of incident AF were inconclusive in HUNT2.

Based on the main model, UK Biobank participants who reported morning chronotype without insomnia symptoms had little evidence of a decreased risk of incident AF (HR 0.96; 95% CI 0.88, 1.05), while those who reported morning chronotype with insomnia symptoms had weak evidence of an increased risk (HR 1.08; 95% CI 0.97, 1.21) and those who reported evening chronotype with insomnia symptoms had strong evidence of an increased risk of incident AF (HR 1.18; 95% CI 1.04, 1.35) when compared to those who reported evening chronotype without insomnia symptoms (Figure 11). However, the joint associations of morning chronotype and short sleep duration, as well as morning chronotype and long sleep duration on the risk of incident AF were inconclusive in UK Biobank.

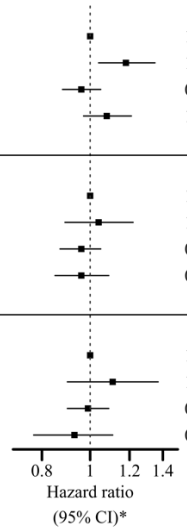
The estimated associations remained fairly unchanged in our additional model (Paper III: Supplementary Tables S5–S9).

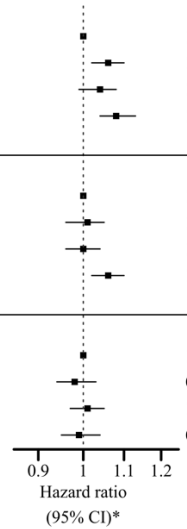
2x2 Factorial MR analysis

In UK Biobank, participants with high genetic risk for insomnia symptoms and high genetic risk for short sleep duration had a slightly higher risk of incident AF (HR 1.03; 95% CI 0.99, 1.07 and HR 1.02; 95% CI 0.97, 1.06, respectively), whereas participants with high genetic risks for both traits had the highest risk (HR 1.08; 95% CI 1.03, 1.12)

Figure 11: Observational and 2x2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of insomnia symptoms/ sleep duration and chronotype on the risk of incident atrial fibrillation in UK Biobank.

Observational analysis[†]	N (incident cases)	Hazard ratio (95% CI)*	Factorial MR analysis[‡]	N (incident cases)	Hazard ratio (95% CI)*
Insomnia symptoms - Chronotype[#]					
No - No	77 439 (3 910)	1 [Reference]	Both GRS ≤ median	80 092 (4 545)	1 [Reference]
Yes - No	29 507 (1 835)	1.18 (1.04, 1.35)	Insomnia GRS > median	85 782 (5 076)	1.06 (1.02, 1.10)
No - Yes	130 675 (7 175)	0.96 (0.88, 1.05)	Chronotype GRS > median	85 782 (5 034)	1.04 (0.99, 1.08)
Yes - Yes	49 731 (3 272)	1.08 (0.97, 1.21)	Both GRS > median	80 092 (4 875)	1.08 (1.04, 1.13)
Short sleep - Chronotype[#]					
No - No	73 714 (3 761)	1 [Reference]	Both GRS ≤ median	77 263 (4 313)	1 [Reference]
Yes - No	24 173 (1 285)	1.04 (0.89, 1.22)	Short sleep GRS > median	75 883 (4 306)	1.01 (0.96, 1.05)
No - Yes	125 264 (6 914)	0.96 (0.87, 1.05)	Chronotype GRS > median	75 883 (4 258)	1.00 (0.96, 1.04)
Yes - Yes	42 794 (2 573)	0.96 (0.85, 1.09)	Both GRS > median	77 263 (4 621)	1.06 (1.02, 1.10)
Long sleep - Chronotype[#]					
No - No	73 714 (3 761)	1 [Reference]	Both GRS ≤ median	71 916 (4 209)	1 [Reference]
Yes - No	9 059 (699)	1.11 (0.90, 1.37)	Long sleep GRS > median	54 640 (3 122)	0.98 (0.94, 1.03)
No - Yes	125 264 (6 914)	0.99 (0.90, 1.09)	Chronotype GRS > median	76 031 (4 484)	1.01 (0.97, 1.05)
Yes - Yes	12 348 (960)	0.93 (0.77, 1.11)	Both GRS > median	50 524 (2 923)	0.99 (0.95, 1.04)





CI, confidence interval; MR, Mendelian randomization; GRS, genetic risk score.
In the observational analysis for each sleep trait combination, 'No – No' represents the absence of both sleep traits in combination, 'Yes – No' represents the presence of the first sleep trait and absence of the second, 'No – Yes' represents the absence of first sleep trait and presence of second, and 'Yes – Yes' represents the presence of both sleep traits.
In the factorial MR analysis for each sleep trait combination, both GRS ≤ median represents low genetic risks for both sleep traits in combination, sleep trait 1 GRS > median represents high genetic risk for sleep trait 1 only, sleep trait 2 GRS > median represents high genetic risk for sleep trait 2 only, and both GRS > median represents high genetic risks for both sleep traits.
[†] Estimates from the main model adjusted for age, gender, marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend Deprivation Index (for UK Biobank only), shift work, and employment status.
[‡] Estimates adjusted for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
[*] Derived using the unweighted genetic risk score for each sleep trait in UK Biobank and using the weighted genetic risk score for each sleep trait in HUNT2.
[#] Chronotype - morning vs. evening preference; or chronotype genetic risk score calculated using the alleles for morning preference.

(Figure 10); however, there was no evidence of interaction (RERI 0.03; 95% CI -0.03, 0.09). This pattern was however not consistent in HUNT2, showing imprecise estimates. The joint effects of insomnia symptoms and long sleep duration on the risk of incident AF were inconclusive in both UK Biobank and HUNT2 (Figure 10).

Additionally, UK Biobank participants with high genetic risk for insomnia symptoms and high genetic risk for morning chronotype had a slightly higher risk of incident AF (HR 1.06; 95% CI 1.02, 1.10 and HR 1.04; 95% CI 0.99, 1.08, respectively), whereas participants with high genetic risks for both sleep traits had the highest risk (HR 1.08; 95% CI 1.04, 1.13) (Figure 11). Notably, there was no evidence of interaction (RERI -0.01; 95% CI -0.07, 0.04). The UK Biobank participants with high genetic risk for short sleep duration and high genetic risk for morning chronotype had no increased risk of incident AF (HR 1.01; 95% CI 0.96, 1.05 and HR 1.00; 95% CI 0.96, 1.04, respectively), whereas participants with high genetic risks for both had an increased risk (HR 1.06; 95% CI 1.02, 1.10), but there was no statistical evidence of interaction (RERI 0.06; 95% CI -0.01, 0.12). The joint effects of long sleep duration and morning chronotype in the UK Biobank were inconclusive (Figure 11).

Sensitivity analyses

Using the uwGRS for the sleep traits in HUNT2, the one-sample MR estimates changed markedly for short and long sleep duration (HR 1.97; 95% CI 0.68, 5.72 and HR 1.19; 95% CI 0.64, 2.23, respectively), but only slightly for insomnia symptoms and 24-hour sleep duration (Paper III: Supplementary Table S20). However, the 2x2 factorial MR estimates remained fairly unchanged (Paper III: Supplementary Figure S1).

Upon correction for multiple comparisons, several confounding factors were associated with sleep trait GRS in both UK Biobank and HUNT2 (Paper III: Supplementary Tables S21 and S22). Furthermore, adjusting for these confounding factors in the one-sample MR analysis showed slightly weaker adverse causal effects of insomnia symptoms (HR 1.05; 95% CI 0.97, 1.13) and short sleep duration (HR 1.09; 95% CI 0.98, 1.22) in UK Biobank (Paper III: Supplementary Table S23). Moreover, the effect estimate for sleep duration attenuated slightly and was less precise in UK Biobank (HR 0.82; 95% CI 0.67, 1.00).

The causal estimates obtained using the MR-Egger, the weighted median, and weighted mode-based methods attenuated slightly and were less precise (Paper III: Supplementary Tables S24 and S25, as well as Figures S2–S6). Furthermore, the post hoc one-sample MR analysis using insomnia symptom SNPs from Lane *et al*. [219] provided similar estimates, where the TSPS estimates from HUNT2 showed a suggestive adverse causal effect (HR 1.11; 95% CI 0.87, 1.41) (Paper III: Supplementary Table S26 and Figure S7).

The causal estimates attenuated slightly when using GRS comprising 116 insomnia SNPs (one missing in the HUNT imputed dataset) and 72 chronotype SNPs, which replicated at a genome-wide significance level ($P < 5x10^{-8}$) in the independent 23andMe dataset (Paper III: Supplementary Tables S27 and S28).

The estimates from factorial MR analysis using sleep trait GRS as quantitative traits (per standard deviation increase) and their product term inferred similar effects when compared to estimates from 2x2 factorial MR (Paper III: Supplementary Figure S8). In UK Biobank, the GRSs for insomnia symptoms and short sleep duration were independently linked to an increased risk of incident AF (HR 1.03; 95% CI 1.01, 1.04 and HR 1.02; 95% CI 1.00, 1.03, respectively), with no evidence of interaction (RERI 0.01; 95% CI -0.01, 0.02). Similarly, the GRSs for insomnia symptoms and morning chronotype were independently associated with an increased risk of incident AF (HR 1.03; 95% CI 1.02, 1.05 and HR 1.01; 95% CI 1.00, 1.03, respectively); however, there was no evidence of interaction (RERI -0.01; 95% CI -0.02, 0.01). Additionally, the GRSs for short sleep duration and morning chronotype were both independently linked to an increased risk of incident AF (HR 1.02; 95% CI 1.00, 1.04 and HR 1.01; 95% CI 1.00, 1.03, respectively), with no evidence of interaction (RERI 0.01; 95% CI -0.01, 0.02).

5 Discussion

The overall aim of this thesis was to investigate the individual and joint causal influence of sleep traits on the risk of incident AMI and AF. We used different causal inference approaches and followed the principle of triangulation. We emphasize our findings from the UK Biobank due to its large sample, and consequently high statistical power. Our findings are summarized as follows:

Aim I. We found that participants with insomnia symptoms, short sleep duration, long sleep duration and evening chronotype had an increased risk of incident AMI when compared to participants without these sleep traits in UK Biobank. A similar trend was observed in HUNT2 for insomnia symptoms and short sleep duration. Participants who exhibited combinations of insomnia symptoms with short sleep duration, insomnia symptoms with long sleep duration, insomnia symptoms with evening chronotype, short sleep duration with evening chronotype, and long sleep duration with evening chronotype had higher risks of incident AMI than participants who exhibited only one sleep trait in UK Biobank, where we found evidence of interaction (assessed using RERI) for insomnia symptoms with long sleep duration. We detected similar trends for the combination of insomnia symptoms with short sleep duration and an increased risk of incident AMI in HUNT2, but no evidence of interaction.

Aim II. In our MR analyses, we found evidence of an increased risk of incident AMI from insomnia symptoms, weak evidence of an increased risk from short sleep duration, and evidence of a decreased risk from long sleep duration in UK Biobank. However, these causal risks of short and long sleep duration were not observed in HUNT2. Participants in UK Biobank with high genetic risks for two sleep traits in certain combinations (i.e., insomnia symptoms with short sleep duration, insomnia symptoms with morning chronotype, and short sleep duration with morning chronotype) had higher risks of incident AMI than participants with high genetic risk for only one sleep trait, but there was no evidence of interaction. These results were not replicated in HUNT2.

Aim III. We found that participants with insomnia symptoms had an increased risk of incident AF when compared to those without these symptoms; however, we found no evidence of associations between short or long sleep duration or chronotype and the risk of incident AF in UK Biobank or HUNT2. Participants who exhibited insomnia symptoms with long sleep duration and insomnia symptoms with evening chronotype had higher risks of incident AF than those

who exhibited only one sleep trait in UK Biobank; however, there was no evidence of interaction. These results were not replicated in HUNT2.

Aim IV. In our MR analyses, we found evidence of an increased risk of incident AF from insomnia symptoms and short sleep duration in UK Biobank. However, these causal risks were not replicated in HUNT2. Participants in UK Biobank with high genetic risks for two sleep traits in certain combinations (i.e., insomnia symptoms with short sleep duration, insomnia symptoms with morning chronotype, and short sleep duration with morning chronotype) had higher risks of incident AF than participants with high genetic risk for only one sleep trait, but there was no evidence of interaction. These results were not replicated in HUNT2.

5.1 Methodological considerations

This thesis incorporated both prospective cohort and MR study designs. It is important to acknowledge that the findings presented in this thesis could be influenced by random error, which decreases the precision of the estimates, and by systematic error, which interferes with the validity of the results. In the following sections, I address these topics through a discussion of random error, internal validity, and external validity.

5.1.1 Random error and statistical precision

Random error, also referred to as chance variation, is the deviation in the observed value from the true value resulting from unexplained variability in the data [221]. It is imperative to measure, limit, and account for random error. Variance is a measure of random error, and its reciprocal, statistical precision, can be estimated through CIs. In this thesis, we reported 95% CIs as a measure of the precision of our effect estimates. A narrow CI indicates high statistical precision and a low possibility of random error. However, it is important to note that 95% CIs do not consider systematic errors. Therefore, narrow 95% CIs do not ascertain that the effect estimates are accurate or the associations are true [221].

A common way to minimize random error and enhance precision in epidemiological studies is to increase the sample size [221]. We had availability of data from two large population health studies — UK Biobank and HUNT2. We also had a fairly large number of participants included in our studies, thus providing sufficient precision. However, some exceptions included stratified analyses (in Paper I), where stratification of data reduced precision in estimates despite a large overall sample. In such scenarios, the ability to detect effect modification might be poor, and these estimates should thus be interpreted with care.

The power of a statistical test is the likelihood of the test correctly rejecting the null hypothesis when it indeed is false [221]. The statistical power of an MR study is attributed to the sample size and the strength of the association between the instrument and risk factor [171]. In Papers II and III, we only considered genetic variants associated with the sleep traits of interest at a genome-wide significance level ($P < 5x10^{-8}$) to mitigate random error [222]. However, since a single genetic variant typically explains a small proportion of variance for a given trait (referred to as a weak instrument), statistical power poses a significant challenge in MR analyses. The statistical power is lower in an MR analysis than an equivalent observational analysis [223]. Consequently, a large sample size was required to detect causal effects in MR analysis and avoid bias due to weak instruments [222, 224]. Furthermore, the 2x2 factorial MR analysis required individual-level genotype data and was based on the dichotomization of GRSs, which implies a low power to detect additive interactions [177, 223]. Thus, it required an even large sample size to detect an interaction effect with sufficient power. We acknowledge the low power concerns, which can partially be overcome by the use of strong instruments [178]. We also had fairly large individual-level genotyped data from the UK Biobank, which can alleviate some of the low power concerns in our analyses.

The replication of findings across independent cohorts further ensures the robustness of the findings and reduces the likelihood of chance findings (random error) [222]. We leveraged data from the UK Biobank and HUNT2, which provided an opportunity to replicate the findings across these cohorts. Although the sample size of HUNT2 is only about 10% of the size of UK Biobank, we believe HUNT2 can still offer valuable insights for cross-validation purposes, acknowledging that its limited statistical power may potentially overlook any weak effects.

As recommended by Burgess *et al.* [211], we applied bootstrapping methods to calculate corrected standard errors (SEs) for the TSPS estimates, as described for the one-sample MR analyses in Paper II. We found that no adjustments were required to the 95% CIs for any sleep trait estimates in the UK Biobank sample, but observed considerably wider 95% CIs for short and long sleep duration estimates in the HUNT2 sample. Importantly, simulations have shown that the use of bootstrapping is limited to the availability of strong instruments [225]. This implies that the validity of the 95% CIs obtained using bootstrapping for the TSPS estimates on the risk of incident AMI (in Paper II) may be questionable and based on the strength of instruments. As a result, we decided to restrict the application of the bootstrapping methods solely to the one-sample MR analyses conducted in Paper II.

5.1.2 Systematic error and internal validity

Unlike random error, systematic error is not affected by the study sample size [221]. Systemic error is commonly referred to as bias and interferes with the internal validity of the study results. If unaccounted for, systematic errors can result in inaccurate effect estimates or the emergence of spurious associations [221].

5.1.2.1 Selection bias

Selection bias refers to systematic errors that arise due to the procedures employed to select study participants and the factors that influence their participation [221]. This results in differences in exposure-outcome associations between those who participate in the study and those who do not participate. In this thesis, it is plausible that selection bias may have arisen due to non-participation, missing data, and loss to follow-up.

Non-participation

We used data from the UK Biobank and HUNT2 cohorts in all three papers. Participation in these cohorts was contingent upon individuals being alive, residing in the target area and being willing to participate. Among more than 9.2 million eligible individuals in the UK Biobank, the participation rate was only 5.5%, indicating a low response [184, 185]. In comparison, 69.5% of the 93 898 individuals eligible for HUNT2 participated [153, 186]. Although the participation rate in HUNT2 was higher than that of UK Biobank, which may suggest a reduced likelihood of selection bias in HUNT2, it was not possible to rule out that bias due to non-participation could have affected the findings in either cohort. A study comparing UK Biobank participants with its source population reported that participants were less likely to be obese, smoke or drink alcohol, and had fewer self-reported health conditions [226]. Additionally, participants aged 70–74 years had a lower rate of all-cause mortality, which highlights evidence of a healthy volunteer participation bias. A non-participation study following HUNT2 found that the main reasons for not participating were lack of time and interest [188]. However, among non-participants aged 70 years or more, the main reason for not participating was their regular health follow-ups during general practitioner or hospital visits, implying possible health differences between participants and non-participants in this age group.

Missing data

Prospective studies often have missing data on covariates, which can lead to selection bias and reduce statistical power [221]. We used complete-case analyses by excluding participants with missing data on covariates for our observational analyses in Papers I and III. Missing data can occur randomly or non-randomly. Randomly missing data would lead to no systematic differences between what was missing and what was observed, while non-randomly missing data can bias the results [227]. Additionally, missing data can also arise

due to the genotype quality control process, where genotyped samples with low quality were removed to ensure high-quality data [184, 189, 190]. Genotyping was performed by experienced personnel who were unaware of the participants' phenotype status. Therefore, we believe that any missing genotype data would likely be random. However, we cannot rule out that missing data in our analyses would have affected the results.

Loss to follow-up and the competing risks of death

Loss to follow-up can introduce selection bias when participants migrate to different areas and can no longer be followed [221]. However, the likelihood of this type of selection bias is anticipated to be low since only a small proportion of participants included in the studies (approximately 0.2% in UK Biobank and 3–5% in HUNT2) emigrated and thus could not be followed. Additionally, the estimates may be influenced by competing risks resulting from death among participants who were being followed for the occurrence of AMI and AF [228]. However, it is worth noting that the UK Biobank had a shorter follow-up period (~11 years compared to ~21 years in HUNT2) and involved younger participants, which resulted in a low proportion of competing deaths. As a result, competing deaths are less likely to alter the estimates in UK Biobank.

Furthermore, selection bias can affect the validity of the genetic instrument in the MR when selection into the study sample is influenced by a collider between the genetic instrument and confounder(s) of the exposure-outcome association [229]. This artificially induces an association between the genetic instrument and confounder(s), thereby compromising the validity of the instrument.

5.1.2.2 Information bias

Information bias refers to systematic errors that arise due to inaccuracies or errors in the measurement or classification of the exposure, outcome or other variables [221]. Measurement error in discrete variables is defined as misclassification and can be differential if the error depends on the value of other variables, and non-differential if independent of the value of other variables. Differential misclassification of the exposure dependent on the outcome, or vice versa, can under- or overestimate the effect. The non-differential misclassification of dichotomous exposure generally causes an underestimation of the effect; however, non-differential misclassification of exposure with more than two categories can lead to under- or overestimation of the effect. Measurement error or the misclassification of a confounding variable hampers its proper adjustment in the analysis [221].

Misclassification due to self-reported data on exposure

In our studies, sleep traits were based on self-reporting and not confirmed using objective measures of sleep, such as polysomnography or actigraphy. Although our definition of

insomnia symptoms did not encompass all insomnia complaints (i.e., difficulty falling asleep, night awakenings, waking up early, and daytime impairments), these complaints cannot be captured objectively or evaluated by polysomnography [230]. The use of self-reported sleep duration did not ascertain whether it accurately reflects time in bed or actual sleep time; however, actigraphy often tends to overestimate sleep duration [81]. Moreover, validated measures assessing chronotypes (e.g., the MEQ and MCTQ) have been proposed [85, 86], the chronotype in UK Biobank was assessed using a single question.

When using questionnaires, participants may misunderstand the questions and thereby become misclassified for sleep traits. Consequently, this may reduce the power to detect an association between sleep traits and genetic variants [231]. We used insomnia symptoms, sleep duration, and chronotype GWASs conducted by Jansen *et al.* [191], Dashti *et al.* [192], and Jones *et al.* [193], respectively, to identify SNPs for the MR analyses. These sleep phenotypes were based on self-reported data and align with our respective definitions. We also used the insomnia symptom SNPs from Lane *et al.* [219] in the post hoc analyses, which represent SNPs for any insomnia symptoms ('Sometimes'/'Usually' as cases vs. 'Never/rarely' as controls) or frequent insomnia symptoms ('Usually' as cases vs. 'Never/rarely' as controls).

The use of questionnaire information carries inherent limitations in that participants may misunderstand the questions or may under- or overreport traits. Also, questionnaire information is susceptible to bias due to recall [221]. It is important to acknowledge these errors since they cannot be completely ruled out. However, the questionnaires were completed prior to the design of these studies and these errors would not be related to the participants' outcomes for AMI/AF, thereby minimizing any possibility of differential misclassification and would only bias our results towards the null.

24-hour sleep duration was a discrete variable (i.e., hours in integers) and other sleep traits were dichotomized in our studies, which are likely to be coarsened approximations of the true underlying latent exposures [232]. There can exist a genetically driven variation in the true latent exposure within the levels of a discrete/binary exposure. In the context of MR, this may open up alternate pathways from the genetic instrument to the outcome that do not pass through binary exposure and violate the exclusion restriction assumption. As a result, this may under- or overestimate the effect estimate but will not influence the direction of the effect [232].

Misclassification of outcome

The misclassification of outcome is mostly non-differential and biases the estimates towards the null. However, when the outcome specificity is 100% (i.e., no false positives), the ratio estimates will not be biased, regardless of the sensitivity [221]. For this thesis, we

relied on linkages to medical and mortality records for information on the diagnosis of AMI/AF, where outcomes were identified using ICD codes. ICD codes are considered high quality, which implies that there is a low likelihood of outcome misclassification. However, misclassification can still occur due to changes in coding practices, regulatory guidelines, and traditions over time. Moreover, variations in coding practices between the UK and Norway, as well as among different medical doctors, can contribute to outcome misclassification. Since the medical information data used were limited to specific regions or countries for the included cohorts, any records of study participants admitted to hospitals outside of these regions or countries might have been missed. While it is not possible to completely ignore outcome misclassification, we do not believe that this would have considerably affected the estimates in our setting.

5.1.2.3 Confounding

Confounding is a distortion of effects when the apparent association of an exposure on an outcome is caused by a third factor known as a confounder [221]. The confounder is identified as a variable associated with — but not a consequence of — the exposure and is a cause of the outcome. It is crucial to account for confounding in epidemiological studies since it may result in an under- or overestimation of the effect of the exposure. However, confounders are not always measured. Randomization, which forms the basis of RCTs, is one of the most effective ways to account for confounding, where the confounders (measured or unmeasured) are equally distributed across the groups being compared. Restriction, stratification, and statistical modelling are other ways to deal with confounding [221].

Confounding in observational studies

Confounders should not be confused with mediators or colliders [221]. Mediators are variables on the causal pathway that convey some or all effect of the exposure on the outcome. Colliders are common consequences of exposure and the outcome. Mediators and colliders should not be controlled for since this would lead to biased estimates [221]. Both the UK Biobank and HUNT2 had information on a large number of socio-demographic, lifestyle, and clinical data on the participants. In this thesis, the selection of potential confounders was performed based on *a priori* knowledge about the factors associated with both the exposure and the outcome.

In Papers I and III, we controlled for age and gender in our crude models for all observational analyses and further adjusted for important demographics, lifestyle factors, and some established cardiovascular risk factors in our main models. We additionally controlled for factors that may either be confounders or mediators for the associations

under study in our additional models. Nevertheless, controlling for these additional factors did not considerably alter our effect estimates.

Another way to mitigate confounding is by estimating associations within homogeneous categories (e.g., age group, gender, etc.) [221]. In Paper I, we stratified our analyses by age, gender, shift work, depression, and anxiety. Furthermore, since chronic disorders correlate with sleep traits, we adjusted for chronic disorder(s) in our models as the sensitivity analyses.

Despite our considerable efforts to limit potential confounding in the observational analyses (Papers I and III), it is important to acknowledge that we could not rule out residual confounding due to measurement errors or misclassification of the confounders, as well as confounding from unknown factors in our studies [221]. An important example of an unknown factor is OSA, which is a well-established risk factor for CVD [233]. While daytime sleepiness is a common characteristic of sleep apnea syndrome, individuals with OSA often experience difficulties initiating sleep, difficulties maintaining sleep, and experience early-morning awakenings [234]. However, a large European population-based study suggested that the prevalence of other sleep disorders, including OSA, is only ~5% among those who have insomnia symptoms [235]. For any such factor(s) to be able to substantially influence our estimates, it must be unrelated to other covariates controlled for in the models, while also being strongly associated with both the exposure and the outcome. In the case of OSA, it is known to be correlated with age, BMI, BP, and depression [234, 236]. By adjusting for these related variables in our analyses, we have partially accounted for some of the confounding that could arise from OSA. Thus, it seems unlikely that OSA alone could explain any increased risk of AMI/AF observed among participants with different sleep traits or their combinations in our studies.

Confounding in MR studies

Due to the nature of the observational study designs, residual and unmeasured confounding could not be eliminated entirely [221]. MR is an important alternative design used to determine evidence of causality [166, 167]. Since genetic variants segregate randomly at conception and are not influenced by any lifestyle/environmental factors, the MR is less susceptible to residual or unmeasured confounding than the observational study designs. We investigated the association between sleep trait instruments and measured potential confounders of the exposure-outcome relationship to assess the independence assumption of MR. However, it is important to acknowledge that confounding of the genetic variants with the outcome can still exist due to population and familial effects (e.g., population stratification, dynastic effects, and assortative mating) by violating the independence assumption [222, 237].

Population stratification arises due to systematic variations in allele frequencies related to traits of interest across different groups [238]. These are usually controlled by adjusting for top principal components in the analyses. However, residual population stratification from geographic or regional differences cannot be solely controlled via principal components [239, 240]. Dynastic effects occur when the parental genotype directly influences the offspring phenotype independent of their shared genotype [237]. It is logical that parents create family environments that align with their own genotypes, which in turn influences the development of traits in their offspring [241]. Moreover, other familial relationships such as siblings, grandparents, aunts/uncles, and cousins may also have a small potential influence on the phenotype of the offspring, akin to a weaker form of dynastic effects [237, 241]. Assortative mating refers to the phenomenon where individuals choose partners who share similar phenotypes to a greater extent than would be expected by chance [242]. When assortative mating leads individuals with a specific genetic predisposition to select mates with certain genetically influenced phenotypes, it could introduce spurious genetic associations that can bias the MR estimates [243].

5.1.2.4 Reverse causation

It is crucial to define the temporal sequence of events in the observational study designs to accurately determine the direction of causation [221]. Reverse causation is a situation wherein the outcome of interest influences the exposure rather than the exposure influencing the outcome. This can lead to incorrect conclusions regarding the causal relationship between exposure and outcome [221]. In Paper I, we repeated the primary analyses after excluding the first 2 years of follow-up, which allowed us to establish the temporal sequence of AMI incidence, thereby minimizing the impact of potential reverse causation. These results did not change when compared to the primary analyses. In Papers II and III, we employed MR designs that are robust to bias resulting from reverse causation since the genetic variants utilized as instruments for the sleep traits are determined at conception and precede the incidence of AMI/AF.

5.1.2.5 Pleiotropy

Genetic pleiotropy is a natural phenomenon whereby a single genetic variant can have effects on multiple traits [244]. There are two forms of pleiotropy: vertical pleiotropy and horizontal pleiotropy. Vertical pleiotropy occurs when the genetic variant influences a trait downstream of the exposure (i.e., a mediator of the exposure of interest). However, the genetic variant exerts its effect on the outcome solely through exposure, thus adhering to the exclusion restriction assumption of MR. In contrast, horizontal pleiotropy occurs when the genetic variant independently influences another trait (or other traits), thereby introducing alternative pathway(s) through which it affects the outcome (not through exposure). Notably, this violates the exclusion restriction assumption. The pleiotropy is

balanced, and MR estimates are unbiased when the horizontal pleiotropic effects of the genetic variants are equally positive and negative, whereas unbalanced (directional) pleiotropy threatens the validity of MR estimates.

The use of a large number of genetic variants as instruments increases the likelihood of pleiotropy and false positive effects [176]. To investigate horizontal pleiotropy, we applied pleiotropy robust methods such as the MR-Egger, the weighted median, and weighted mode-based methods, as sensitivity analyses in Papers II and III. These results were largely consistent across various MR methods, except for insomnia symptoms on the risk of incident AMI, which showed evidence of directional pleiotropy in the MR-Egger analysis. However, simulations have demonstrated the potential unreliability of MR-Egger estimates in the one-sample setting [214]. Moreover, the genetic risk of insomnia symptoms was previously found to be strongly associated with BMI, smoking status, depression, and education among other covariates [191], which may be indicative of confounding, mediation or horizontal pleiotropy. Another approach to control for horizontal pleiotropy is to apply multivariable MR [179]. Previous studies have shown only mild attenuation of the causal effects of insomnia symptoms on CAD/AF risk when adjusting for BMI, smoking, depression, and education in multivariable MR [96, 98].

5.1.2.6 Weak instrument bias

As previously mentioned, it is typical for a single genetic variant to only explain a small proportion of variability in a given trait, which might lead to weak instrument bias [171]. In accordance with recommendations [173], we constructed GRS by aggregating multiple genetic variants associated with a trait to create a single instrument for use in the MR analyses conducted in Papers II and III. This also improves the statistical power of the MR analyses [173]. F-statistics were calculated to assess exposure instruments' strength to determine the likelihood of weak instrument bias in our MR analyses [245]. As a rule of thumb, the F-statistic >10 is generally desirable [171]. Given that some of the F-statistics reported here were <10, we observed indications of weak instrument bias for short and long sleep duration in HUNT2. As a result, our MR findings for individual or joint causal effects including short or long sleep duration in HUNT2 might not be reliable.

5.1.2.7 Winner's curse

Winner's curse, also known as the Beavis effect, refers to a phenomenon in which the estimated associations of the reported significant variants for a trait in the discovery samples of a GWAS are likely to be overestimated [246]. It occurs due to the nature of hypothesis testing and the multiple testing in a GWAS. When numerous genetic variants are tested simultaneously, among the initially identified associations, only those with larger effect sizes are more likely to surpass the significance threshold and be reported.

Consequently, the effect sizes of these reported variants tend to be exaggerated [246]. Thus, replication studies and meta-analyses are crucial for validating and providing more accurate estimates of genetic associations [246, 247]. Jansen *et al.* [191] and Jones *et al.* [193] replicated their GWAS results and meta-analyzed the effect sizes from the UK Biobank and 23andMe, whereas Dashti *et al.* [192] could not replicate their GWAS findings from UK Biobank. In the context of one-sample MR, the use of the same sample as a discovery analysis for genetic variants can bias the MR estimates towards the null [223, 248]. To mitigate this potential bias in Papers II and III, we employed the unweighted GRSs of sleep traits as instruments for our primary analyses of UK Biobank.

5.1.3 Generalizability (external validity)

The external validity of a study refers to the generalizability of its findings to diverse populations and subgroups beyond the specific population that was investigated [221]. The analyses in this thesis included one cohort from the UK (UK Biobank) and another from Norway (HUNT2), which potentially limits the generalizability of these results to other populations and ethnicities. UK Biobank is not representative of the wider UK population [226], whereas HUNT2 is modestly representative of the Norwegian population [188]. This reality further limits the generalizability of UK Biobank findings to the general population of the UK.

Moreover, differences across the cohorts included in the analyses may also impact the generalizability of our findings to each other:

a) The mean age at baseline was higher in UK Biobank (56 years) than in HUNT2 (48 years).
b) The participants were older in HUNT2 (aged 20 years or older) than in UK Biobank (aged 40 to 69 years).
c) The questionnaires were collected more than 10 years apart in HUNT2 (1995–97) and UK Biobank (2006–08), which may have caused differences due to time-related trends.
d) Different lengths of follow-up (approximately 12 years in UK Biobank and approximately 21 years in HUNT2).
e) UK Biobank (5.5% response rate) may represent a healthier sample [226], whereas HUNT2 (69.5% response rate) may be a more representative sample [188], which may have resulted in stronger selection bias in UK Biobank.
f) More seasonal variation in daylight in Trøndelag (Norway) than in the UK; however, no evidence of seasonal variation in reports of insomnia symptoms or time in bed was found in HUNT2 [249].

5.2 Appraisal of the findings

Due to the fundamental difference between the nature of inherited genetic variants and observed sleep behaviors, comparisons between MR and observational findings should be done with caution. MR estimates reflect the lifelong influence of genetic variants on sleep behaviors, while observational estimates capture the sleep behaviors measured at one time point. In the following sections, I discuss the results obtained from our observational and MR analyses in relation to relevant prior studies.

5.2.1 Sleep traits and the risk of acute myocardial infarction (Papers I and II)

Individual sleep traits and the risk of incident AMI

Insomnia symptoms. In our observational and MR analyses, we found evidence suggesting that insomnia symptoms increased the risk of incident AMI in UK Biobank. These findings are consistent across much of the prior observational [121, 250–253] and MR studies [96–98, 106, 219]. However, we only found weak evidence of increased risk of incident AMI from our observational and MR analyses in HUNT2, which may be attributed to low statistical power from a smaller sample. Similar to our study, a previous observational study on insomnia and AMI in HUNT2 found that individual insomnia symptom(s) and the cumulative number of insomnia symptoms were associated with an increased risk of incident AMI [121]. In the present study, we have a longer follow-up of approximately 21 years for the HUNT2 participants as compared to 11.4 years in the prior study.

Short sleep duration. Our observational analysis found evidence suggesting that short sleep duration increased the risk of incident AMI in UK Biobank. Although we only found weak evidence from the MR analysis to support this notion, these findings are in line with most existing observational [100, 122] and MR studies [104, 122]. The strength of the observational association was weaker in HUNT2, whereas our MR analysis in HUNT2 found no causal effect of short sleep duration on the risk of incident AMI. This could be due to low statistical power from a smaller sample and/or weak instrument for short sleep duration in HUNT2.

Long sleep duration. Our observational findings suggesting that long sleep duration moderately increased the risk of incident AMI in UK Biobank are consistent with findings from prior observational studies [100, 122]. However, there was no association of long sleep duration and the risk of incident AMI in HUNT2. As previously mentioned, this may be due to notable differences in the two cohorts. Moreover, the dominance of short sleepers in UK Biobank and long sleepers in HUNT2 — possibly due to a general time-related trend towards short sleep duration from 1995–97 (HUNT2) to 2006–10 (UK Biobank) [254] — makes it challenging to directly compare the findings across these two cohorts. In contrast,

our MR findings provide evidence of a protective causal effect of long sleep duration on the risk of incident AMI in UK Biobank, which aligns with the weak concordant effect presented in another MR study [104]. This indicates that long sleep duration may be indicative of poor health status since it is closely associated with factors such as depression, poor sleep quality, sedentary lifestyles, and underlying comorbid conditions [147, 255]. Therefore, it is possible that residual confounding or reverse causation may have biased any observational findings of long sleep duration. Again, our MR findings were not replicated in HUNT2, which could be due to its smaller sample and/or weak instrument for long sleep duration.

Chronotype. Our observational findings for evening chronotype and increased risk of incident AMI in UK Biobank are consistent with some prior observational studies [108, 123]. On the contrary, our MR findings suggested weak evidence of the causal effect of morning chronotype and increased risk of incident AMI in UK Biobank. This suggests that the observational findings may have been influenced by bias common in conventional observational studies. Thus, further studies are required to confirm any speculations of a possible causal relationship between morning/evening chronotype and the risk of incident AMI.

Combination of sleep traits and the risk of incident AMI

Insomnia symptoms with short sleep duration. Our observational findings for the joint association of insomnia symptoms with short sleep duration and the moderately increased risk of incident AMI in UK Biobank are consistent with prior observational studies on AMI [124] and CHD [113, 115]. Similarly, a prospective study by Bertisch *et al.* on 4 437 CVD-free participants from the SHHS followed for a median of 11.4 years reported a 29% increased risk of incident CVD (HR 1.29; 95% CI 1.00, 1.66) for those who had insomnia symptoms with polysomnographic short sleep duration [112]. These findings further align with our findings from the MR analysis in UK Biobank, which showed that participants with high genetic risks for both insomnia symptoms and short sleep duration had a higher risk of incident AMI than participants with high genetic risk for only one sleep trait. However, this was not observed in HUNT2, possibly due to low statistical power from a smaller sample and/or weak instrument for short sleep duration. Some consistency in these findings across observational and MR designs suggests a causal relationship. Moreover, the lack of interaction effect (assessed using RERI) between insomnia symptoms and short sleep duration on the risk of incident AMI are consistent across both observational and MR findings.

Insomnia symptoms with long sleep duration. Our observational analysis show evidence of interaction between insomnia symptoms and long sleep duration on the risk of incident AMI. Additionally, the observed increase in the risk of incident AMI from the combination

of insomnia symptoms and long sleep duration in UK Biobank is consistent with a prospective study on this phenotype and incident CHD [111]. Sands-Lincoln *et al.* followed 86 329 postmenopausal women aged 50–79 years from the Women's Health Initiative (WHI) Observational Study for a mean of 10.3 years. They found that women at a high risk of insomnia symptoms (defined as having a score ≥9 on the WHI Insomnia Rating Scale (WHIIRS)) with ≥10 h sleep duration had a 93% increased risk of incident CHD (HR 1.93; 95% CI 1.06, 3.51) when compared to those at low risk of insomnia symptoms (defined as having a score <9 on the WHIIRS) with 7–8 h sleep duration [111]. Since this study exclusively involved postmenopausal women aged 50–79 years, the observed association may not be generalizable to the broader population. Notably, no such association of this phenotype and incident AMI was found in our observational analysis in HUNT2, and these inconsistent findings across UK Biobank and HUNT2 cohorts could be attributed to possible differences in the two cohorts, as previously mentioned. In contrast, our MR findings showed no interaction (assessed using RERI) between insomnia symptoms and long sleep duration in relation to the risk of incident AMI, neither in UK Biobank nor HUNT2. As previously mentioned, our MR findings in UK Biobank suggest a protective effect of genetic predisposition to long sleep duration on incident AMI, which was not affected by additionally being genetically predisposed to insomnia symptoms. This further suggests that the observational findings might have been influenced by bias common in conventional observational studies. Poor health could be a confounder that would lead to false indications of the harmful consequences of prolonged sleep in observational studies [147, 255].

Chronotype with insomnia symptoms or short/long sleep duration. We are unaware of any previous observational or MR studies that have investigated the joint causal influence of chronotype with insomnia symptoms or short/long sleep duration on the risk of incident AMI. Our observational findings from UK Biobank indicate that in combination with insomnia symptoms or short sleep duration, evening chronotype is more deleterious than morning chronotype in terms of the risk of incident AMI. In contrast, our MR findings indicate that UK Biobank participants with high genetic risks for both insomnia symptoms and morning chronotype, as well as those with high genetic risks for both short sleep duration and morning chronotype had higher risks of incident AMI than participants with high genetic risk for only one sleep trait. Although there was no interaction (assessed using RERI), these MR findings may suggest that the weak adverse effect of genetic predisposition to morning chronotype on incident AMI could be partly explained by a concomitant genetic predisposition to insomnia symptoms or short sleep duration. Moreover, our MR findings suggesting that UK Biobank participants with high genetic risks for both long sleep duration and morning chronotype likely had a lower risk of incident AMI are inconsistent with our observational findings, where long sleep duration

together with morning chronotype was associated with an increased risk. Again, there was no interaction and — if anything — our MR findings suggest a protective effect of genetic predisposition to long sleep duration on incident AMI, which was not affected by additionally being genetically predisposed to morning chronotype.

5.2.2 Sleep traits and the risk of atrial fibrillation (Paper III)

Individual sleep traits and the risk of incident AF

Insomnia symptoms. In our observational and MR analyses in UK Biobank, we found evidence suggesting that insomnia symptoms increased the risk of incident AF. These findings are consistent across most existing observational [125, 126] and MR studies [96–98, 106]. Our observational analysis in HUNT2 found weak evidence of an increased risk of incident AF, whereas our one-sample MR analysis showed no effect of insomnia symptoms on the risk of incident AF. This lack of replication in HUNT2 could be due to low statistical power from its smaller sample. Moreover, the estimated effect of insomnia symptoms using fewer SNPs from Lane *et al.* [219] showed the tendency toward an increased risk of incident AF in participants genetically predisposed to insomnia symptoms (HR 1.11; 95% CI 0.87, 1.41) in HUNT2.

Sleep duration. Our observational findings of no association of 24-hour sleep duration on the risk of incident AF in any of the cohorts could indicate the presence of an underlying U-shaped association, as evident from previous observational studies noting that both short and long sleep durations increase the risk of incident AF [126, 127, 256–258]. Surprisingly, we observed no associations of short or long sleep durations when assessed separately in the observational analysis. On the other hand, the one-sample MR findings in UK Biobank suggested a protective effect per hour increase in sleep duration and an adverse effect of short sleep duration on the risk of incident AF, which are in line with previous MR analyses [104, 128]. However, these were not replicated in HUNT2, which again may be attributed to a smaller sample and/or weak instrument for short sleep duration.

Chronotype. Our observational and MR findings of no association of chronotype on the risk of incident AF in the UK Biobank align with prior research [126].

Combination of sleep traits and the risk of incident AF

Insomnia symptoms with short sleep duration. Our observational analysis found no evidence of the joint association of insomnia symptoms with short sleep duration on the risk of incident AF in UK Biobank or HUNT2. This is in contrast to a prior observational study in UK Biobank that found that healthy sleep patterns (represented by morning chronotype, adequate sleep duration (7–8 h), no frequent insomnia symptoms, no snoring, and no frequent daytime sleepiness) were associated with a lower risk of AF [126]. On the other hand, our MR analysis found that UK Biobank participants with high genetic risks for

both insomnia symptoms and short sleep duration had higher risk of incident AF than participants with high genetic risk for only one sleep trait. Although there was no evidence of interaction (assessed using RERI), this suggests a potential causal relationship that was not evident in the conventional observational method due to inherent bias. However, this was not replicated in HUNT2, possibly due to low power from a smaller sample and/or weak instrument for short sleep duration.

Insomnia symptoms with long sleep duration. Our observational analysis found the joint association of insomnia symptoms with long sleep duration and an increased risk of incident AF in UK Biobank. This aligns with a prior study in UK Biobank that found healthy sleep patterns (represented by morning chronotype, adequate sleep duration (7–8 h), no frequent insomnia symptoms, no snoring, and no frequent daytime sleepiness) were associated with a lower risk of AF [126]. However, this could not be replicated in our observational analysis in HUNT2, which can be attributed to its small sample size. On the other hand, our MR analysis found no evidence of an increased risk of incident AF in participants with high genetic risks for both insomnia symptoms and long sleep duration, thus suggesting that the observed joint association of insomnia symptoms and long sleep duration on the risk of incident AF in UK Biobank may not be causal.

Chronotype with insomnia symptoms or short/long sleep duration. Our observational analysis found the joint association of insomnia symptoms with evening chronotype and an increased risk of incident AF in UK Biobank. However, our MR analysis indicate that UK Biobank participants with high genetic risks for both insomnia symptoms and morning chronotype had a higher risk of incident AF than those with high genetic risk for only one sleep trait, though there was no evidence of interaction (assessed using RERI). Observed inconsistencies across the observational and MR findings suggest that the observational association may not be causal. Our observational findings suggested no joint association of short sleep duration with morning/evening chronotype on the risk of incident AF in UK Biobank. However, our MR findings showed that UK Biobank participants with high genetic risks for both short sleep duration and morning chronotype had a higher risk of incident AF than those with high genetic risk for only one of these sleep traits, though there was no evidence of interaction (assessed using RERI). Our observational and MR findings on the combination of long sleep duration with morning/evening chronotype and the risk of incident AF showed no effects.

6 Conclusions and future perspectives

In this thesis, we have investigated both prospectively and using MR, sleep traits and their interplay as risk factors for the development of AMI and AF. This thesis contributes new knowledge about the joint effects of sleep traits on the risk of incident AMI and AF. The factorial MR findings suggesting that the genetic predisposition for two sleep traits in certain combinations (i.e., insomnia symptoms with short sleep duration, insomnia symptoms with morning chronotype, and short sleep duration with morning chronotype) make individuals more susceptible to develop AMI and AF are novel. Although there was no interaction (assessed using RERI), our results add to the body of evidence regarding the adverse health effects of certain sleep traits and their combinations. For instance, the importance of a healthy sleep — free from insomnia symptoms with adequate sleep duration (7–8 h) — in reducing the risk of incident AMI and AF could be relevant for preventive initiatives.

Although these findings were not entirely consistent in the observational settings, we observed that individuals exhibiting two sleep traits in all combinations had higher risks of incident AMI than those exhibiting only one sleep trait — showing interaction for insomnia symptoms with long sleep duration. Also, the observational findings suggest that individuals who had insomnia symptoms with long sleep duration and insomnia symptoms with evening chronotype had higher risks of incident AF than those who had only one sleep trait, showing no interaction. This may indicate that some of these observed joint associations may be due to bias evident in conventional observational studies. Despite that we included data from UK Biobank which constitutes one of the largest cohorts to study interactions using MR, it is possible that our MR analysis — especially factorial MR — is underpowered in detecting the interaction effects.

Notably, we could not triangulate the findings between the observational and MR study designs in this thesis, which makes it difficult to synthesize similar conclusions. Thus, further research is warranted, particularly research addressing the underlying mechanisms for these associations.

Although MR is considered superior to observational study designs, MR studies suffer from bias attributed to population stratification, dynastic effects, and assortative mating. Through the use of within-family designs, it is possible to mitigate confounding arising from population and familial effects [259]. Since MR methods are emerging for within-family and between-sibling analyses, the results presented here would be an ideal candidate for replication with these analyses [237, 259]. These approaches can effectively account for family structure by utilizing sibling data or parent-offspring trio data [237, 259]. Such designs would account for any bias in our MR findings. However, the limited sample sizes

of the available sibling or parent-offspring datasets pose a major challenge in attaining sufficient statistical power for such analyses.

Additionally, our use of self-reported sleep traits is prone to measurement error. This challenge underscores the need to quantify sleep duration at home and over longer periods using objective measurements such as wearable sensor technology (e.g., actigraphy). Moreover, the application of factorial MR designs is limited to a single-sample setting. The extensions of multivariable MR that use a two-stage least-squares estimator to estimate additive interactions between two continuous exposures on a continuous outcome have been proposed to have greater power [177]. However, further work is required to test and develop novel approaches for binary outcomes [178]. The use of this approach to assess interaction within a two-sample MR setting can be challenging since this would require the association of genetic variants and their cross-terms with the risk factors and their product [177]. Notably, GWAS studies using the product of the two risk factors as a single phenotype are uncommon.

References

1. Roth GA, Mensah GA, Fuster V. The Global Burden of Cardiovascular Diseases and Risks: A Compass for Global Action. Journal of the American College of Cardiology. 2020;76:2980–1.

2. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. Journal of the American College of Cardiology. 2017;70:1–25.

3. Roth T. Insomnia: definition, prevalence, etiology, and consequences. J Clin Sleep Med. 2007;3 5 Suppl:S7–10.

4. Calem M, Bisla J, Begum A, Dewey M, Bebbington PE, Brugha T, et al. Increased prevalence of insomnia and changes in hypnotics use in England over 15 years: analysis of the 1993, 2000, and 2007 National Psychiatric Morbidity Surveys. Sleep. 2012;35:377–84.

5. Pallesen S, Sivertsen B, Nordhus IH, Bjorvatn B. A 10-year trend of insomnia prevalence in the adult Norwegian population. Sleep Medicine. 2014;15:173–9.

6. Ariansen I, Selmer R, Graff-Iversen S, Egeland GM, Sakshaug S. Cardiovascular diseases in Norway. In: Public Health Report - Health Status in Norway. Oslo: Norwegian Institute of Public Health [updated (24 Jan 2020)]. https://www.fhi.no/en/op/hin/health-disease/cardiovascular-disease-in-norway---/?term=&h=1. Accessed 24 May 2023.

7. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth Universal Definition of Myocardial Infarction (2018). Circulation. 2018;138:e618–51.

8. Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868–74.

9. Dégano IR, Salomaa V, Veronesi G, Ferriéres J, Kirchberger I, Laks T, et al. Twenty-five-year trends in myocardial infarction attack and mortality rates, and case-fatality, in six European populations. Heart. 2015;101:1413–21.

10. Sulo G, Igland J, Nygård O, Vollset SE, Ebbing M, Tell GS. Favourable trends in incidence of AMI in Norway during 2001–2009 do not include younger adults: a CVDNOR project. European Journal of Preventive Cardiology. 2014;21:1358–64.

11. Yeh RW, Sidney S, Chandra M, Sorel M, Selby JV, Go AS. Population trends in the incidence and outcomes of acute myocardial infarction. N Engl J Med. 2010;362:2155–65.

12. Penman I, Ralston S, Strachan M, Hobson R. Davidson's Principles and Practice of Medicine - 24th Edition. 24th edition. Elsevier; 2022.

13. Arrhythmias - What is an Arrhythmia? | National Heart, Lung and Blood Institute, National Institutes of Health. 2022. https://www.nhlbi.nih.gov/health/arrhythmias. Accessed 24 May 2023.

14. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart Disease and Stroke Statistics—2019 Update: A Report From the American Heart Association. Circulation. 2019;139:e56–528.

15. Kornej J, Börschel CS, Benjamin EJ, Schnabel RB. Epidemiology of Atrial Fibrillation in the 21st Century. Circulation Research. 2020;127:4–20.

16. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. International Journal of Stroke. 2021;16:217–21.

17. Kjerpeseth LJ, Igland J, Selmer R, Ellekjær H, Tveit A, Berge T, et al. Prevalence and incidence rates of atrial fibrillation in Norway 2004–2014. Heart. 2021;107:201–7.

18. Laske TG, Iaizzo PA. The Cardiac Conduction System. In: Iaizzo PA, editor. Handbook of Cardiac Anatomy, Physiology, and Devices. Totowa, NJ: Humana Press; 2005. p. 123–36.

19. Iwasaki Y, Nishida K, Kato T, Nattel S. Atrial Fibrillation Pathophysiology. Circulation. 2011;124:2264–74.

20. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. European Heart Journal. 2021;42:373–498.

21. Dilaveris PE, Kennedy HL. Silent atrial fibrillation: epidemiology, diagnosis, and clinical impact. Clinical Cardiology. 2017;40:413–8.

22. Gutierrez C, Blanchard DG. Diagnosis and Treatment of Atrial Fibrillation. Am Fam Physician. 2016;94:442–52.

23. Jackson SL, Tong X, Yin X, George MG, Ritchey MD. Emergency Department, Hospital Inpatient, and Mortality Burden of Atrial Fibrillation in the United States, 2006 to 2014. The American Journal of Cardiology. 2017;120:1966–73.

24. Wasmer K, Eckardt L, Breithardt G. Predisposing factors for atrial fibrillation in the elderly. J Geriatr Cardiol. 2017;14:179–84.

25. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med. 1976;85:447–52.

26. Pedersen LR, Frestad D, Michelsen MM, Mygind ND, Rasmusen H, Suhrs HE, et al. Risk Factors for Myocardial Infarction in Women and Men: A Review of the Current Literature. Curr Pharm Des. 2016;22:3835–52.

27. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of Premature Natural and Surgical Menopause With Incident Cardiovascular Disease. JAMA. 2019;322:2411–21.

28. Scheuner MT, Wang SJ, Raffel LJ, Larabell SK, Rotter JI. Family history: a comprehensive genetic risk assessment method for the chronic conditions of adulthood. Am J Med Genet. 1997;71:315–24.

29. Miyazawa K, Ito K, Ito M, Zou Z, Kubota M, Nomura S, et al. Cross-ancestry genome-wide analysis of atrial fibrillation unveils disease biology and enables cardioembolic risk prediction. Nat Genet. 2023;55:187–97.

30. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364:937–52.

31. Elliott AD, Middeldorp ME, Van Gelder IC, Albert CM, Sanders P. Epidemiology and modifiable risk factors for atrial fibrillation. Nat Rev Cardiol. 2023;20:404–17.

32. Moliterno DJ, Willard JE, Lange RA, Negus BH, Boehrer JD, Glamann DB, et al. Coronary-Artery Vasoconstriction Induced by Cocaine, Cigarette Smoking, or Both. N Engl J Med. 1994;330:454–9.

33. Mostofsky E, van der Bom JG, Mukamal KJ, Maclure M, Tofler GH, Muller JE, et al. Risk of Myocardial Infarction Immediately After Alcohol Consumption. Epidemiology. 2015;26:143–50.

34. Aune D, Schlesinger S, Norat T, Riboli E. Tobacco smoking and the risk of atrial fibrillation: A systematic review and meta-analysis of prospective studies. Eur J Prev Cardiol. 2018;25:1437–51.

35. Goette A, Lendeckel U, Kuchenbecker A, Bukowska A, Peters B, Klein HU, et al. Cigarette smoking induces atrial fibrosis in humans via nicotine. Heart. 2007;93:1056–63.

36. Ettinger PO, Wu CF, Cruz CDL, Weisse AB, Sultan Ahmed S, Regan TJ. Arrhythmias and the “Holiday Heart”: Alcohol associated cardiac rhythm disorders. American Heart Journal. 1978;95:555–62.

37. Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, et al. Physical Inactivity Rapidly Induces Insulin Resistance and Microvascular Dysfunction in Healthy Volunteers. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007;27:2650–6.

38. Wu H, Ballantyne CM. Metabolic Inflammation and Insulin Resistance in Obesity. Circulation Research. 2020;126:1549–64.

39. Ofman P, Khawaja O, Rahilly-Tierney CR, Peralta A, Hoffmeister P, Reynolds MR, et al. Regular Physical Activity and Risk of Atrial Fibrillation. Circulation: Arrhythmia and Electrophysiology. 2013;6:252–6.

40. Newman W, Parry-Williams G, Wiles J, Edwards J, Hulbert S, Kipourou K, et al. Risk of atrial fibrillation in athletes: a systematic review and meta-analysis. Br J Sports Med. 2021;55:1233–8.

41. Wang TJ, Parise H, Levy D, D'Agostino RB, Wolf PA, Vasan RS, et al. Obesity and the Risk of New-Onset Atrial Fibrillation. JAMA. 2004;292:2471–7.

42. Willett WC. Dietary fats and coronary heart disease. Journal of Internal Medicine. 2012;272:13–24.

43. Watanabe H, Tanabe N, Watanabe T, Darbar D, Roden DM, Sasaki S, et al. Metabolic Syndrome and Risk of Development of Atrial Fibrillation. Circulation. 2008;117:1255–60.

44. Olafiranye O, Zizi F, Brimah P, Jean-louis G, Makaryus AN, McFarlane S, et al. Management of Hypertension among Patients with Coronary Heart Disease. Int J Hypertens. 2011;2011:653903.

45. Healey JS, Connolly SJ. Atrial fibrillation: hypertension as a causative agent, risk factor for complications, and potential therapeutic target. The American Journal of Cardiology. 2003;91:9–14.

46. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. European Heart Journal. 2017;38:2459–72.

47. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. The American Journal of Medicine. 1977;62:707–14.

48. Liang F, Wang Y. Coronary heart disease and atrial fibrillation: a vicious cycle. American Journal of Physiology-Heart and Circulatory Physiology. 2021;320:H1–12.

49. La Sala L, Prattichizzo F, Ceriello A. The link between diabetes and atherosclerosis. Eur J Prev Cardiolog. 2019;26 2_suppl:15–24.

50. Karam BS, Chavez-Moreno A, Koh W, Akar JG, Akar FG. Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes. Cardiovascular Diabetology. 2017;16:120.

51. Hu B, Li W, Wang X, Liu L, Teo K, Yusuf S. Marital Status, Education, and Risk of Acute Myocardial Infarction in Mainland China: The INTER-HEART Study. J Epidemiol. 2012;22:123–9.

52. Tetzlaff J, Tetzlaff F, Geyer S, Sperlich S, Epping J. Widening or narrowing income inequalities in myocardial infarction? Time trends in life years free of myocardial infarction and after incidence. Population Health Metrics. 2021;19:47.

53. Rozanski A, Blumenthal JA, Kaplan J. Impact of Psychological Factors on the Pathogenesis of Cardiovascular Disease and Implications for Therapy. Circulation. 1999;99:2192–217.

54. May AM, Van Wagoner DR, Mehra R. OSA and Cardiac Arrhythmogenesis: Mechanistic Insights. Chest. 2017;151:225–41.

55. Lévy S. Factors predisposing to the development of atrial fibrillation. Pacing Clin Electrophysiol. 1997;20 10 Pt 2:2670–4.

56. Brain Basics: Understanding Sleep | National Institute of Neurological Disorders and Stroke, National Institutes of Health. https://www.ninds.nih.gov/health-information/public-education/brain-basics/brain-basics-understanding-sleep. Accessed 26 May 2023.

57. Jung CM, Melanson EL, Frydendall EJ, Perreault L, Eckel RH, Wright KP. Energy expenditure during sleep, sleep deprivation and sleep following sleep deprivation in adult humans. J Physiol. 2011;589 Pt 1:235–44.

58. Frank MG. The Function of Sleep. In: Sleep: A Comprehensive Handbook. 2005. p. 45–8.

59. Porkka-Heiskanen T, Alanko L, Kalinchuk A, Stenberg D. Adenosine and sleep. Sleep Medicine Reviews. 2002;6:321–32.

60. Vitaterna MH, Takahashi JS, Turek FW. Overview of Circadian Rhythms. Alcohol Res Health. 2001;25:85–93.

61. Natural Patterns of Sleep | Healthy Sleep. A resource from the Division of Sleep Medicine at Harvard Medical School. https://healthysleep.med.harvard.edu/healthy/science/what/sleep-patterns-rem-nrem. Accessed 26 May 2023.

62. Berry R. Fundamentals of Sleep Medicine. Elsevier; 2012.

63. Buysse DJ. Sleep Health: Can We Define It? Does It Matter? Sleep. 2014;37:9–17.

64. Vgontzas AN, Fernandez-Mendoza J, Liao D, Bixler EO. Insomnia with objective short sleep duration: The most biologically severe phenotype of the disorder. Sleep Medicine Reviews. 2013;17:241–54.

65. American Academy of Sleep Medicine. International Classification of Sleep Disorders, 3rd ed. (ICSD-3). Darien, IL: American Academy of Sleep Medicine; 2014.

66. Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed. Arlington, VA, US: American Psychiatric Publishing, Inc.; 2013.

67. Baglioni C, Altena E, Bjorvatn B, Blom K, Bothelius K, Devoto A, et al. The European Academy for Cognitive Behavioural Therapy for Insomnia: An initiative of the European

Insomnia Network to promote implementation and dissemination of treatment. Journal of Sleep Research. 2020;29:e12967.

68. Riemann D, Baglioni C, Bassetti C, Bjorvatn B, Dolenc Groselj L, Ellis JG, et al. European guideline for the diagnosis and treatment of insomnia. Journal of Sleep Research. 2017;26:675–700.

69. Sivertsen B. Sleep problems in Norway. In: Public Health Report - Health Status in Norway. Oslo: Norwegian Institute of Public Health [updated (20 May 2019)]. https://www.fhi.no/en/op/hin/mental-health/sleep-problems/. Accessed 27 May 2023.

70. Sivertsen B, Pallesen S, Friborg O, Nilsen KB, Bakke ØK, Goll JB, et al. Sleep patterns and insomnia in a large population-based study of middle-aged and older adults: The Tromsø study 2015–2016. Journal of Sleep Research. 2021;30:e13095.

71. Sivertsen B, Vedaa Ø, Harvey AG, Glozier N, Pallesen S, Aarø LE, et al. Sleep patterns and insomnia in young adults: A national survey of Norwegian university students. Journal of Sleep Research. 2019;28:e12790.

72. Grandner MA, Chakravorty S. Insomnia in Primary Care: Misreported, Mishandled, and Just Plain Missed. J Clin Sleep Med. 2017;13:937–9.

73. Morin CM, Belleville G, Bélanger L, Ivers H. The Insomnia Severity Index: Psychometric Indicators to Detect Insomnia Cases and Evaluate Treatment Response. Sleep. 2011;34:601–8.

74. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28:193–213.

75. Pallesen S, Bjorvatn B, Nordhus IH, Sivertsen B, Hjørnevik M, Morin CM. A New Scale for Measuring Insomnia: The Bergen Insomnia Scale. Percept Mot Skills. 2008;107:691–706.

76. Chaput J-P, Dutil C, Sampasa-Kanyinga H. Sleeping hours: what is the ideal number and how does age impact this? Nat Sci Sleep. 2018;10:421–30.

77. Horne J. Why we sleep: The functions of sleep in humans and other mammals. New York, NY, US: Oxford University Press; 1988.

78. Bonnet MH, Arand DL. We are Chronically Sleep Deprived. Sleep. 1995;18:908–11.

79. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation's updated sleep duration recommendations: final report. Sleep Health: Journal of the National Sleep Foundation. 2015;1:233–43.

80. Consensus Conference Panel, Watson Nathaniel F., Badr M. Safwan, Belenky Gregory, Bliwise Donald L., Buxton Orfeu M., et al. Recommended Amount of Sleep for a Healthy

Adult: A Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society. Journal of Clinical Sleep Medicine. 11:591–2.

81. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. Sleep Medicine. 2001;2:389–96.

82. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian Typology: A Comprehensive Review. Chronobiology International. 2012;29:1153–75.

83. Fischer D, Lombardi DA, Marucci-Wellman H, Roenneberg T. Chronotypes in the US - Influence of age and sex. PLoS One. 2017;12:e0178782.

84. Duffy JF, Zitting K-M, Chinoy ED. Aging and Circadian Rhythms. Sleep Med Clin. 2015;10:423–34.

85. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4:97–110.

86. Roenneberg T, Wirz-Justice A, Merrow M. Life between Clocks: Daily Temporal Patterns of Human Chronotypes. J Biol Rhythms. 2003;18:80–90.

87. Berne RM, Levy MN. Cardiovascular Physiology. 8th Edition. St. Louis: Mosby Inc.; 2001.

88. Vlahandonis A, Walter LM, Yiallourou SR, Horne RSC. Autonomic Cardiovascular Regulation During Sleep. In: Kheirandish-Gozal L, Gozal D, editors. Sleep Disordered Breathing in Children: A Comprehensive Clinical Guide to Evaluation and Treatment. Totowa, NJ: Humana Press; 2012. p. 85–103.

89. Boudreau P, Yeh W-H, Dumont GA, Boivin DB. Circadian Variation of Heart Rate Variability Across Sleep Stages. Sleep. 2013;36:1919–28.

90. Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N, et al. Autonomic activity during human sleep as a function of time and sleep stage. Journal of Sleep Research. 2001;10:253–64.

91. Carrington MJ, Barbieri R, Colrain IM, Crowley KE, Kim Y, Trinder J. Changes in cardiovascular function during the sleep onset period in young adults. Journal of Applied Physiology. 2005;98:468–76.

92. Loredo JS, Nelesen R, Ancoli-Israel S, Dimsdale JE. Sleep Quality and Blood Pressure Dipping in Normal Adults. Sleep. 2004;27:1097–103.

93. Murali NS, Svatikova A, Somers VK. Cardiovascular physiology and sleep. Front Biosci. 2003;8:s636-652.

94. Sofi F, Cesari F, Casini A, Macchi C, Abbate R, Gensini GF. Insomnia and risk of cardiovascular disease: a meta-analysis. European Journal of Preventive Cardiology. 2014;21:57–64.

95. Ge L, Guyatt G, Tian J, Pan B, Chang Y, Chen Y, et al. Insomnia and risk of mortality from all-cause, cardiovascular disease, and cancer: Systematic review and meta-analysis of prospective cohort studies. Sleep Medicine Reviews. 2019;48:101215.

96. Larsson SC, Markus HS. Genetic Liability to Insomnia and Cardiovascular Disease Risk. Circulation. 2019;140:796–8.

97. Liu X, Li C, Sun X, Yu Y, Si S, Hou L, et al. Genetically Predicted Insomnia in Relation to 14 Cardiovascular Conditions and 17 Cardiometabolic Risk Factors: A Mendelian Randomization Study. J Am Heart Assoc. 2021;10:e020187.

98. Yuan S, Mason AM, Burgess S, Larsson SC. Genetic liability to insomnia in relation to cardiovascular diseases: a Mendelian randomisation study. Eur J Epidemiol. 2021;36:393–400.

99. Wang D, Li W, Cui X, Meng Y, Zhou M, Xiao L, et al. Sleep duration and risk of coronary heart disease: A systematic review and meta-analysis of prospective cohort studies. International Journal of Cardiology. 2016;219:231–9.

100. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. Eur Heart J. 2011;32:1484–92.

101. Itani O, Jike M, Watanabe N, Kaneita Y. Short sleep duration and health outcomes: a systematic review, meta-analysis, and meta-regression. Sleep Medicine. 2017;32:246–56.

102. Jike M, Itani O, Watanabe N, Buysse DJ, Kaneita Y. Long sleep duration and health outcomes: A systematic review, meta-analysis and meta-regression. Sleep Medicine Reviews. 2018;39:25–36.

103. Krittanawong C, Tunhasiriwet A, Wang Z, Zhang H, Farrell AM, Chirapongsathorn S, et al. Association between short and long sleep durations and cardiovascular outcomes: a systematic review and meta-analysis. European Heart Journal Acute Cardiovascular Care. 2019;8:762–70.

104. Ai S, Zhang J, Zhao G, Wang N, Li G, So H-C, et al. Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK Biobank. European Heart Journal. 2021;42:3349–57.

105. Nikbakhtian S, Reed AB, Obika BD, Morelli D, Cunningham AC, Aral M, et al. Accelerometer-derived sleep onset timing and cardiovascular disease incidence: a UK Biobank cohort study. European Heart Journal - Digital Health. 2021. https://doi.org/10.1093/ehjdh/ztab088.

106. Yang Y, Fan J, Shi X, Wang Y, Yang C, Lian J, et al. Causal associations between sleep traits and four cardiac diseases: a Mendelian randomization study. ESC Heart Failure. 2022;9:3160–6.

107. Knutson KL, Turek FW. The U-shaped association between sleep and health: the 2 peaks do not mean the same thing. Sleep. 2006;29:878–9.

108. Merikanto I, Lahti T, Puolijoki H, Vanhala M, Peltonen M, Laatikainen T, et al. Associations of Chronotype and Sleep With Cardiovascular Diseases and Type 2 Diabetes. Chronobiology International. 2013;30:470–7.

109. Makarem N, Paul J, Giardina E-GV, Liao M, Aggarwal B. Evening chronotype is associated with poor cardiovascular health and adverse health behaviors in a diverse population of women. Chronobiology International. 2020;37:673–85.

110. Fan M, Sun D, Zhou T, Heianza Y, Lv J, Li L, et al. Sleep patterns, genetic susceptibility, and incident cardiovascular disease: a prospective study of 385 292 UK biobank participants. European Heart Journal. 2020;41:1182–9.

111. Sands-Lincoln M, Loucks EB, Lu B, Carskadon MA, Sharkey K, Stefanick ML, et al. Sleep Duration, Insomnia, and Coronary Heart Disease Among Postmenopausal Women in the Women's Health Initiative. J Womens Health (Larchmt). 2013;22:477–86.

112. Bertisch SM, Pollock BD, Mittleman MA, Buysse DJ, Bazzano LA, Gottlieb DJ, et al. Insomnia with objective short sleep duration and risk of incident cardiovascular disease and all-cause mortality: Sleep Heart Health Study. Sleep. 2018;41.

113. Westerlund A, Bellocco R, Sundström J, Adami H-O, Åkerstedt T, Trolle Lagerros Y. Sleep characteristics and cardiovascular events in a large Swedish cohort. Eur J Epidemiol. 2013;28:463–73.

114. Chien K-L, Chen P-C, Hsu H-C, Su T-C, Sung F-C, Chen M-F, et al. Habitual Sleep Duration and Insomnia and the Risk of Cardiovascular Events and All-cause Death: Report from a Community-Based Cohort. Sleep. 2010;33:177–84.

115. Chandola T, Ferrie JE, Perski A, Akbaraly T, Marmot MG. The effect of short sleep duration on coronary heart disease risk is greatest among those with sleep disturbance: a prospective study from the Whitehall II cohort. Sleep. 2010;33:739–44.

116. Vgontzas AN, Liao D, Pejovic S, Calhoun S, Karataraki M, Bixler EO. Insomnia With Objective Short Sleep Duration Is Associated With Type 2 Diabetes: A population-based study. Diabetes Care. 2009;32:1980–5.

117. Bathgate CJ, Edinger JD, Wyatt JK, Krystal AD. Objective but Not Subjective Short Sleep Duration Associated with Increased Risk for Hypertension in Individuals with Insomnia. Sleep. 2016;39:1037–45.

118. Fernandez-Mendoza J, Vgontzas AN, Liao D, Shaffer ML, Vela-Bueno A, Basta M, et al. Insomnia With Objective Short Sleep Duration and Incident Hypertension. Hypertension. 2012;60:929–35.

119. Johann AF, Hertenstein E, Kyle SD, Baglioni C, Feige B, Nissen C, et al. Insomnia with objective short sleep duration is associated with longer duration of insomnia in the Freiburg Insomnia Cohort compared to insomnia with normal sleep duration, but not with hypertension. PLOS ONE. 2017;12:e0180339.

120. Dean YE, Shebl MA, Rouzan SS, Bamousa BAA, Talat NE, Ansari SA, et al. Association between insomnia and the incidence of myocardial infarction: A systematic review and meta-analysis. Clinical Cardiology. 2023;46:376–85.

121. Laugsand LE, Vatten LJ, Platou C, Janszky I. Insomnia and the Risk of Acute Myocardial Infarction. Circulation. 2011;124:2073–81.

122. Daghlas I, Dashti HS, Lane J, Aragam KG, Rutter MK, Saxena R, et al. Sleep Duration and Myocardial Infarction. J Am Coll Cardiol. 2019;74:1304–14.

123. Fan Y, Wu Y, Peng Y, Zhao B, Yang J, Bai L, et al. Sleeping Late Increases the Risk of Myocardial Infarction in the Middle-Aged and Older Populations. Frontiers in Cardiovascular Medicine. 2021;8.

124. Kalmbach DA, Pillai V, Arnedt JT, Drake CL. DSM-5 Insomnia and Short Sleep: Comorbidity Landscape and Racial Disparities. Sleep. 2016;39:2101–11.

125. Lee H-H, Chen Y-C, Chen J-J, Lo S-H, Guo Y-L, Hu H-Y. Insomnia and the Risk of Atrial Fibrillation: A Population-Based Cohort Study. Acta Cardiol Sin. 2017;33:165–72.

126. Li X, Zhou T, Ma H, Huang T, Gao X, Manson JE, et al. Healthy Sleep Patterns and Risk of Incident Arrhythmias. Journal of the American College of Cardiology. 2021;78:1197–207.

127. Arafa A, Kokubo Y, Shimamoto K, Kashima R, Watanabe E, Sakai Y, et al. Sleep duration and atrial fibrillation risk in the context of predictive, preventive, and personalized medicine: the Suita Study and meta-analysis of prospective cohort studies. EPMA J. 2022;13:77–86.

128. Zhao J, Yang F, Zhuo C, Wang Q, Qu Z, Wang Q, et al. Association of Sleep Duration With Atrial Fibrillation and Heart Failure: A Mendelian Randomization Analysis. Frontiers in Genetics. 2021;12.

129. Hsieh CG, Martin JL. Short Sleep, Insomnia, and Cardiovascular Disease. Curr Sleep Med Rep. 2019;5:234–42.

130. Grimaldi D, Carter JR, Van Cauter E, Leproult R. Adverse Impact of Sleep Restriction and Circadian Misalignment on Autonomic Function in Healthy Young Adults. Hypertension. 2016;68:243–50.

131. Castro-Diehl C, Diez Roux AV, Redline S, Seeman T, McKinley P, Sloan R, et al. Sleep Duration and Quality in Relation to Autonomic Nervous System Measures: The Multi-Ethnic Study of Atherosclerosis (MESA). Sleep. 2016;39:1927–40.

132. Jarrin DC, Ivers H, Lamy M, Chen IY, Harvey AG, Morin CM. Cardiovascular autonomic dysfunction in insomnia patients with objective short sleep duration. Journal of Sleep Research. 2018;27:e12663.

133. Vgontzas AN, Zoumakis E, Bixler EO, Lin H-M, Follett H, Kales A, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab. 2004;89:2119–26.

134. Broussard JL, Kilkus JM, Delebecque F, Abraham V, Day A, Whitmore HR, et al. Elevated ghrelin predicts food intake during experimental sleep restriction. Obesity (Silver Spring). 2016;24:132–8.

135. Kjeldsen JS, Hjorth MF, Andersen R, Michaelsen KF, Tetens I, Astrup A, et al. Short sleep duration and large variability in sleep duration are independently associated with dietary risk factors for obesity in Danish school children. Int J Obes (Lond). 2014;38:32–9.

136. Broussard JL, Ehrmann DA, Van Cauter E, Tasali E, Brady MJ. Impaired insulin signaling in human adipocytes after experimental sleep restriction: a randomized, crossover study. Ann Intern Med. 2012;157:549–57.

137. Lanfranchi PA, Pennestri M-H, Fradette L, Dumont M, Morin CM, Montplaisir J. Nighttime blood pressure in normotensive subjects with chronic insomnia: implications for cardiovascular risk. Sleep. 2009;32:760–6.

138. Fernandez-Mendoza J, He F, Vgontzas AN, Liao D, Bixler EO. Objective short sleep duration modifies the relationship between hypertension and all-cause mortality. J Hypertens. 2017;35:830–6.

139. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications. Vascular Pharmacology. 2016;77:1–7.

140. Kanagasabai T, Ardern CI. Contribution of Inflammation, Oxidative Stress, and Antioxidants to the Relationship between Sleep Duration and Cardiometabolic Health. Sleep. 2015;38:1905–12.

141. D'Aurea C, Poyares D, Piovezan RD, Passos GS, Tufik S, Mello MT de. Objective short sleep duration is associated with the activity of the hypothalamic-pituitary-adrenal axis in insomnia. Arq Neuro-Psiquiatr. 2015;73:516–9.

142. Charloux A, Gronfier C, Lonsdorfer-Wolf E, Piquard F, Brandenberger G. Aldosterone release during the sleep-wake cycle in humans. American Journal of Physiology-Endocrinology and Metabolism. 1999;276:E43–9.

143. Xu Y, Sharma D, Li G, Liu Y. Atrial remodeling: New pathophysiological mechanism of atrial fibrillation. Medical Hypotheses. 2013;80:53–6.

144. Guo Y, Lip GYH, Apostolakis S. Inflammation in Atrial Fibrillation. Journal of the American College of Cardiology. 2012;60:2263–70.

145. Chen P-S, Chen LS, Fishbein MC, Lin S-F, Nattel S. Role of the Autonomic Nervous System in Atrial Fibrillation. Circulation Research. 2014;114:1500–15.

146. Zhang L, Hou Y, Po SS. Obstructive Sleep Apnoea and Atrial Fibrillation. Arrhythm Electrophysiol Rev. 2015;4:14–8.

147. Grandner MA, Drummond SPA. Who Are the Long Sleepers? Towards an Understanding of the Mortality Relationship. Sleep Med Rev. 2007;11:341–60.

148. Chouchou F, Pichot V, Pépin JL, Tamisier R, Celle S, Maudoux D, et al. Sympathetic overactivity due to sleep fragmentation is associated with elevated diurnal systolic blood pressure in healthy elderly subjects: the PROOF-SYNAPSE study. European Heart Journal. 2013;34:2122–31.

149. Vallat R, Shah VD, Redline S, Attia P, Walker MP. Broken sleep predicts hardened blood vessels. PLoS Biol. 2020;18:e3000726.

150. Khanji MY, Karim S, Cooper J, Chahal A, Aung N, Somers VK, et al. Impact of Sleep Duration and Chronotype on Cardiac Structure and Function: The UK Biobank Study. Current Problems in Cardiology. 2023;48:101688.

151. Porta M. A dictionary of epidemiology. Oxford university press; 2014.

152. Conroy M, Sellors J, Effingham M, Littlejohns TJ, Boultwood C, Gillions L, et al. The advantages of UK Biobank's open-access strategy for health research. J Intern Med. 2019;286:389–97.

153. Krokstad S, Langhammer A, Hveem K, Holmen T, Midthjell K, Stene T, et al. Cohort Profile: The HUNT Study, Norway. International Journal of Epidemiology. 2013;42:968–77.

154. Andersson C, Johnson AD, Benjamin EJ, Levy D, Vasan RS. 70-year legacy of the Framingham Heart Study. Nat Rev Cardiol. 2019;16:687–98.

155. Hill AB. The Environment and Disease: Association or Causation? Proc R Soc Med. 1965;58:295–300.

156. Krieger N, Davey Smith G. The tale wagged by the DAG: broadening the scope of causal inference and explanation for epidemiology. Int J Epidemiol. 2016;45:1787–808.

157. Davey Smith G. Post–Modern Epidemiology: When Methods Meet Matter. American Journal of Epidemiology. 2019;188:1410–9.

158. Hannan EL. Randomized Clinical Trials and Observational Studies: Guidelines for Assessing Respective Strengths and Limitations. JACC: Cardiovascular Interventions. 2008;1:211–7.

159. Grimes DA, Schulz KF. Bias and causal associations in observational research. The Lancet. 2002;359:248–52.

160. Jones DS, Podolsky SH. The history and fate of the gold standard. The Lancet. 2015;385:1502–3.

161. Zabor EC, Kaizer AM, Hobbs BP. Randomized Controlled Trials. Chest. 2020;158:S79–87.

162. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nat Genet. 2018;50:1412–25.

163. Sollis E, Mosaku A, Abid A, Buniello A, Cerezo M, Gil L, et al. The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. Nucleic Acids Research. 2023;51:D977–85.

164. Maher BS. Polygenic Scores in Epidemiology: Risk Prediction, Etiology, and Clinical Utility. Curr Epidemiol Rep. 2015;2:239–44.

165. Dudbridge F. Polygenic Epidemiology. Genet Epidemiol. 2016;40:268–72.

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

167. Davey Smith G, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. International Journal of Epidemiology. 2004;33:30–42.

168. Hingorani A, Humphries S. Nature's randomised trials. The Lancet. 2005;366:1906–8.

169. Swanson SA, Tiemeier H, Ikram MA, Hernán MA. Nature as a trialist? Deconstructing the analogy between Mendelian Randomization and randomized trials. Epidemiology. 2017;28:653–9.

170. VanderWeele TJ, Tchetgen Tchetgen EJ, Cornelis M, Kraft P. Methodological Challenges in Mendelian Randomization. Epidemiology. 2014;25:427.

171. Davies NM, Holmes MV, Smith GD. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362:k601.

172. Lawlor DA, Wade K, Borges MC, Palmer T, Hartwig FP, Hemani G, et al. A Mendelian Randomization dictionary: Useful definitions and descriptions for undertaking, understanding and interpreting Mendelian Randomization studies. OSF Preprints. 2019.

173. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian randomization. International Journal of Epidemiology. 2013;42:1134–44.

174. Pierce BL, Burgess S. Efficient Design for Mendelian Randomization Studies: Subsample and 2-Sample Instrumental Variable Estimators. American Journal of Epidemiology. 2013;178:1177–84.

175. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013;37:658–65.

176. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23:R89-98.

177. Rees JMB, Foley CN, Burgess S. Factorial Mendelian randomization: using genetic variants to assess interactions. International Journal of Epidemiology. 2020;49:1147–58.

178. North T-L, Davies NM, Harrison S, Carter AR, Hemani G, Sanderson E, et al. Using Genetic Instruments to Estimate Interactions in Mendelian Randomization Studies. Epidemiology. 2019;30:e33–5.

179. Burgess S, Thompson SG. Multivariable Mendelian Randomization: The Use of Pleiotropic Genetic Variants to Estimate Causal Effects. American Journal of Epidemiology. 2015;181:251–60.

180. Stampfer MJ, Buring JE, Willett W, Rosner B, Eberlein K, Hennekens CH. The 2 x 2 factorial design: its application to a randomized trial of aspirin and carotene in U.S. physicians. Stat Med. 1985;4:111–6.

181. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. International Journal of Epidemiology. 2016;45:1866–86.

182. UK Biobank: Protocol for a large-scale prospective epidemiological resource. 2006. https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us. Accessed 25 Oct 2021.

183. Hewitt J, Walters M, Padmanabhan S, Dawson J. Cohort profile of the UK Biobank: Diagnosis and characteristics of cerebrovascular disease. BMJ Open. 2016;6:e009161.

184. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018;562:203–9.

185. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLOS Medicine. 2015;12:e1001779.

186. Åsvold BO, Langhammer A, Rehn TA, Kjelvik G, Grøntvedt TV, Sørgjerd EP, et al. Cohort Profile Update: The HUNT Study, Norway. International Journal of Epidemiology. 2023;52:e80–91.

187. Haugen MO. Trøndelag. Store norske leksikon. 2023.

188. Holmen J, Midthjell K, Krüger Ø, Langhammer A, Holmen TL, Bratberg GH, et al. The Nord-Trøndelag Health Study 1995–97 (HUNT 2): objectives, contents, methods and participation. Norsk epidemiologi. 2003;13:19–32.

189. Mitchell RE, Hemani G, Dudding T, Corbin L, Harrison S, Paternoster L. UK Biobank Genetic Data: MRC-IEU Quality Control, version 2. 2019. https://data.bris.ac.uk/data/dataset/1ovaau5sxunp2cv8rcy88688v.

190. Brumpton BM, Graham S, Surakka I, Skogholt AH, Løset M, Fritsche LG, et al. The HUNT study: A population-based cohort for genetic research. Cell Genomics. 2022;2:100193.

191. Jansen PR, Watanabe K, Stringer S, Skene N, Bryois J, Hammerschlag AR, et al. Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways. Nat Genet. 2019;51:394–403.

192. Dashti HS, Jones SE, Wood AR, Lane JM, van Hees VT, Wang H, et al. Genome-wide association study identifies genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates. Nat Commun. 2019;10:1100.

193. Jones SE, Lane JM, Wood AR, van Hees VT, Tyrrell J, Beaumont RN, et al. Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms. Nat Commun. 2019;10:343.

194. World Health Organization (WHO). International Classification of Diseases (ICD). https://www.who.int/standards/classifications/classification-of-diseases. Accessed 4 Jun 2023.

195. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381–95.

196. International Physical Activity Questionnaire (IPAQ): Guidelines for data processing analysis of the International Physical Activity Questionnaire (IPAQ) - Short and long forms. 2005. https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=540. Accessed 25 Oct 2021.

197. International Physical Activity Questionnaire (IPAQ): IPAQ scoring protocol. 2005. https://sites.google.com/site/theipaq/scoring-protocol. Accessed 25 Oct 2021.

198. Brumpton BM, Langhammer A, Ferreira MAR, Chen Y, Mai X-M. Physical activity and incident asthma in adults: the HUNT Study, Norway. BMJ Open. 2016;6:e013856.

199. Kurtze N, Rangul V, Hustvedt BE, Flanders WD. Reliability and validity of self-reported physical activity in the Nord-Trøndelag Health Study (HUNT 2). Eur J Epidemiol. 2007;22:379–87.

200. Townsend P. Poverty in the United Kingdom: A Survey of Household Resources and Standards of Living. London: Allen Lane and Penguin Books; 1979.

201. Elliott P, Peakman TC, UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International Journal of Epidemiology. 2008;37:234–44.

202. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67:361–70.

203. Skapinakis P. Hospital Anxiety and Depression Scale (HADS). In: Michalos AC, editor. Encyclopedia of Quality of Life and Well-Being Research. Dordrecht: Springer Netherlands; 2014. p. 2930–3.

204. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res. 2002;52:69–77.

205. Mykletun A, Stordal E, Dahl AA. Hospital Anxiety and Depression (HAD) scale: factor structure, item analyses and internal consistency in a large population. Br J Psychiatry. 2001;179:540–4.

206. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol. 2005;20:575–9.

207. Hosmer DW, Lemeshow S. Confidence Interval Estimation of Interaction. Epidemiology. 1992;3:452.

208. Knol MJ, VanderWeele TJ, Groenwold RHH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. Eur J Epidemiol. 2011;26:433–8.

209. VanderWeele TJ, Knol MJ. A Tutorial on Interaction. Epidemiologic Methods. 2014;3.

210. de Mutsert R, Jager KJ, Zoccali C, Dekker FW. The effect of joint exposures: examining the presence of interaction. Kidney International. 2009;75:677–81.

211. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. Stat Methods Med Res. 2017;26:2333–55.

212. Burgess S, Labrecque JA. Mendelian randomization with a binary exposure variable: interpretation and presentation of causal estimates. Eur J Epidemiol. 2018;33:947–52.

213. Koyanagi A, Garin N, Olaya B, Ayuso-Mateos JL, Chatterji S, Leonardi M, et al. Chronic Conditions and Sleep Problems among Adults Aged 50 years or over in Nine Countries: A Multi-Country Study. PLoS One. 2014;9:e114742.

214. Minelli C, Del Greco M. F, van der Plaat DA, Bowden J, Sheehan NA, Thompson J. The use of two-sample methods for Mendelian randomization analyses on single large datasets. International Journal of Epidemiology. 2021;50:1651–9.

215. Barry C, Liu J, Richmond R, Rutter MK, Lawlor DA, Dudbridge F, et al. Exploiting collider bias to apply two-sample summary data Mendelian randomization methods to one-sample individual level data. PLOS Genetics. 2021;17:e1009703.

216. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. International Journal of Epidemiology. 2015;44:512–25.

217. 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;40:304–14.

218. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. International Journal of Epidemiology. 2017;46:1985–98.

219. Lane JM, Jones SE, Dashti HS, Wood AR, Aragam KG, van Hees VT, et al. Biological and clinical insights from genetics of insomnia symptoms. Nat Genet. 2019;51:387–93.

220. Knol MJ, van der Tweel I, Grobbee DE, Numans ME, Geerlings MI. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. Int J Epidemiol. 2007;36:1111–8.

221. Rothman K, Greenland S, Lash TL. Modern Epidemiology. 3rd Edition. Philadelphia, US: Lippincott Williams & Wilkins; 2007.

222. Sanderson E, Glymour MM, Holmes MV, Kang H, Morrison J, Munafò MR, et al. Mendelian randomization. Nature Reviews Methods Primers. 2022;2.

223. Zheng J, Baird D, Borges M-C, Bowden J, Hemani G, Haycock P, et al. Recent Developments in Mendelian Randomization Studies. Curr Epidemiol Rep. 2017;4:330–45.

224. Pierce BL, Ahsan H, VanderWeele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. International Journal of Epidemiology. 2011;40:740–52.

225. Moreira MJ, Porter JR, Suarez GA. Bootstrap validity for the score test when instruments may be weak. Journal of Econometrics. 2009;149:52–64.

226. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. American Journal of Epidemiology. 2017;186:1026–34.

227. Little R, Rubin D. Statistical Analysis with Missing Data. 3rd Edition. Hoboken, NJ, USA: Wiley; 2019.

228. Puddu PE, Amaduzzi PL, Ricci B. Coronary heart disease incidence and competing risks: an important issue. J Geriatr Cardiol. 2017;14:425–9.

229. Gkatzionis A, Burgess S. Contextualizing selection bias in Mendelian randomization: how bad is it likely to be? International Journal of Epidemiology. 2019;48:691–701.

230. Littner M, Hirshkowitz M, Kramer M, Kapen S, Anderson WM, Bailey D, et al. Practice parameters for using polysomnography to evaluate insomnia: an update. Sleep. 2003;26:754–60.

231. Edwards BJ, Haynes C, Levenstien MA, Finch SJ, Gordon D. Power and sample size calculations in the presence of phenotype errors for case/control genetic association studies. BMC Genetics. 2005;6:18.

232. Tudball MJ, Bowden J, Hughes RA, Ly A, Munafò MR, Tilling K, et al. Mendelian randomisation with coarsened exposures. Genetic Epidemiology. 2021;45:338–50.

233. Hung J, Whitford EG, Hillman DR, Parsons RW. Association of sleep apnoea with myocardial infarction in men. The Lancet. 1990;336:261–4.

234. Benetó A, Gomez-Siurana E, Rubio-Sanchez P. Comorbidity between sleep apnea and insomnia. Sleep Medicine Reviews. 2009;13:287–93.

235. Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. Sleep Medicine Reviews. 2002;6:97–111.

236. Krell SB, Kapur VK. Insomnia complaints in patients evaluated for obstructive sleep apnea. Sleep Breath. 2005;9:104–10.

237. Brumpton B, Sanderson E, Heilbron K, Hartwig FP, Harrison S, Vie GÅ, et al. Avoiding dynastic, assortative mating, and population stratification biases in Mendelian randomization through within-family analyses. Nat Commun. 2020;11:3519.

238. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;38:904–9.

239. Haworth S, Mitchell R, Corbin L, Wade KH, Dudding T, Budu-Aggrey A, et al. Apparent latent structure within the UK Biobank sample has implications for epidemiological analysis. Nat Commun. 2019;10:333.

240. Lawson DJ, Davies NM, Haworth S, Ashraf B, Howe L, Crawford A, et al. Is population structure in the genetic biobank era irrelevant, a challenge, or an opportunity? Hum Genet. 2020;139:23–41.

241. Kong A, Thorleifsson G, Frigge ML, Vilhjalmsson BJ, Young AI, Thorgeirsson TE, et al. The nature of nurture: Effects of parental genotypes. Science. 2018;359:424–8.

242. Robinson MR, Kleinman A, Graff M, Vinkhuyzen AAE, Couper D, Miller MB, et al. Genetic evidence of assortative mating in humans. Nat Hum Behav. 2017;1:1–13.

243. Hartwig FP, Davies NM, Davey Smith G. Bias in Mendelian randomization due to assortative mating. Genetic Epidemiology. 2018;42:608–20.

244. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in Mendelian randomization studies. Human Molecular Genetics. 2018;27:R195–208.

245. Burgess S, Thompson SG, CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. International Journal of Epidemiology. 2011;40:755–64.

246. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet. 2003;33:177–82.

247. Zhong H, Prentice RL. Correcting “winner’s curse” in odds ratios from genomewide association findings for major complex human diseases. Genetic Epidemiology. 2010;34:78–91.

248. Taylor AE, Davies NM, Ware JJ, VanderWeele T, Smith GD, Munafò MR. Mendelian randomization in health research: Using appropriate genetic variants and avoiding biased estimates. Economics & Human Biology. 2014;13:99–106.

249. Sivertsen B, Øverland S, Krokstad S, Mykletun A. Seasonal Variations in Sleep Problems at Latitude 63°–65° in Norway: The Nord-Trøndelag Health Study, 1995–1997. American Journal of Epidemiology. 2011;174:147–53.

250. Zheng B, Yu C, Lv J, Guo Y, Bian Z, Zhou M, et al. Insomnia symptoms and risk of cardiovascular diseases among 0.5 million adults: A 10-year cohort. Neurology. 2019;93:e2110–20.

251. Hsu C-Y, Chen Y-T, Chen M-H, Huang C-C, Chiang C-H, Huang P-H, et al. The Association Between Insomnia and Increased Future Cardiovascular Events: A Nationwide Population-Based Study. Psychosomatic Medicine. 2015;77:743.

252. Chung W-S, Lin C-L, Chen Y-F, Chiang JY, Sung F-C, Chang Y-J, et al. Sleep Disorders and Increased Risk of Subsequent Acute Coronary Syndrome in Individuals without Sleep Apnea: A Nationwide Population-Based Cohort Study. Sleep. 2013;36:1963–8.

253. Meisinger C, Heier M, Löwel H, Schneider A, Döring A. Sleep Duration and Sleep Complaints and Risk of Myocardial Infarction in Middle-aged Men and Women from the General Population: The MONICA/KORA Augsburg Cohort Study. Sleep. 2007;30:1121–7.

254. Ford ES, Cunningham TJ, Croft JB. Trends in Self-Reported Sleep Duration among US Adults from 1985 to 2012. Sleep. 2015;38:829–32.

255. Stamatakis KA, Punjabi NM. Long sleep duration: A risk to health or a marker of risk? Sleep medicine reviews. 2007;11:337.

256. Khawaja O, Sarwar A, Albert CM, Gaziano JM, Djoussé L. Sleep Duration and Risk of Atrial Fibrillation (from the Physicians' Health Study). The American Journal of Cardiology. 2013;111:547–51.

257. Genuardi MV, Ogilvie RP, Saand AR, DeSensi RS, Saul MI, Magnani JW, et al. Association of Short Sleep Duration and Atrial Fibrillation. Chest. 2019;156:544–52.

258. Song Q, Liu X, Hu W, Zhou W, Liu A, Wang X, et al. Long Sleep Duration Is an Independent Risk Factor for Incident Atrial Fibrillation in a Chinese Population: A Prospective Cohort Study. Sci Rep. 2017;7:3679.

259. Davies NM, Howe LJ, Brumpton B, Havdahl A, Evans DM, Davey Smith G. Within family Mendelian randomization studies. Human Molecular Genetics. 2019;28:R170–9.

Papers I – III and appendices

Arora N, Richmond RC, Brumpton BM, Åsvold BO, Dalen H, Skarpsno ES, Strand LB.

Self-reported insomnia symptoms, sleep duration, chronotype and the risk of acute myocardial infarction (AMI): a prospective study in the UK Biobank and the HUNT Study

Eur J Epidemiol. 2023 Jun;38(6):643-656
doi: 10.1007/s10654-023-00981-x

Paper I

Paper I

European Journal of Epidemiology (2023) 38:643–656
https://doi.org/10.1007/s10654-023-00981-x

CARDIOVASCULAR DISEASE

Self-reported insomnia symptoms, sleep duration, chronotype and the risk of acute myocardial infarction (AMI): a prospective study in the UK Biobank and the HUNT Study

Nikhil Arora[1,2] · Rebecca Claire Richmond[2,3] · Ben Michael Brumpton[1,4,5] · Bjørn Olav Åsvold[1,5,6] · Håvard Dalen[7,8,9] · Eivind Schjelderup Skarpsno[10,11] · Linn Beate Strand[10]

Received: 8 August 2022 / Accepted: 25 February 2023 / Published online: 27 March 2023

Abstract
Insomnia and short/long sleep duration increase the risk of AMI, but their interaction with each other or with chronotype is not well known. We investigated the prospective joint associations of any two of these sleep traits on risk of AMI. We included 302 456 and 31 091 participants without past AMI episodes from UK Biobank (UKBB; 2006–10) and the Trøndelag Health Study (HUNT2; 1995–97), respectively. A total of 6 833 and 2 540 incident AMIs were identified during an average 11.7 and 21.0 years follow-up, in UKBB and HUNT2, respectively. Compared to those who reported normal sleep duration (7–8 h) without insomnia symptoms, the Cox proportional hazard ratios (HRs) for incident AMI in UKBB among participants who reported normal, short and long sleep duration with insomnia symptoms were 1.07 (95% CI 0.99, 1.15), 1.16 (95% CI 1.07, 1.25) and 1.40 (95% CI 1.21, 1.63), respectively. The corresponding HRs in HUNT2 were 1.09 (95% CI 0.95, 1.25), 1.17 (95% CI 0.87, 1.58) and 1.02 (95% CI 0.85, 1.23). The HRs for incident AMI in UKBB among evening chronotypes were 1.19 (95% CI 1.10, 1.29) for those who had insomnia symptoms, 1.18 (95% CI 1.08, 1.29) for those with short sleep duration, and 1.21 (95% CI 1.07, 1.37) for those with long sleep duration, compared to morning chronotypes without another sleep symptom. The relative excess risk for incident AMI in UKBB due to interaction between insomnia symptoms and long sleep duration was 0.25 (95% CI 0.01, 0.48). Insomnia symptoms with long sleep duration may contribute more than just an additive effect of these sleep traits on the risk of AMI.

Keywords Insomnia · Sleep duration · Chronotype · Myocardial infarction · Prospective study

✉ Nikhil Arora
nikhil.arora@ntnu.no; docnikhilarora@gmail.com

[1] K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway

[2] Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

[3] MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK

[4] Department of Medicine, St. Olavs Hospital, Trondheim, Norway

[5] HUNT Research Centre, Department of Public Health and Nursing, Norwegian University of Science and Technology, Levanger, Norway

[6] Department of Endocrinology, Clinic of Medicine, St. Olavs Hospital, Trondheim, Norway

[7] Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway

[8] Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway

[9] Clinic of Cardiology, St. Olavs Hospital, Trondheim, Norway

[10] Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway

[11] Department of Neurology and Clinical Neurophysiology, St. Olavs Hospital, Trondheim, Norway

Introduction

Globally, more than 8.9 million people die of coronary heart disease (CHD) each year [1]. Some well-known modifiable factors that increase the risk of CHD incidence are dyslipidaemia, hypertension, obesity, diabetes and cigarette smoking [2]. A substantial proportion of CHD, including acute myocardial infarction (AMI) cannot be explained by these known risk factors, and its global burden makes it important to detect novel risk factors [3]. Sleep plays an important role in maintaining health and well-being [4]. Sleep disorders have been associated with several adverse health conditions, including those related to cardiovascular health such as hypertension [5–7], obesity [8, 9], and dyslipidemia [10].

It is estimated that 33% of the population suffer from one or more insomnia symptoms, i.e. trouble falling asleep, frequent awakenings during night, or too early awakening [11, 12], and its prevalence is increasing [13]. We have previously found in the second wave of the Trøndelag Health Study (HUNT2) that individual insomnia symptom(s) and number of insomnia symptoms are associated with incident AMI [14]. Long and short sleep durations have also been found to be associated with increased risk of incident AMI [15], thus indicating the presence of a U-shaped association [16]. Chronotype referred to an individuals' preference for sleep timing, where a morning person prefers to get up and go to bed early (early bird), while an evening person prefers to get up and go to bed late (night owl) [17], has also been suggested as potential risk factor for AMI [18]. Only a few studies have investigated this association, and the evidence is not consistent with both morning and evening chronotypes found to be at risk of cardiovascular disease (CVD) [19–21].

Although these sleep traits (insomnia symptoms, sleep duration, and chronotype) are interrelated [22–24], most epidemiological studies have evaluated them as distinct entities without consideration of the others. Insomnia symptoms with objective short sleep duration, suggested to be the most biologically severe insomnia disorder phenotype [25], is associated with higher risk of CVD incidence [26]. Moreover, a study reported higher frequency of insomnia symptoms, and long or short sleep duration among evening than morning chronotypes, suggesting evening chronotypes may be more predisposed to sleep disturbances and/or its related consequences [27]. Despite the availability of self-reported sleep traits from large epidemiological studies and the evidence highlighting the complex nature of coexisting sleep traits phenotypes [22–24], the associations of coexisting sleep traits on incident AMI are not well explored.

Given the complex relationship between insomnia symptoms, sleep duration and chronotype and the scarce amount of research on the joint associations of these risk factors on AMI, we prospectively investigated the joint associations of any two self-reported sleep traits together (i.e., insomnia symptoms and sleep duration; insomnia symptoms and chronotype; and chronotype and sleep duration) on subsequent risk of incident AMI in two large population-based cohorts – the UK Biobank and the HUNT2. We also investigated the associations of these sleep traits individually on the risk of incident AMI in the same study samples.

Methods

Study population

UK Biobank (UKBB)

UKBB is a population-based prospective study of middle-aged adults (ranging from 40 to 69 years) recruited during March 2006–July 2010 and living within 25 miles of one of the 22 study assessment centres located throughout England, Scotland and Wales.

More than 9.2 million individuals were invited and 502 460 participated. The participants signed an electronic consent and completed a touchscreen questionnaire along with a brief computer-assisted interview. They provided detailed information about their lifestyle, physical measures and had blood, urine and saliva samples collected and stored for future analysis, as described elsewhere [28].

UKBB received ethical approval from the National Health Service (NHS) Research Ethics Service (reference number 11/NW/0382). The UKBB database was formed in accordance with the Declaration of Helsinki.

HUNT2

All inhabitants aged 20 years or older were invited to participate during a four-phase population-based health survey (the HUNT Study) in the Trøndelag County of Norway, first in 1984–86 (HUNT1), then in 1995–97 (HUNT2), and 2006–08 (HUNT3), and last in 2017–19 (HUNT4). This study is based on data from HUNT2.

In total, 94 187 individuals were invited during 1995–97 and 65 228 (69.3%) participated [29]. The invitation letter was sent by mail along with a self-administered questionnaire. The participants attended examination stations where clinical examination was performed, and blood samples were drawn by trained personnel. A second questionnaire was handed out at the examination site. Detailed information regarding the HUNT2 study has been published elsewhere [30].

The HUNT Study was approved by the Data Inspectorate of Norway and recommended by the Regional Committee for Ethics in Medical Research (REK; reference number 152/95/AH/JGE). The ethical approval for conducting this study was also obtained from the Regional Committee for Ethics in Medical Research (REK nord; reference number 2020/47206). The HUNT Study was conducted in accordance with the Declaration of Helsinki.

Exposures

Insomnia symptoms

In both UKBB and HUNT2, insomnia symptoms were defined as difficulty falling asleep, difficulty maintaining sleep or waking up too early without any related daytime impairment. Thus, our definition of insomnia is not aligned with established frameworks for classification of insomnia [31].

In the UKBB, the participants were asked: "Do you have trouble falling asleep at night or do you wake up in the middle of the night?" with response options "Never/rarely", "Sometimes", "Usually" or "Prefer not to answer", and 500 956 participants (99.7%) answered this insomnia symptoms question. Participants were classified as having insomnia symptoms if they answered "Usually", otherwise they were classified as having no insomnia symptoms.

In HUNT2, insomnia symptoms were assessed by the following two questions: "Have you had difficulty falling asleep in the last month?" and "During the last month, have you woken too early and not been able to get back to sleep?" with response options "Never", "Sometimes", "Often" or "Almost every night", and 54 322 participants (83.3%) answered one or both of these insomnia symptom questions. Participants who responded "Often" or "Almost every night" to at least one of these questions were classified as having insomnia symptoms. For the participants in HUNT2 answering only one of the insomnia symptom questions, we did the following: (1) if they answered "Often" or "Almost every night" to one of the questions, but did not answer the other, they were classified as having insomnia symptoms, and (2) if they answered "Never" or "Sometimes" to one of the questions, but did not answer the other, they were excluded to avoid possible misclassification.

Sleep duration

In UKBB, participants were asked about sleep duration the last four weeks: "About how many hours sleep do you get in every 24 h? (please include naps)". In HUNT2, participants were asked about sleep duration: "How many hours do you usually spend lying down (i.e. sleeping and/or napping) during a 24-h period?". In UKBB and HUNT2, 498 245 (99.2%) and 53 203 (81.6%) participants reported their sleep duration, respectively. A three-level categorical variable was created defining sleep duration as "Short" (6 h or less), "Normal" (7–8 h) or "Long" (9 h or more) as per recommendations from the National Sleep Foundation [32]. Extreme responses of less than 3 h or more than 18 h were excluded to avoid improbable short or long sleep durations confounded by poor health.

Chronotype

In UKBB, 496 281 (98.8%) participants reported chronotype ("Do you consider yourself to be?" with response options "Definitely a 'morning' person", "More a 'morning' than 'evening' person", "More an 'evening' than a 'morning' person", "Definitely an 'evening' person", "Do not know", or "Prefer not to answer"). Chronotype was not reported in HUNT2. Chronotype was dichotomized categorizing the alternatives "Definitely a 'morning' person" or "More a 'morning' than 'evening' person" as "Morning" chronotype; and "More an 'evening' than a 'morning' person" or "Definitely an 'evening' person" as "Evening" chronotype. The alternatives "Do not know" or "Prefer not to answer" were excluded.

Outcome ascertainment

In UKBB, follow-up for AMI incidence was conducted via linkage to the Hospital Episode Statistics (HES) for England, Scottish Morbidity Record (SMR) and Patient Episode Database for Wales (PEDW) where health-related outcomes had been defined by International Classification of Diseases (ICD)-9 and ICD-10 codes (Field IDs: 41270, 41271, 41280 and 41281). Mortality information was obtained from NHS Digital for participants in England and Wales and from the NHS Central Register (part of the National Records of Scotland) for participants in Scotland where cause of death had been defined by ICD-10 codes (Field IDs: 40001 and 40000).

In HUNT2, the participants were followed up for incident AMI, either identified at hospitals or by the National Cause of Death Registry. Hospitalizations for AMI were identified through a linkage with medical records from the three hospitals (St. Olavs Hospital, Levanger Hospital and Namsos Hospital) of Trøndelag County in which health-related outcomes had been defined by ICD-9 and ICD-10 codes. Death records were identified by a linkage with the National Cause of Death Registry where cause of death had been defined by ICD-10 codes.

Incident AMI was defined as the first occurrence of either hospitalization or death due to AMI between date of participation and end of follow-up. All participants with any episode(s) of AMI before their date of participation were

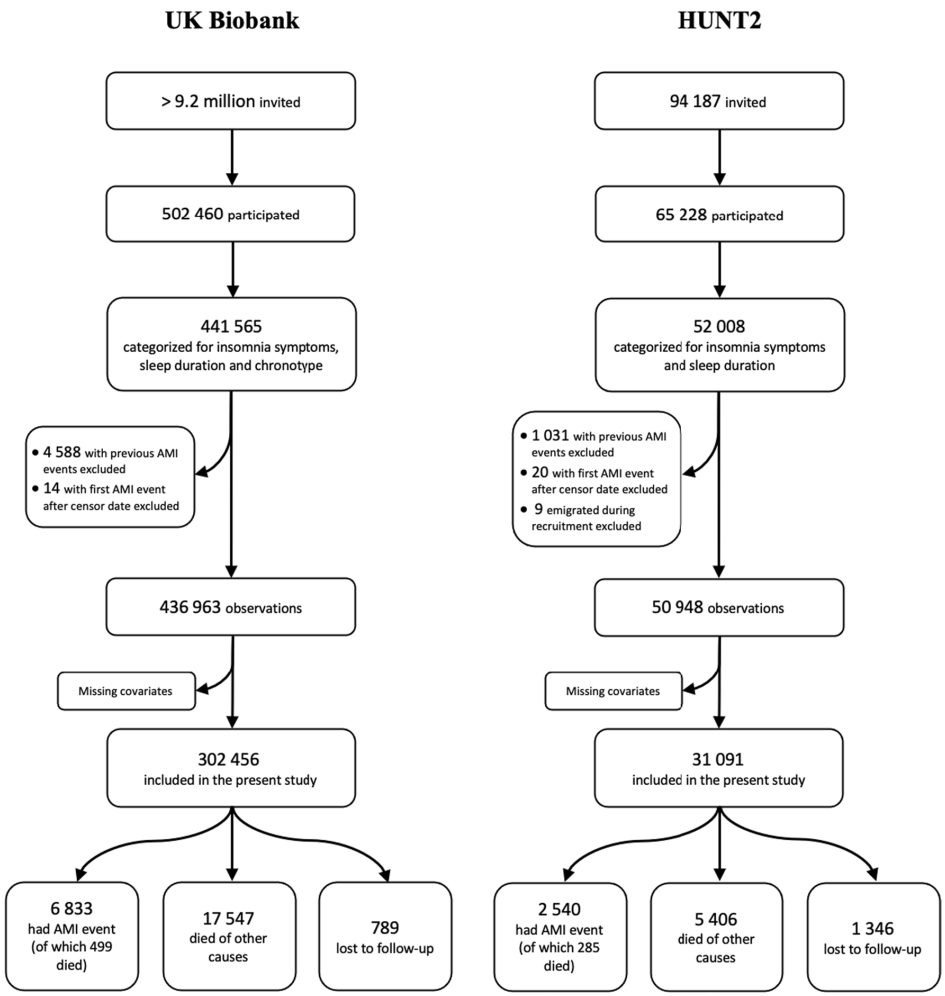


Fig. 1 Flow chart of the participant selection process

excluded. We used ICD-9 code 410 and ICD-10 codes I21 and I22 to identify hospitalizations and deaths due to AMI before and after baseline. A flow chart of participant selection process for our analyses is summarized in Fig. 1.

Covariates

Information on socio-demographic (i.e. age, gender, marital status, ethnicity (for UKBB only), education and employment status) and lifestyle factors (i.e. smoking, alcohol intake, shift work, physical activity and use of sleep medication(s)) was collected by means of a self-administered questionnaire. A clinical examination was conducted by trained staff where measurements on weight, height, and blood pressure were recorded and blood samples were collected.

For UKBB, marital status was categorized as "Married" or "Unmarried", while for HUNT2 it was categorized as "Married", "Unmarried" or "Separated/Divorced/Widowed". The information on alcohol intake frequency was categorized for "Never", "Monthly", "Weekly" or "Daily" alcohol intake. Smoking status of the participants were categorized as "Never", "Previous" or "Current" smoker. Body mass index (BMI) was computed by dividing weight (in kgs) by the squared value of height (in metres) and was analysed as a continuous variable. The information on physical

activity was categorized as "Low/inactive", "Moderate" or "High" level of physical activity based on International Physical Activity Questionnaire (IPAQ) grouping. Education attainment was categorized as "Primary" (10 years or less), "Secondary" (11–13 years) or "Tertiary" (14 years or more) level of education. Information on shift work/night shifts was analysed as a dichotomous "Yes" or "No" variable. Employment status was categorized as "Employed" or "Not employed". For UKBB, ethnic background was categorized as "White", "Mixed", "Asian/Asian British", "Black/Black British", "Chinese" or "Other" ethnic groups, to account for ethnic heterogeneity. We did not adjust for ethnicity in HUNT2, since the population of Nord-Trøndelag is mostly white (less than 3% non-Caucasians) [30]. For UKBB, the Townsend deprivation index (TDI) was used as a continuous variable to account for varying socioeconomic disparities and urban-rural mix within the UK. In HUNT2, education attainment was used to capture any socioeconomic differences. Systolic blood pressure, blood cholesterol levels and blood glucose levels were collected on clinical and laboratory examination and were analysed as continuous variables. Fasting time for UKBB and time since last meal for HUNT2 were used as a continuous variable. Use of sleep medication(s) was ascertained from self-reported use of medications and was analysed as a dichotomous "Yes" or "No" variable. For UKBB, depression and anxiety were categorized as "Yes" or "No" based on diagnosis mapped as ICD-10 codes until the summer of 2019 based on hospital, primary care or self-reported health records, while the Hospital Anxiety and Depression score (HADS) was used on an ordinal scale for HUNT2.

Further details on how the covariates were handled are provided in the supplementary material.

Statistical analysis

We analysed UKBB and HUNT2 separately. We used Cox proportional hazard models to examine the prospective associations of self-reported insomnia symptoms, sleep duration and chronotype individually on the subsequent risk of incident AMI. We then assessed the joint associations of two sleep traits together i.e., insomnia symptoms and sleep duration; insomnia symptoms and chronotype; and chronotype and sleep duration on the risk of incident AMI. Each participant was followed until either first incident AMI, death, loss to follow-up or until end of follow-up (March 23, 2021 for UKBB and December 31, 2020 for HUNT2). We calculated the number of incident AMI events, person-years at risk and hazard ratios (HRs) with 95% confidence intervals (CIs) using different models adjusting for potential confounding factors.

We reviewed the literature and created Directed Acyclic Graphs (DAGs) to select covariates that could cause both sleep disturbances and AMI. First, we adjusted for age and gender (Model 1). In our main model (Model 2), we further adjusted for marital status, alcohol intake, smoking status, BMI, physical activity, education, shift work/night shifts and employment status. We also adjusted for TDI and ethnicity in Model 2 for our analyses on UKBB. Lastly in Model 3, we additionally included systolic blood pressure, blood cholesterol levels, blood glucose levels, use of sleep medication(s), depression, and anxiety which may be both confounders and/or mediators for the associations under study. Because blood samples were non-fasting, blood laboratory investigations especially for cholesterol and glucose levels could be influenced by time between last meal and venepuncture, so we also adjusted for time since last meal in Model 3.

We tested the proportionality of hazards using log-log curves and Schoenfeld residuals test. The joint associations of any two sleep traits together on the subsequent risk of incident AMI were assessed by using the relative excess risk due to interaction (RERI) with 95% CIs [33]. In brief, RERI > 0 and the lower limit of 95% CI > 0 suggests a synergistic effect of two sleep traits together on incident AMI, i.e., their joint effect on incident AMI is even greater than the sum of their individual effects [34].

We did formal tests for interaction between each sleep trait and their combinations with age (above and below 65 years) and gender. After reviewer comments, we also examined interaction due to shift work, depression (HADS-Depression score above and below 8 in HUNT2) and anxiety (HADS-Anxiety score above and below 8 in HUNT2). In addition, analyses stratified by age, gender, shift work, depression and anxiety were conducted.

We performed several sensitivity analyses to assess the robustness of our findings. To reduce the possibility of reverse causality as an explanation for the observed associations, we repeated the analyses after excluding the first two years of follow-up. In another sensitivity analyses, we adjusted for any self-reported chronic disorders in Models 2 and 3, as sleep disturbances co-exist with certain illnesses and chronic pain [35]. We repeated the same analyses within UKBB restricting only to the White British sample. To compare the findings from UKBB and HUNT2 with the same study follow-up duration, we repeated the analyses in HUNT2 with end of follow-up until December 31, 2008.

The statistical analyses were conducted using R version 4.1.1 for Mac OS (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics of the study population according to insomnia symptoms status are displayed in Table 1. The baseline characteristics according to sleep duration

Table 1 Baseline characteristics of participants from UK Biobank and HUNT2 according to self-reported insomnia symptoms

	UK Biobank		HUNT2	
	Insomnia symptoms (n = 302 456)		Insomnia symptoms (n = 31 091)	
	No	Yes	No	Yes
Total, % (n)	72.7 (219 784)	27.3 (82 672)	87.5 (27 196)	12.5 (3 895)
Variables, % (n)				
Male	49.2 (108 116)	39.6 (32 777)	47.7 (12 982)	39.6 (1 542)
Married	74.5 (163 730)	71.0 (58 717)	61.2 (16 634)	61.2 (2 385)
Weekly alcohol intake	50.9 (111 836)	46.7 (38 612)	26.6 (7 235)	25.6 (996)
Current smokers	10.0 (21 965)	11.0 (9 051)	28.3 (7 706)	34.8 (1 354)
Highly physically active	41.2 (90 468)	38.5 (31 836)	38.6 (10 513)	28.9 (1 126)
Tertiary education	48.6 (106 732)	42.7 (35 291)	25.7 (6 984)	18.6 (725)
Shift workers	5.6 (12 261)	4.9 (4 015)	18.5 (5 042)	15.7 (612)
Employed	62.2 (136 694)	52.8 (43 635)	74.2 (20 183)	57.0 (2 222)
Use of sleep medication(s)	0.5 (1 036)	2.0 (1 654)	3.2 (872)	24.9 (970)
Suffering from depression	9.7 (21 210)	16.4 (13 572)	–	–
Suffering from anxiety	5.3 (11 651)	8.8 (7 282)	–	–
Variables, mean (SD)				
Age, *years*	55.87 (8.22)	57.25 (7.70)	45.10 (15.04)	51.52 (15.82)
TDI	−1.49 (2.97)	−1.29 (3.09)	–	–
BMI, kg/m^2	27.14 (4.54)	27.68 (5.01)	26.08 (3.94)	26.47 (4.32)
SBP, mmHg	137.5 (18.61)	137.8 (18.43)	134.10 (19.47)	136.70 (21.15)
Time since last meal, h	3.76 (2.37)	3.81 (2.48)	2.14 (1.91)	2.22 (1.95)
Serum cholesterol, mmol/L	5.70 (1.12)	5.74 (1.16)	5.72 (1.21)	6.00 (1.24)
Blood glucose, mmol/L	5.08 (1.16)	5.16 (1.31)	5.32 (1.31)	5.45 (1.42)
HADS-D scores	–	–	2.92 (2.67)	5.12 (3.65)
HADS-A scores	–	–	3.80 (2.95)	6.51 (4.08)

SD indicates standard deviation; TDI, Townsend deprivation index; BMI, body mass index; SBP, systolic blood pressure; HADS – D scores, Hospital Anxiety and Depression Score – Depression scores; and HADS – A scores, Hospital Anxiety and Depression Score – Anxiety scores

and chronotype are displayed in Tables S1 and S2, respectively. In UKBB and HUNT2, the prevalence of insomnia symptoms was 27.3% and 12.5%, respectively. The mean (SD) hours of sleep duration for UKBB and HUNT2 were 7.17 (1.07) hours and 7.94 (1.17) hours, respectively. The prevalence of short sleep duration and long sleep duration was 23.9% and 7.4%, respectively in UKBB; and 6.2% and 23.7%, respectively in HUNT2. The prevalence of evening chronotype in UKBB was 37.2%. Participants who reported insomnia symptoms, long sleep duration or morning chronotype were older and were more likely to be women than men. In HUNT2, males were more likely to sleep for short duration compared to females. Participants who reported insomnia symptoms or long sleep duration were more likely unemployed. In HUNT2, the use of sleep medication(s) was more common among participants who reported insomnia symptoms or long sleep duration. In both cohorts, depression or anxiety was more frequent among participants who reported insomnia symptoms.

Among 302 456 UKBB participants without previous AMI, a total of 6 833 incident AMIs were observed during a mean (SD) follow-up period of 11.7 (1.9) years. Among 31 091 HUNT2 participants without previous AMI, a total of 2 540 incident AMIs were identified during a mean (SD) follow-up of 21.0 (6.5) years.

Associations of self-reported individual sleep trait(s) and incident AMI

The age- and gender-adjusted HRs and multivariable adjusted HRs with 95% CIs for incident AMI in relation to self-reported insomnia symptoms, sleep duration and chronotype are presented in Table 2. After adjusting for potential confounders (Model 2), the participants who reported insomnia symptoms had a HR of 1.11 (95% CI 1.05, 1.16) and 1.09 (95% CI 0.98, 1.21) for incident AMI in UKBB and HUNT2, respectively, compared to those without insomnia symptoms. Compared to participants who reported normal sleep duration (7–8 h), the HRs for incident AMI in UKBB were 1.09 (95% CI 1.04, 1.16) and 1.14 (95% CI 1.05, 1.24) for those who reported short sleep duration (6 h or less) and long sleep duration (9 h or more), respectively.

Table 2 Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to self-reported insomnia symptoms, sleep duration and chronotype in UK Biobank (UKBB) and HUNT2

		Insomnia symptoms		**Sleep duration**			**Chronotype**	
		No	Yes	Short	Normal	Long	Morning	Evening
UK Biobank (n = 302 456)	AMI events/ Person-years	4 784/ 2 583 503	2 049/ 964 868	1 794/ 844 021	4 365/ 2 445 890	674/ 258 460	4 206/ 2 229 937	2 627/ 1 318 434
	Model 1	Ref.	1.19 (1.13, 1.25)	1.20 (1.14, 1.27)	Ref.	1.29 (1.19, 1.40)	Ref.	1.14 (1.08, 1.19)
	Model 2	Ref.	1.11 (1.05, 1.16)	1.09 (1.04, 1.16)	Ref.	1.14 (1.05, 1.24)	Ref.	1.08 (1.03, 1.13)
	Model 3	Ref.	1.08 (1.03, 1.14)	1.09 (1.03, 1.15)	Ref.	1.10 (1.01, 1.19)	Ref.	1.06 (1.01, 1.12)
HUNT2 (n = 31 091)	AMI events/ Person-years	2 120/ 577 219	420/ 76 391	151/ 41 306	1 668/ 470 344	721/ 141 960	–	–
	Model 1	Ref.	1.17 (1.06, 1.30)	1.15 (0.97, 1.35)	Ref.	1.05 (0.96, 1.15)	–	–
	Model 2	Ref.	1.09 (0.98, 1.21)	1.05 (0.89, 1.24)	Ref.	0.97 (0.88, 1.06)	–	–
	Model 3	Ref.	1.08 (0.96, 1.21)	1.09 (0.93, 1.29)	Ref.	0.94 (0.85, 1.03)	–	–

Model 1, adjusted for age and gender

Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index (for UKBB), ethnicity (for UKBB), shift work, and employment status

Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety

Table 3 Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and sleep duration in UK Biobank (UKBB) and HUNT2.

		No insomnia symptoms			**Insomnia symptoms**		
		Sleep duration			**Sleep duration**		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 302 456)	AMI events/ Person-years	900/ 427 642	3 395/ 1 951 096	489/ 204 765	894/ 416 379	970/ 494 794	185/ 53 695
	Model 1	1.16 (1.08, 1.25)	Ref.	1.22 (1.11, 1.34)	1.32 (1.23, 1.42)	1.12 (1.05, 1.21)	1.72 (1.49, 2.00)
	Model 2	1.07 (0.99, 1.15)	Ref.	1.09 (0.99, 1.20)	1.16 (1.07, 1.25)	1.07 (0.99, 1.15)	1.40 (1.21, 1.63)
	Model 3	1.08 (1.00, 1.16)	Ref.	1.05 (0.96, 1.16)	1.13 (1.04, 1.21)	1.05 (0.98, 1.13)	1.32 (1.14, 1.54)
HUNT2 (n = 31 091)	AMI events/ Person-years	106/ 32 967	1 420/ 420 985	594/ 123 267	45/ 8 339	248/ 49 360	127/ 18 692
	Model 1	1.10 (0.90, 1.34)	Ref.	1.06 (0.96, 1.17)	1.38 (1.03, 1.86)	1.17 (1.03, 1.34)	1.19 (0.99, 1.43)
	Model 2	1.02 (0.84, 1.24)	Ref.	0.97 (0.88, 1.07)	1.17 (0.87, 1.58)	1.09 (0.95, 1.25)	1.02 (0.85, 1.23)
	Model 3	1.08 (0.89, 1.32)	Ref.	0.94 (0.85, 1.04)	1.18 (0.87, 1.60)	1.08 (0.94, 1.25)	0.97 (0.80, 1.19)

Model 1, adjusted for age and gender

Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index (for UKBB), ethnicity (for UKBB), shift work, and employment status

Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety

Table 4 Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and chronotype in UK Biobank

		No insomnia symptoms		Insomnia symptoms	
		Chronotype		Chronotype	
		Morning	Evening	Morning	Evening
UK Biobank	AMI events/	2 953/	1 831/	1 253/	796/
(n = 302 456)	Person-years	1 625 404	958 099	604 533	360 335
	Model 1	Ref.	1.12 (1.06, 1.19)	1.17 (1.10, 1.25)	1.36 (1.26, 1.47)
	Model 2	Ref.	1.08 (1.02, 1.15)	1.11 (1.04, 1.18)	1.19 (1.10, 1.29)
	Model 3	Ref.	1.07 (1.01, 1.14)	1.09 (1.02, 1.17)	1.14 (1.06, 1.24)

Model 1, adjusted for age and gender

Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, ethnicity, shift work, and employment status

Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety

The corresponding HRs in HUNT2 were similar for those who reported short sleep duration (HR 1.05; 95% CI 0.89, 1.24), but not for those who reported long sleep duration (HR 0.97; 95% CI 0.88, 1.06). Compared to morning chronotypes, the HR for incident AMI was 1.08 (95% CI 1.03, 1.13) for evening chronotypes in UKBB.

Joint associations of self-reported sleep traits and incident AMI

Table 3 presents HRs with 95% CIs for incident AMI in relation to the joint association of self-reported insomnia symptoms and sleep duration within UKBB and HUNT2. Compared to participants who reported normal sleep duration without insomnia symptoms, the multi-adjusted HR for incident AMI in UKBB was 1.07 (95% CI 0.99, 1.15) for those who reported normal sleep duration with insomnia symptoms, whereas the HR increased to 1.16 (95% CI 1.07, 1.25) for those who reported short sleep duration with insomnia symptoms and 1.40 (95% CI 1.21, 1.63) for those who reported long sleep duration with insomnia symptoms. The corresponding HRs in HUNT2 were similar for those who reported normal sleep duration with insomnia symptoms (HR 1.09; 95% CI 0.95, 1.25), and who reported short sleep duration with insomnia symptoms (HR 1.17; 95% CI 0.87, 1.58), but not for those who reported long sleep duration with insomnia symptoms (HR 1.02; 95% CI 0.85, 1.23). In UKBB, we found statistical evidence for biological interaction beyond additivity for long sleep duration with insomnia symptoms (relative excess risk due to interaction (RERI) 0.25; 95% CI 0.01, 0.48), but no such evidence for short sleep duration with insomnia symptoms (RERI 0.02; 95% CI -0.11, 0.15). In HUNT2, we did not find evidence of interaction beyond additivity for short sleep duration (RERI 0.06; 95% CI -0.36, 0.48) or long sleep duration (RERI -0.04; 95% CI -0.28, 0.20) with insomnia symptoms.

HRs with 95% CIs for incident AMI in relation to the joint association of self-reported insomnia symptoms and chronotype within UKBB are presented in Table 4. Compared to morning chronotypes without insomnia symptoms, the HRs for incident AMI were 1.08 (95% CI 1.02, 1.15) for evening chronotypes without insomnia symptoms, and 1.11 (95% CI 1.04, 1.18) for morning chronotypes with insomnia symptoms, whereas the HR increased to 1.19 (95% CI 1.10, 1.29) for evening chronotypes with insomnia symptoms. There was no evidence of interaction beyond additivity for evening chronotype with insomnia symptoms (RERI -0.01; 95% CI -0.12, 0.12).

Table 5 presents HRs with 95% CIs for incident AMI in relation to the joint association of self-reported chronotype and sleep duration within UKBB. Compared to participants who reported normal sleep duration with morning chronotype, the HR for incident AMI was 1.08 (95%

Table 5 Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported chronotype and sleep duration in UK Biobank

		Morning chronotype			Evening chronotype		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 302 456)	AMI events/ Person-years	1 141/ 539 398	2 689/ 1 540 774	376/ 149 766	653/ 304 623	1 676/ 905 117	298/ 108 694
	Model 1	1.21 (1.12, 1.29)	Ref.	1.28 (1.15, 1.42)	1.37 (1.26, 1.49)	1.13 (1.06, 1.20)	1.46 (1.29, 1.64)
	Model 2	1.10 (1.03, 1.18)	Ref.	1.14 (1.03, 1.27)	1.18 (1.08, 1.29)	1.08 (1.02, 1.15)	1.21 (1.07, 1.37)
	Model 3	1.09 (1.02, 1.17)	Ref.	1.12 (1.00, 1.24)	1.16 (1.06, 1.27)	1.07 (1.01, 1.14)	1.15 (1.01, 1.29)

Model 1, adjusted for age and gender

Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, ethnicity, shift work, and employment status

Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety

CI 1.02, 1.15) for those who reported normal sleep duration with evening chronotype, whereas the HR increased to 1.18 (95% CI 1.08, 1.29) for those who reported short sleep duration with evening chronotype and 1.21 (95% CI 1.07, 1.37) for those who reported long sleep duration with evening chronotype. There was no evidence of interaction beyond additivity for short sleep duration (RERI -0.01; 95% CI -0.14, 0.12) or long sleep duration (RERI -0.02; 95% CI -0.21, 0.18) with evening chronotype.

We found no strong statistical evidence of interaction by age for any individual sleep traits in both cohorts (Table S3). However, for the combination of insomnia and chronotype, we found that young or middle-aged adults (< 65 years), who were evening chronotypes without insomnia symptoms or morning chronotypes with insomnia symptoms had an increased risk of AMI compared to morning chronotypes without insomnia symptoms. We did not find the same increased risk of AMI in these phenotypes among the older participants (≥ 65 years). Additionally, we found no statistical evidence of interaction by gender, shift work, depression or anxiety (Tables S4–S7).

Sensitivity analyses

When excluding the first two years of follow-up, a total of 6 089 and 2 390 AMI events were reported within UKBB and HUNT2, respectively, and the estimated associations remained fairly unchanged, but were less precise (Tables S8–S11). When adjusting for the presence of any chronic disorders, the effect estimates remained essentially unchanged, but were less precise (Tables S12–S15). When restricting the analyses to "White British" participants in UKBB (n = 269 375), similar findings were reported (Tables S16–S19). A total of 1 144 AMI events were reported in HUNT2 until December 31, 2008 (i.e., the mean (SD) follow-up period of 11.6 (2.5) years), and the estimated associations remained fairly unchanged, but were less precise compared to the complete follow-up period of 21.0 years (Tables S20–S21).

Discussion

This is a population-based study of self-reported insomnia symptoms, sleep duration, and chronotype involving two large European cohorts. In UKBB, we found that those who had insomnia symptoms, short/long sleep duration, or evening chronotype had an increased risk for incident AMI, compared to those who had no insomnia symptoms, normal sleep duration or morning chronotype, respectively. Although participants with the combinations of two sleep traits (i.e., insomnia symptoms, sleep duration, and chronotype) had the greatest risk of incident AMI in UKBB, we

found a synergistic association only for insomnia symptoms with long sleep duration. In HUNT2, we observed similar trends for incident AMI among those who had insomnia symptoms, short sleep duration or their combination, with less precise effect estimates possibly due to lack of power. We found no evidence of synergistic association due to the interaction between these sleep traits in HUNT2.

Our findings for insomnia symptoms and risk of incident AMI are in line with that of a prior study on HUNT2 participants with an average of 11.4 years of follow-up, where individual insomnia symptom(s) and cumulative number of insomnia symptoms were associated with increased risk of incident AMI [14]. Compared to this study, we have a longer follow-up for HUNT2 participants (21.0 years) and have combined the insomnia symptoms to match the UKBB definition.

Our findings that short and long sleep duration moderately increased the risk of incident AMI, compared to normal sleep duration in UKBB, are consistent with findings from a prior study on UKBB with median 7.0 years of follow-up [15]. We have a slightly longer follow-up for UKBB participants (11.7 years) and a normal sleep duration reference group (7–8 h instead of 6–9 h) as per the sleep duration recommendations [32]. We found no association between long sleep duration and risk of AMI in HUNT2. These inconsistent findings might be explained by notable differences in the two cohorts. The lower participation rate in UKBB (5.5%) compared to HUNT2 (69.3%) might have caused selection bias. Moreover, the dominance of short sleepers in UKBB and long sleepers in HUNT2 is possibly due to a general time trend towards short sleep duration from 1995–97 (HUNT2) to 2006–10 (UKBB) [36], making the comparison between the two cohorts difficult.

Our findings for evening chronotype and increased risk of incident AMI are consistent with evidence by Fan et al. [18], that followed 4 576 AMI-free participants for a mean of 10.6 years from the Sleep Heart Health Study (SHHS). They reported that participants with sleep onset later than 12 midnight had 62% increased risk of AMI, compared to those with sleep onset between 10:01 PM and 11:00 PM [18]. In our study, we used self-reported information on chronotype that captured not only early/late sleep onset behaviours, but also early/late morning wake-up behaviours which may more accurately depict time of the day when sleep occurs.

Our findings for the joint association of insomnia symptoms with short sleep duration and moderately increased risk of incident AMI are consistent with evidence from a cross-sectional study by Kalmbach et al. involving 3 911 subjects from Evolution of Pathways to Insomnia Cohort (EPIC) study [22]. They found that subjects who had self-reported insomnia disorder with short sleep duration had three times the odds for AMI (Odds ratio 3.23; 95% CI 1.45, 7.21), compared to those who never had insomnia with 6 h or more of sleep duration [22]. Since this was a cross-sectional study, reverse causation is likely as sleep problems are common in patients with CHD [37]. Moreover, they considered only few potential confounders (age, sex and obesity) in their fully-adjusted model. Similarly, a prospective study by Bertisch et al. involving 4 437 CVD-free participants from SHHS followed for a median of 11.4 years, found a 29% increased risk of incident CVD (HR 1.29; 95% CI 1.00, 1.66) for those who had insomnia symptoms with polysomnographic short sleep duration, compared to those who had no insomnia symptoms with 6 h or more of sleep duration [26].

Our findings showing a synergistic association of insomnia symptoms with long sleep duration and increased risk of incident AMI in UKBB are consistent with a prospective study on this phenotype and incident CHD. Sands-Lincoln et al. followed 86 329 postmenopausal women aged 50–79 years from Women's Health Initiative (WHI) Observational Study for a mean of 10.3 years. They found that women at high risk of insomnia symptoms, defined as WHI Insomnia Rating Scale (WHIIRS) ≥ 9, with 10 h or more sleep duration had 93% increased risk of incident CHD (HR 1.93; 95% CI 1.06, 3.51), compared to those at low risk of insomnia symptoms, defined as WHIIRS < 9, with 7–8 h of sleep duration [23]. Since this study only involved postmenopausal women aged 50–79 years, the reported association of insomnia symptoms with long sleep duration on the risk of CHD may not be generalizable to the general population. Moreover, the observed inconsistencies in the association of this phenotype and incident AMI in HUNT2 and UKBB might be due to possible differences in the two cohorts, as explained above.

To the best of our knowledge, this is the first study to investigate the joint associations of chronotype with insomnia symptoms or short/long sleep duration on the risk of incident AMI and to investigate the statistical evidence for biological interaction beyond additivity due to the conjunct presence of these sleep traits. The conjunct presence of insomnia symptoms with long sleep duration may be a vulnerable phenotype contributing to a greater risk of AMI, than simply an additive effect of insomnia symptoms and long sleep duration.

Sleep debt, which occurs through insomnia and short sleep duration, may result in glucose intolerance, decreased thyrotropin secretion, increased cortisol concentration, increased sympathetic nervous activity [38], and elevated C-reactive protein (CRP) levels [39], which are pathophysiological in the development of hypertension [7], and CVD events [40, 41]. Evening chronotype is associated with abdominal obesity independent of BMI [42], and with altered secretion of adipokines [43], which is directly involved in the pathogenesis of arterial hypertension and an increased cardiometabolic risk [19]. Although evidence on the biological mechanisms involving long sleep duration are

limited, the association of long sleep duration on the risk of AMI may be explained by poor sleep quality, depression or other underlying comorbidities [44]. People reporting long sleep duration are more likely to have poor sleep quality due to fragmented sleep with repeated awakenings [44]. Poor sleep quality may also increase sympathetic activity [45] and activate an inflammatory response [46]. The aftermath activation of CRP may inhibit endothelium-dependent vasodilation and nitric oxide synthesis, suggestive to cause arterial stiffness [47] and trigger atherosclerosis [46, 48]. Insomnia symptoms and long sleep duration may have unique and independent biological pathways through which they cause increase in risk of incident AMI, and this could be the cause of their synergistic effect due to interaction.

The strengths of the current study include the use of two large cohorts with information on self-reported insomnia symptoms, sleep duration and chronotype, making it possible to examine the joint association of these traits on the risk of AMI. Incident AMIs were ascertained using linkages of the cohorts through hospital records and death certificates which minimizes the chance of misclassification. Moreover, we had rich information on possible confounders (e.g., sociodemographic, lifestyle, clinical and biochemical factors).

The current study has several limitations. Sleep traits were not assessed objectively using validated measures such as actigraphy or polysomnography, which may have caused some measurement error. It remains therefore uncertain whether self-reported sleep duration in the present study represents time in bed or actual sleep time. However, sleep duration tends to be overestimated by actigraphy [49], and polysomnography is not routinely used for the evaluation of insomnia, because symptoms of trouble falling asleep, frequent awakenings during night, or too early awakenings may not be captured objectively [50]. Insomnia is a highly subjective disorder and is primarily defined by the nature of the complaint, thus relying on medical records could potentially cause misclassification as it is often misreported or not reported in medical records [51]. Furthermore, neither the questionnaire used to collect the sleep complaints was validated in the two cohorts, nor does our definition of insomnia symptoms comply with the established frameworks for classification of insomnia [31]. For instance, we lack information about some night time symptoms (waking up earlier in UKBB and difficulty maintaining sleep in HUNT2), nor did we have information about daytime impairment or if the symptoms occurred at least three times per week for at least 3 months. This may have biased our estimates towards the null as people with clinically diagnosed insomnia may have been misclassified as not having insomnia. Moreover, since the Trøndelag County is located near the Arctic circle, seasonal variations in the amount of daylight could have caused seasonal fluctuations in the sleep habits. However, a prior study on HUNT2 found no evidence of seasonal variation in reports of insomnia symptoms characterized by difficulty falling asleep and maintaining sleep [52]. We did not have information about sleep apnoea or other sleep disorders in our study. However, a European population-based study suggested that the prevalence of other sleep disorders, including obstructive sleep apnoea is only ~ 5% among those who have insomnia symptoms [12]. Also, we adjusted for age, BMI, blood pressure and depression in our analyses, that are some of the strong correlates of both sleep apnoea and CVD [53]. Thus it appears unlikely that sleep apnoea alone could explain the higher risk of AMI among participants with different sleep traits or their combinations in our study. Lastly, our findings from the two cohorts should be carefully compared due to cohort differences as: (1) the low participation rate in UKBB (5.5%) compared to HUNT2 (69.3%) which might have led to selection bias; (2) the self-reported sleep traits were collected more than 10 years apart in the two cohorts; (3) the mean age at baseline were higher in UKBB (56.6 years) than in HUNT2 (48.3 years); and (4) the difference in prevalence of the sleep duration categories in the two cohorts, where short sleepers dominated in UKBB and long sleepers in HUNT2.

Conclusion

Our study suggests that individual sleep traits i.e., insomnia symptoms, sleep duration and chronotype alone are important phenotypes associated with an increased risk of AMI, and we found evidence of an excess risk due to interaction for insomnia symptoms with long sleep duration. Insomnia symptoms with long sleep duration may be a vulnerable phenotype that needs to be further explored. Thus, subsequent studies investigating sleep problems on the risk of CHD/CVD should consider interaction between insomnia symptoms and long sleep duration, as investigating only one element may provide a partial recognition of clinically relevant sleep phenotype leading to their out of sight health consequences. We would also suggest further studies to apply a Mendelian randomization design using genetic variants as instrument variables for the sleep traits. Such studies could rule out limitations due to residual confounding and reverse causation. Also, studies aimed at exploring potential vascular and metabolic mechanisms behind insomnia, short/long sleep duration and chronotype are warranted to better understand association underlying AMI risk.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10654-023-00981-x.

Acknowledgements This research has been conducted using data from UK Biobank, a major biomedical database (www.ukbiobank.ac.uk), under application number 40135. The Trøndelag Health Study (HUNT) is a collaboration between HUNT Research Centre (Faculty

of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU)), Trøndelag County Council, Central Norway Regional Health Authority, and the Norwegian Institute of Public Health. We want to thank clinicians and other employees at Nord-Trøndelag Hospital Trust for their support and for contributing to data collection in this research project.

Author contributions NA interpreted and analysed the data, interpreted the findings, and wrote the paper; LBS, and RCR had the original idea for this study, interpreted the data, and critically revised the paper; ESS, BMB, and BOÅ had the original idea for this study, and critically revised the paper; and HD assisted with interpreting data on acute myocardial infarction from medical records assessed through hospitals in the Trøndelag County, and critically revised the paper.

Funding Open access funding provided by Norwegian University of Science and Technology (including St. Olavs Hospital - Trondheim University Hospital), Trondheim, Norway. This study was made possible with the financial support from Nasjonalforeningen for folkehelsen (The National Association for Public Health) in Norway, having Project ID – 19479.

Declarations

Competing interests The authors declare that there is no conflict of interest. The authors of this manuscript have certified that they comply with the principles of ethical publishing.

Ethical approval and consent UK Biobank received ethical approval from the National Health Service (NHS) Research Ethics Service (reference number 11/NW/0382). The HUNT Study was approved by the Data Inspectorate of Norway and recommended by the Regional Committee for Ethics in Medical Research (REK; reference number 152/95/AH/JGE). The ethical approval for conducting this study was also obtained from the Regional Committee for Ethics in Medical Research (REK nord; reference number 2020/47206).

Informed consent was obtained from all individual participants of both the cohorts included in this study.

References

1. Roth GA, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the global burden of Disease Study 2017. The Lancet. 2018;392:1736–88.
2. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, et al. Prevalence of conventional risk factors in patients with Coronary Heart Disease. JAMA. 2003;290:898–904.
3. Mozaffarian D, Wilson PWF, Kannel WB. Beyond established and novel risk factors. Circulation. 2008;117:3031–8.
4. Cappuccio FP, Miller MA. Sleep and cardio-metabolic disease. Curr Cardiol Rep. 2017;19:110. 2017/09/21.
5. Bathgate CJ, Edinger JD, Wyatt JK, Krystal AD. Objective but not subjective short sleep duration associated with increased risk for hypertension in individuals with Insomnia. Sleep. 2016;39:1037–45.
6. Vgontzas AN, Liao D, Bixler EO, Chrousos GP, Vela-Bueno A. Insomnia with objective short sleep duration is associated with a high risk for hypertension. Sleep. 2009;32:491–7.
7. Gangwisch JE, Heymsfield SB, Boden-Albala B, Buijs RM, Kreier F, Pickering TG, et al. Short sleep duration as a risk factor for hypertension. Hypertension. 2006;47:833–9.
8. Wu Y, Zhai L, Zhang D. Sleep duration and obesity among adults: a meta-analysis of prospective studies. Sleep Med. 2014;15:1456–62.
9. Cappuccio FP, Taggart FM, Kandala NB, Currie A, Peile E, Stranges S, et al. Meta-analysis of short sleep duration and obesity in children and adults. Sleep. 2008;31:619–26.
10. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag, Chronotype, and cardiometabolic risk. J Clin Endocrinol Metabolism. 2015;100:4612–20.
11. Roth T. Insomnia: definition, prevalence, etiology, and consequences. J Clin Sleep Med. 2007;3:7–10.
12. Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev. 2002;6:97–111.
13. Calem M, Bisla J, Begum A, Dewey M, Bebbington PE, Brugha T, et al. Increased prevalence of insomnia and changes in hypnotics use in England over 15 years: analysis of the 1993, 2000, and 2007 National Psychiatric Morbidity Surveys. Sleep. 2012;35:377–84.
14. Laugsand LE, Vatten LJ, Platou C, Janszky I. Insomnia and the risk of Acute myocardial infarction. Circulation. 2011;124:2073–81.
15. Daghlas I, Dashti HS, Lane J, Aragam KG, Rutter MK, Saxena R, et al. Sleep duration and myocardial infarction. J Am Coll Cardiol. 2019;74:1304–14.
16. Knutson KL, Turek FW. The U-shaped association between sleep and health: the 2 peaks do not mean the same thing. Sleep. 2006;29:878–9.
17. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian typology: a comprehensive review. Volume 29. Chronobiology International. Taylor & Francis; 2012. pp. 1153–75.
18. Fan Y, Wu Y, Peng Y, Zhao B, Yang J, Bai L et al. Sleeping late increases the risk of myocardial infarction in the middle-aged and older populations. Front Cardiovasc Med. 2021;8.
19. Merikanto I, Lahti T, Puolijoki H, Vanhala M, Peltonen M, Laatikainen T, et al. Associations of chronotype and sleep with cardiovascular diseases and type 2 diabetes. Chronobiol Int. 2013;30:470–7.
20. Hulsegge G, Picavet HSJ, van der Beek AJ, Verschuren WMM, Twisk JW, Proper KI. Shift work, chronotype and the risk of cardiometabolic risk factors. Eur J Public Health. 2019;29:128–34.
21. Nikbakhtian S, Reed AB, Obika BD, Morelli D, Cunningham AC, Aral M et al. Accelerometer-derived sleep onset timing and cardiovascular disease incidence: a UK Biobank cohort study. Eur Heart J Digit Health. 2021. https://doi.org/10.1093/ehjdh/ztab088
22. Kalmbach DA, Pillai V, Arnedt JT, Drake CL. DSM-5 insomnia and short sleep: comorbidity landscape and racial disparities. Sleep. 2016;39:2101–11.
23. Sands-Lincoln M, Loucks EB, Lu B, Carskadon MA, Sharkey K, Stefanick ML, et al. Sleep duration, insomnia, and coronary

heart disease among postmenopausal women in the women's health initiative. J Womens Health (Larchmt). 2013;22:477–86.
24. Chien K-L, Chen P-C, Hsu H-C, Su T-C, Sung F-C, Chen M-F, et al. Habitual sleep duration and insomnia and the risk of Cardiovascular events and all-cause death: report from a community-based cohort. Sleep. 2010;33:177–84.
25. Vgontzas AN, Fernandez-Mendoza J, Liao D, Bixler EO. Insomnia with objective short sleep duration: the most biologically severe phenotype of the disorder. Sleep Med Rev. 2013;17:241–54.
26. Bertisch SM, Pollock BD, Mittleman MA, Buysse DJ, Bazzano LA, Gottlieb DJ et al. Insomnia with objective short sleep duration and risk of incident cardiovascular disease and all-cause mortality: sleep heart health study. Sleep. 2018;41.
27. Merikanto I, Kronholm E, Peltonen M, Laatikainen T, Lahti T, Partonen T. Relation of chronotype to sleep complaints in the general Finnish population. Chronobiol Int. 2012;29:311–7.
28. UK Biobank: Protocol for a large-scale prospective epidemiological resource. [Internet]. Stockport, Cheshire, UK: UK Biobank Coordinating Centre; 2006 [cited 2021 Oct 25]. Available from: https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us
29. Åsvold BO, Langhammer A, Rehn TA, Kjelvik G, Grøntvedt TV, Sørgjerd EP et al. Cohort Profile Update: The HUNT Study, Norway. Epidemiology; 2021. https://doi.org/10.1101/2021.10.12.21264858
30. Holmen J, Midthjell K, Krüger Ø, Langhammer A, Holmen TL, Bratberg GH, et al. The Nord-Trøndelag Health Study 1995–97 (HUNT 2): objectives, contents, methods and participation. Norsk epidemiologi. 2003;13:19–32.
31. Riemann D, Baglioni C, Bassetti C, Bjorvatn B, Dolenc Groselj L, Ellis JG, et al. European guideline for the diagnosis and treatment of insomnia. J Sleep Res. 2017;26:675–700.
32. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation's sleep time duration recommendations: methodology and results summary. Sleep Health. 2015;1:40–3.
33. Rothman K, Greenland S, Lash TL. Modern Epidemiology. 3rd ed. Research Triangle Park: RTI International; 2007.
34. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol. 2005;20:575–9.
35. Koyanagi A, Garin N, Olaya B, Ayuso-Mateos JL, Chatterji S, Leonardi M, et al. Chronic conditions and sleep problems among adults aged 50 years or over in nine countries: a multi-country study. PLoS ONE. 2014;9:e114742.
36. Ford ES, Cunningham TJ, Croft JB. Trends in self-reported sleep duration among US adults from 1985 to 2012. Sleep. 2015;38:829–32.
37. Frøjd LA, Munkhaugen J, Moum T, Sverre E, Nordhus IH, Papageorgiou C, et al. Insomnia in patients with coronary heart disease: prevalence and correlates. J Clin Sleep Med. 2021;17:931–8.
38. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. The Lancet. 1999;354:1435–9.
39. Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol. 2004;43:678–83.
40. Kwok CS, Kontopantelis E, Kuligowski G, Gray M, Muhyaldeen A, Gale CP, et al. Self-reported sleep duration and quality and cardiovascular disease and mortality: a dose-response meta-analysis. J Am Heart Assoc. 2018;7:e008552.
41. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. Eur Heart J. 2011;32:1484–92.
42. De Amicis R, Galasso L, Leone A, Vignati L, De Carlo G, Foppiani A, et al. Is abdominal fat distribution associated with chronotype in adults independently of lifestyle factors? Nutrients. 2020;12:592.
43. Després J-P. Abdominal obesity: the most prevalent cause of the metabolic syndrome and related cardiometabolic risk. Eur Heart J Suppl. 2006;8:B4–12.
44. Grandner MA, Drummond SPA. Who are the long sleepers? Towards an understanding of the mortality relationship. Sleep Med Rev. 2007;11:341–60.
45. Chouchou F, Pichot V, Pépin JL, Tamisier R, Celle S, Maudoux D, et al. Sympathetic overactivity due to sleep fragmentation is associated with elevated diurnal systolic blood pressure in healthy elderly subjects: the PROOF-SYNAPSE study. Eur Heart J. 2013;34:2122–31.
46. Vallat R, Shah VD, Redline S, Attia P, Walker MP. Broken sleep predicts hardened blood vessels. PLoS Biol. 2020;18:e3000726.
47. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. Arterioscler Thromb Vasc Biol. 2004;24:969–74.
48. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: pathophysiologic mechanisms and emerging clinical indications. Vasc Pharmacol. 2016;77:1–7.
49. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. Sleep Med. 2001;2:389–96.
50. Littner M, Hirshkowitz M, Kramer M, Kapen S, Anderson WM, Bailey D, et al. Practice parameters for using polysomnography to evaluate insomnia: an update. Sleep. 2003;26:754–60.
51. Grandner MA, Chakravorty S. Insomnia in primary care: misreported, mishandled, and just plain missed. J Clin Sleep Med. 2017;13:937–9.
52. Sivertsen B, Øverland S, Krokstad S, Mykletun A. Seasonal variations in sleep problems at Latitude 63°–65° in Norway: the Nord-Trøndelag Health Study, 1995–1997. Am J Epidemiol. 2011;174:147–53.
53. Romero-Corral A, Caples SM, Lopez-Jimenez F, Somers VK. Interactions between obesity and obstructive sleep apnea. Chest. 2010;137:711–9.

Supplementary material, Paper I

The supplementary material for Paper I can also be found from the following link, as part of the published paper:

https://link.springer.com/article/10.1007/s10654-023-00981-x#additional-information

Details on handling of covariates

Clinical information

Information on socio-demographic (i.e., sex, age, marital status, ethnicity (for UKBB only), education and employment status) and lifestyle factors (i.e., smoking, alcohol intake, shift work, physical activity, and use of sleep medication(s)) was collected by means of a self-administered questionnaire. A clinical examination was conducted by trained staff and measurements on weight, height, and blood pressure were collected.

In UKBB, the information on marital status was categorized as “Married” for participants who live with their husband/wife/partner and as “Unmarried” for participants not living with their husband/wife/partner. Additionally, the information on numbers living in a household was used to categorize living alone as “Unmarried” for observations having missing information on marital status. In HUNT2, the information on marital status was categorized into “Unmarried”, “Married” and “Separated/Divorced/Widowed”.

In UKBB, participants were categorized based on their ethnicity as “White”, “Mixed”, “Asian/ Asian British”, “Black/Black British”, “Chinese” or “Other”.

Within UKBB and HUNT2, education was categorized into whether participants had attained “10 years or less” (primary and lower secondary school), “11 - 13 years” (upper secondary school) or “14 years or more” (university/college) of education.

The information on working status from UKBB and HUNT2 was used to create a binary employment status variable with categories “Employed” or “Not employed”.

The information on smoking status was categorized as “Never”, “Previous” or “Current” smoker for UKBB and HUNT2.

In UKBB and HUNT2, the participants were asked about alcohol intake frequency and were categorized as “Never/rarely” for non-drinkers or special occasion drinkers, “Monthly” for those drinking 1 - 3 times a month, “Weekly” for those drinking 1- 4 times a week or “Daily/almost daily” for those drinking even more frequently. In addition, the information concerning alcohol never drinker for HUNT2 was used to categorize never had alcohol as “Never/rarely” for observations having missing information on alcohol intake frequency. Thus, this information will be categorized as “Never/rarely”, “Monthly”, “Weekly” or “Daily/almost daily” alcohol intake.

In UKBB, the participants were asked about doing shift work or working night shifts separately and a proxy variable was made merging these two responses and keeping the highest response category as final. The proxy variable was then dichotomized categorizing the highest categories, i.e., “Usually” or “Always” as “Yes”, and “No” otherwise. In HUNT2, working shifts/at night/on call was dichotomized as “Yes” or “No”. Additionally, the information on current employment/work status from both UKBB and HUNT2 was used to categorize those without having any paid employment or self-employed as “No” for observations having missing information on working shifts/at night/on call.

The physical activity (PA) within UKBB was assessed using adapted questions from the validated short International Physical Activity Questionnaire (IPAQ) [1], following rules published for data processing

by IPAQ [2]. IPAQ assessed total physical activity, including walking, moderate, and vigorous PA performed over the last 7 days. The participants were categorized into three mutually exclusive PA categories - “High” (≥ 1h of moderate PA or ≥ ½ h of vigorous PA above basal level of activity on most days), “Moderate” (≥ ½ h of moderate PA above basal level of activity on most days) or “Low/inactive” (anything else) based on a standard scoring criteria [3], where approximately 5000 steps per day was considered as basal activity. In HUNT2, a proxy variable was created based on participants' response to average hours of light and hard PA during leisure time per week in the last year. Light PA was defined as no sweating or not being out of breath, and hard PA as sweating/out of breath. Participants were instructed to include the commute to work as leisure time. We categorized the HUNT2 participants into similar PA categories - “High” defined by ≥ 1h of hard PA regardless of light PA or ≥ 3h of light PA with < 1h of hard PA, “Moderate” defined by ≥ 3h of light PA with no hard PA or < 3h of light PA with < 1h of hard PA, or “Low/inactive” for anything else. A similar categorization strategy for PA was used before by Brumpton *et al* [4]. The reliability and validity of the questions on PA from HUNT2 have been previously reported to be acceptable for hard PA and poor for light PA [5].

In UKBB, the use of sleep medication(s) was ascertained by the self-reported use of medications from the list of sleep medications as used by Daghlas *et al*. [6], along with five other commonly used anxiolytics or sleep medications (list included in Table S22). A dichotomized “Yes” or “No” variable was created for the use of sleep medication(s). In HUNT2, the participants were asked for their use of anxiolytics or sleep medications in the last month and were categorized as “Yes” for daily or weekly intake, and “No” otherwise.

Within UKBB, weight was measured using the Tanita BC-418MA body composition analyzer, to the nearest 0.1kg and height was measured using a Seca 202 height measure. Within HUNT2, weight was measured to the nearest 0.5kg and height was measured to nearest 1cm. The participants for UKBB and HUNT2 wore light clothes and no shoes during these measurements. The body mass index (BMI) was computed by dividing weight (in kgs) by the squared value of height (in meters).

In UKBB, blood pressure (both systolic and diastolic) measurements were recorded automatedly (using Omron HEM-705 IT electronic blood pressure monitor) and/or manually (using manual sphygmomanometer). Two sets of measurements were taken at a one-minute interval and the average of two were used in our analyses. The manual readings were only used if automated readings were unavailable. In HUNT2, blood pressure (both systolic and diastolic) measurements were recorded automatedly using a Dinamap 845XT (Critikon) sphygmomanometer based on oscillometry. Three sets of measurements were taken at a one-minute interval and the average of second and third measurements were used in our analyses.

Townsend Deprivation Index (TDI) as a measure for socioeconomic status was used for UKBB. The index was created from census data on housing, employment, car availability and social class based on postal codes of participants, with higher values indicating greater deprivation. Townsend deprivation index has been validated for use in a UK-based population [7].

Depression and anxiety

For UKBB, hospitals recorded ICD-10 codes - F40 and F41 for anxiety; and F32, F33, F34, F38 and F39 for depression were used to detect participants having anxiety or depression episodes. Two separate binary proxy variables each for anxiety and depression were created using this information categorized as “Yes” or “No”.

For HUNT2, the Hospital Anxiety and Depression Scale (HADS) was used to assess the symptoms of anxiety and depression. The questionnaire consisted of 14 Likert-scaled items (7 for anxiety and 7 for depression) having a four-point scale ranging from 0 (not at all) to 3 (very often). Responses are summed to provide separate scores each for anxiety and depression ranging from 0 to 21. Higher score indicates increased likelihood of anxiety and depression [8]. No somatic items or items regarding sleeping difficulties were included. HADS is a useful tool in the assessment of symptom severity of anxiety and depression both in primary health care and in hospital settings [9]. The psychometric properties of the scale have previously been validated as part of the HUNT study [10].

Laboratory measurements

For UKBB, a random (non-fasting) blood sample was drawn for each participant at the assessment centers as per standard operating procedure for the UKBB and stored in refrigerators between 2 to 8 °C. Fasting time was recorded as the interval between consumption of food or drink and blood sample(s) being taken. Samples were transferred to a central laboratory for storage and analyses on a daily basis. Serum samples were centrifuged for 10 minutes at 2000 RCF. Serum concentrations of glucose, total cholesterol, HDL cholesterol, and triglycerides were analyzed using a Beckman Coulter AU5800 automated analyzer. Glucose was measured using hexokinase analysis. Total cholesterol, HDL cholesterol and triglycerides were measured by CHO-POD analysis, enzyme immunoinhibition analysis and GPO-POD analysis, respectively [11].

For HUNT2, a random (non-fasting) blood sample was drawn for each participant and analyzed at the Central Laboratory, Levanger Hospital, using a Hitachi 911 Autoanalyzer (Hitachi, Mito, Japan). Serum was separated from blood by centrifugation within 2 hours at the screening site and placed in a refrigerator (4 °C). Time between the last meal and venipuncture was recorded and the samples were sent to the laboratory on the same day or within two to three days (for example on weekends). Serum concentrations of glucose, total cholesterol, HDL cholesterol, and triglycerides were analyzed applying reagents from Boehringer Mannheim (Mannheim, Germany). The day-to-day coefficients of variation were 1.3-2.0%, 1.3-1.9%, 2.4%, and 0.7-1.3%, respectively. Glucose was measured using an enzymatic hexokinase method. Total and HDL cholesterol were measured by an enzymatic colorimetric cholesterol esterase method. Measurement of HDL cholesterol was performed after precipitation with phosphor tungsten and magnesium ions. Triglycerides were measured with an enzymatic colorimetric method [12].

References

1. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003/08/06 ed. 2003;35:1381–95.

2. International Physical Activity Questionnaire (IPAQ): Guidelines for data processing analysis of the International Physical Activity Questionnaire (IPAQ) - Short and long forms [Internet]. 2005 [cited 2021 Oct 25]. Available from: https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=540

3. International Physical Activity Questionnaire (IPAQ): IPAQ scoring protocol [Internet]. 2005 [cited 2021 Oct 25]. Available from: https://sites.google.com/site/theipaq/scoring-protocol

4. Brumpton BM, Langhammer A, Ferreira MAR, Chen Y, Mai X-M. Physical activity and incident asthma in adults: the HUNT Study, Norway. BMJ Open. 2016;6:e013856.

5. Kurtze N, Rangul V, Hustvedt BE, Flanders WD. Reliability and validity of self-reported physical activity in the Nord-Trøndelag Health Study (HUNT 2). Eur J Epidemiol. 2007/03/16 ed. 2007;22:379–87.

6. Daghlas I, Dashti HS, Lane J, Aragam KG, Rutter MK, Saxena R, et al. Sleep Duration and Myocardial Infarction. J Am Coll Cardiol. 2019/09/07 ed. 2019;74:1304–14.

7. Townsend P. Poverty in the United Kingdom: A Survey of Household Resources and Standards of Living. London: Allen Lane and Penguin Books; 1979.

8. Skapinakis P. Hospital Anxiety and Depression Scale (HADS). In: Michalos AC, editor. Encyclopedia of Quality of Life and Well-Being Research [Internet]. Dordrecht: Springer Netherlands; 2014. p. 2930–3. Available from: https://doi.org/10.1007/978-94-007-0753-5_1315

9. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res. 2002/02/08 ed. 2002;52:69–77.

10. Mykletun A, Stordal E, Dahl AA. Hospital Anxiety and Depression (HAD) scale: factor structure, item analyses and internal consistency in a large population. Br J Psychiatry. 2001/12/04 ed. 2001;179:540–4.

11. Elliott P, Peakman TC, UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International Journal of Epidemiology. 2008;37:234–44.

12. Holmen J, Midthjell K, Krüger Ø, Langhammer A, Holmen TL, Bratberg GH, et al. The Nord-Trøndelag Health Study 1995–97 (HUNT 2): objectives, contents, methods and participation. Norsk epidemiologi. 2003;13:19–32.

Supplementary tables

Table S1: Baseline characteristics of participants from UK Biobank and HUNT2 according to self-reported sleep duration.

	UK Biobank			HUNT2		
	Sleep duration (n = 302 456)			Sleep duration (n = 31 091)		
	Short (≤ 6 hours)	Normal (7 or 8 hours)	Long (≥ 9 hours)	Short (≤ 6 hours)	Normal (7 or 8 hours)	Long (≥ 9 hours)
Total, % (n)	23.9 (72 216)	68.7 (207 777)	7.4 (22 463)	6.2 (1 928)	70.1 (21 792)	23.7 (7 371)
Variables, % (n)						
Male	47.9 (34 598)	46.4 (96 328)	44.4 (9 967)	57.9 (1 117)	47.8 (10 410)	40.7 (2 997)
Married	68.1 (49 152)	75.6 (157 045)	72.3 (16 250)	55.3 (1 067)	62.9 (13 718)	57.4 (4 234)
Weekly alcohol intake	47.4 (34 203)	51.2 (106 383)	43.9 (9 862)	25.7 (496)	27.7 (6 047)	22.9 (1 688)
Current smokers	12.2 (8 841)	9.4 (19 562)	11.6 (2 613)	37.8 (729)	29.1 (6 333)	27.1 (1 998)
Highly physically active	40.9 (29 502)	40.6 (84 382)	37.5 (8 420)	38.7 (746)	39.4 (8 590)	31.2 (2 303)
Tertiary education	43.7 (31 534)	48.8 (101 431)	40.3 (9 058)	19.1 (368)	27.0 (5 875)	19.9 (1 466)
Shift workers	7.6 (5 474)	4.8 (9 889)	4.1 (913)	23.0 (444)	19.0 (4 131)	14.6 (1 079)
Employed	62.8 (45 343)	61.2 (127 076)	35.2 (7 910)	79.0 (1 523)	78.5 (17 116)	51.1 (3 766)
Use of sleep medication(s)	1.4 (1 035)	0.6 (1 290)	1.6 (365)	5.7 (110)	4.3 (933)	10.8 (799)
Suffering from depression	13.3 (9 622)	10.0 (20 821)	19.3 (4 339)	-	-	-
Suffering from anxiety	7.3 (5 256)	5.6 (11 640)	9.1 (2 037)	-	-	-
Variables, mean (SD)						
Age, *years*	56.11 (7.87)	56.07 (8.16)	58.31 (8.07)	42.93 (13.58)	44.69 (13.86)	50.28 (18.55)
TDI	-1.05 (3.19)	-1.59 (2.91)	-1.21 (3.15)	-	-	-
BMI, kg/m^2	27.85 (4.98)	27.03 (4.51)	27.93 (5.04)	26.34 (4.10)	26.03 (3.89)	26.36 (4.26)
SBP, *mmHg*	137.60 (18.25)	137.40 (18.59)	139.10 (19.15)	132.30 (17.75)	133.40 (18.72)	138.20 (22.41)
Time since last meal, *hours*	3.94 (2.65)	3.71 (2.29)	3.84 (2.52)	2.29 (2.11)	2.15 (1.91)	2.13 (1.89)
Serum cholesterol, *mmol/L*	5.70 (1.13)	5.71 (1.12)	5.68 (1.21)	5.62 (1.14)	5.71 (1.19)	5.92 (1.28)
Blood glucose, *mmol/L*	5.13 (1.26)	5.08 (1.13)	5.26 (1.54)	5.26 (1.17)	5.29 (1.22)	5.49 (1.63)
HADS - D scores	-	-	-	3.66 (3.24)	3.02 (2.75)	3.60 (3.17)
HADS - A scores	-	-	-	4.84 (3.67)	4.05 (3.15)	4.25 (3.38)

SD indicates standard deviation; TDI, Townsend deprivation index; BMI, body mass index; SBP, systolic blood pressure; HADS – D scores, Hospital Anxiety and Depression Scale – Depression scores; and HADS – A scores, Hospital Anxiety and Depression Scale – Anxiety scores.

Table S2: Baseline characteristics of participants from UK Biobank according to self-reported chronotype.

	UK Biobank	
	Chronotype (n = 302 456)	
	Morning	Evening
Total, % (n)	62.8 (189 978)	37.2 (112 478)
Variables, % (n)		
Male	45.9 (87 231)	47.7 (53 662)
Married	75.2 (142 816)	70.8 (79 631)
Weekly alcohol intake	49.7 (94 381)	49.8 (56 067)
Current smokers	8.1 (15 457)	13.8 (15 559)
Highly physically active	42.7 (81 205)	36.5 (41 099)
Tertiary education	46.5 (88 311)	47.8 (53 712)
Shift workers	4.8 (9 164)	6.3 (7 112)
Employed	58.5 (111 179)	61.5 (69 150)
Use of sleep medication(s)	0.8 (1 436)	1.1 (1 254)
Suffering from depression	10.3 (19 525)	13.6 (15 257)
Suffering from anxiety	5.8 (11 094)	7.0 (7 839)
Variables, mean (SD)		
Age, *years*	56.79 (7.95)	55.33 (8.29)
TDI	-1.53 (2.96)	-1.28 (3.08)
BMI, kg/m^2	27.20 (4.63)	27.43 (4.77)
SBP, *mmHg*	138.00 (18.57)	136.90 (18.52)
Time since last meal, *hours*	3.73 (2.29)	3.84 (2.58)
Serum cholesterol, *mmol/L*	5.71 (1.13)	5.71 (1.13)
Blood glucose, *mmol/L*	5.10 (1.17)	5.11 (1.26)

SD indicates standard deviation; TDI, Townsend deprivation index; BMI, body mass index; and SBP, systolic blood pressure.

Table S3: Hazard ratios (95% CIs) for acute myocardial infarction according to insomnia symptoms, sleep duration, chronotype and their joint association stratified by age at 65 years in UK Biobank (UKBB) and HUNT2.*

	UK Biobank			HUNT2		
	Age < 65 y	**Age ≥ 65 y**	**P for interaction**	**Age < 65 y**	**Age ≥ 65 y**	**P for interaction**
Exposures						
Insomnia symptoms						
No	Ref.	Ref.	0.064	Ref.	Ref.	0.190
Yes	1.14 (1.07, 1.21)	1.04 (0.95, 1.14)		1.09 (0.95, 1.25)	1.09 (0.92, 1.29)	
Sleep duration						
Short	1.11 (1.04, 1.18)	1.06 (0.95, 1.17)		0.97 (0.80, 1.18)	1.35 (0.96, 1.89)	
Normal	Ref.	Ref.	0.399	Ref.	Ref.	0.035
Long	1.11 (1.00, 1.24)	1.17 (1.03, 1.32)		0.88 (0.78, 1.01)	1.06 (0.92, 1.21)	
Chronotype						
Morning	Ref.	Ref.	0.219	-	-	
Evening	1.10 (1.03, 1.16)	1.03 (0.94, 1.13)		-	-	
Insomnia symptoms (INS) and Sleep duration (SLD)						
No INS & Short SLD	1.07 (0.98, 1.17)	1.07 (0.92, 1.23)		1.01 (0.81, 1.26)	1.05 (0.66, 1.67)	
No INS & Normal SLD	Ref.	Ref.	0.390	Ref.	Ref.	0.014
No INS & Long SLD	1.06 (0.93, 1.20)	1.11 (0.96, 1.29)		0.87 (0.75, 1.00)	1.10 (0.94, 1.28)	
INS & Short SLD	1.20 (1.10, 1.31)	1.05 (0.92, 1.21)		0.90 (0.62, 1.33)	2.08 (1.29, 3.36)	
INS & Normal SLD	1.10 (1.00, 1.20)	1.02 (0.90, 1.15)		1.09 (0.92, 1.28)	1.15 (0.90, 1.46)	
INS & Long SLD	1.41 (1.16, 1.71)	1.38 (1.09, 1.74)		1.07 (0.81, 1.40)	1.02 (0.78, 1.32)	
Insomnia symptoms (INS) and Chronotype (CT)						
No INS & Morning CT	Ref.	Ref.	<0.001	-	-	
No INS & Evening CT	1.15 (1.07, 1.23)	0.94 (0.85, 1.05)		-	-	
INS & Morning CT	1.21 (1.11, 1.31)	0.94 (0.83, 1.05)		-	-	
INS & Evening CT	1.19 (1.08, 1.31)	1.18 (1.03, 1.35)		-	-	
Chronotype (CT) and Sleep duration (SLD)						
Morning CT & Short SLD	1.15 (1.06, 1.25)	1.00 (0.88, 1.14)		-	-	
Morning CT & Normal SLD	Ref.	Ref.	0.171	-	-	
Morning CT & Long SLD	1.10 (0.95, 1.28)	1.18 (1.01, 1.38)		-	-	
Evening CT & Short SLD	1.18 (1.07, 1.30)	1.19 (1.00, 1.41)		-	-	
Evening CT & Normal SLD	1.13 (1.05, 1.21)	1.00 (0.89, 1.12)		-	-	
Evening CT & Long SLD	1.25 (1.07, 1.46)	1.14 (0.94, 1.39)		-	-	

CI indicates confidence interval; INS, insomnia symptoms; SLD, sleep duration; and CT, chronotype.

* Adjustments were performed as in Model 2, Table 2.

Table S4: Hazard ratios (95% CIs) for acute myocardial infarction according to insomnia symptoms, sleep duration, chronotype and their joint association stratified by gender in UK Biobank (UKBB) and HUNT2.*

	UK Biobank			HUNT2		
	Female	**Male**	**P for interaction**	**Female**	**Male**	**P for interaction**
Exposures						
Insomnia symptoms						
No	Ref.	Ref.	0.795	Ref.	Ref.	0.282
Yes	1.12 (1.02, 1.23)	1.10 (1.03, 1.17)		1.17 (0.99, 1.37)	1.03 (0.89, 1.19)	
Sleep duration						
Short	1.14 (1.02, 1.26)	1.08 (1.01, 1.15)		1.04 (0.76, 1.42)	1.05 (0.86, 1.28)	
Normal	Ref.	Ref.	0.676	Ref.	Ref.	0.801
Long	1.16 (1.00, 1.36)	1.13 (1.02, 1.24)		0.93 (0.79, 1.08)	0.99 (0.88, 1.11)	
Chronotype						
Morning	Ref.	Ref.	0.859	-	-	
Evening	1.08 (0.99, 1.19)	1.08 (1.02, 1.14)		-	-	
Insomnia symptoms (INS) and Sleep duration (SLD)						
No INS & Short SLD	1.19 (1.01, 1.39)	1.04 (0.95, 1.13)		1.12 (0.77, 1.63)	0.99 (0.78, 1.25)	
No INS & Normal SLD	Ref.	Ref.	0.534	Ref.	Ref.	0.596
No INS & Long SLD	1.17 (0.98, 1.40)	1.06 (0.94, 1.18)		0.96 (0.80, 1.14)	0.98 (0.87, 1.11)	
INS & Short SLD	1.17 (1.03, 1.34)	1.15 (1.05, 1.27)		1.04 (0.61, 1.78)	1.25 (0.87, 1.78)	
INS & Normal SLD	1.15 (1.01, 1.30)	1.04 (0.95, 1.13)		1.25 (1.01, 1.54)	0.98 (0.82, 1.18)	
INS & Long SLD	1.33 (0.99, 1.79)	1.43 (1.20, 1.70)		1.04 (0.79, 1.35)	1.02 (0.78, 1.33)	
Insomnia symptoms (INS) and Chronotype (CT)						
No INS & Morning CT	Ref.	Ref.	0.934	-	-	
No INS & Evening CT	1.07 (0.95, 1.20)	1.09 (1.01, 1.16)		-	-	
INS & Morning CT	1.10 (0.98, 1.24)	1.11 (1.03, 1.21)		-	-	
INS & Evening CT	1.22 (1.06, 1.40)	1.17 (1.07, 1.29)		-	-	
Chronotype (CT) and Sleep duration (SLD)						
Morning CT & Short SLD	1.12 (0.98, 1.28)	1.10 (1.01, 1.19)		-	-	
Morning CT & Normal SLD	Ref.	Ref.	0.458	-	-	
Morning CT & Long SLD	1.29 (1.06, 1.57)	1.09 (0.96, 1.24)		-	-	
Evening CT & Short SLD	1.28 (1.09, 1.51)	1.14 (1.03, 1.26)		-	-	
Evening CT & Normal SLD	1.10 (0.98, 1.24)	1.08 (1.00, 1.16)		-	-	
Evening CT & Long SLD	1.10 (0.86, 1.40)	1.26 (1.09, 1.45)		-	-	

CI indicates confidence interval; INS, insomnia symptoms; SLD, sleep duration; and CT, chronotype.

* Adjustments were performed as in Model 2, Table 2.

Table S5: Hazard ratios (95% CIs) for acute myocardial infarction according to insomnia symptoms, sleep duration, chronotype and their joint association stratified by shift work (or night shifts) in UK Biobank (UKBB) and HUNT2.*

	UK Biobank			HUNT2		
	Non-shift workers	**Shift workers**	**P for interaction**	**Non-shift workers**	**Shift workers**	**P for interaction**
Exposures						
Insomnia symptoms						
No	Ref.	Ref.	0.439	Ref.	Ref.	0.399
Yes	1.10 (1.04, 1.16)	1.20 (0.95, 1.51)		1.07 (0.96, 1.20)	1.20 (0.88, 1.64)	
Sleep duration						
Short	1.10 (1.04, 1.16)	1.05 (0.84, 1.30)		1.06 (0.88, 1.28)	0.99 (0.67, 1.45)	
Normal	Ref.	Ref.	0.842	Ref.	Ref.	0.657
Long	1.14 (1.05, 1.24)	1.05 (0.66, 1.66)		0.98 (0.89, 1.08)	0.86 (0.62, 1.19)	
Chronotype						
Morning	Ref.	Ref.	0.519	-	-	
Evening	1.08 (1.03, 1.14)	1.00 (0.81, 1.24)		-	-	
Insomnia symptoms (INS) and Sleep duration (SLD)						
No INS & Short SLD	1.07 (0.99, 1.15)	1.12 (0.86, 1.45)		0.99 (0.79, 1.25)	1.13 (0.74, 1.73)	
No INS & Normal SLD	Ref.	Ref.	0.678	Ref.	Ref.	0.487
No INS & Long SLD	1.09 (0.99, 1.20)	1.02 (0.60, 1.73)		0.98 (0.88, 1.09)	0.91 (0.65, 1.28)	
INS & Short SLD	1.16 (1.07, 1.25)	1.11 (0.80, 1.53)		1.27 (0.92, 1.74)	0.72 (0.30, 1.76)	
INS & Normal SLD	1.06 (0.98, 1.14)	1.39 (1.00, 1.92)		1.05 (0.91, 1.22)	1.39 (0.98, 1.98)	
INS & Long SLD	1.39 (1.20, 1.62)	1.58 (0.64, 3.88)		1.03 (0.85, 1.25)	0.84 (0.34, 2.10)	
Insomnia symptoms (INS) and Chronotype (CT)						
No INS & Morning CT	Ref.	Ref.	0.696	-	-	
No INS & Evening CT	1.08 (1.02, 1.15)	1.06 (0.83, 1.35)		-	-	
INS & Morning CT	1.10 (1.03, 1.18)	1.32 (0.97, 1.80)		-	-	
INS & Evening CT	1.19 (1.10, 1.29)	1.13 (0.80, 1.59)		-	-	
Chronotype (CT) and Sleep duration (SLD)						
Morning CT & Short SLD	1.10 (1.02, 1.18)	1.10 (0.83, 1.47)		-	-	
Morning CT & Normal SLD	Ref.	Ref.	0.825	-	-	
Morning CT & Long SLD	1.14 (1.02, 1.27)	1.30 (0.71, 2.37)		-	-	
Evening CT & Short SLD	1.19 (1.09, 1.30)	1.05 (0.76, 1.45)		-	-	
Evening CT & Normal SLD	1.08 (1.02, 1.15)	1.08 (0.82, 1.42)		-	-	
Evening CT & Long SLD	1.23 (1.09, 1.39)	0.87 (0.42, 1.78)		-	-	

CI indicates confidence interval; INS, insomnia symptoms; SLD, sleep duration; and CT, chronotype.
* Adjustments were performed as in Model 2, Table 2.

Table S6: Hazard ratios (95% CIs) for acute myocardial infarction according to insomnia symptoms, sleep duration, chronotype and their joint association stratified by depression in UK Biobank (UKBB) and HUNT2.*

	UK Biobank			HUNT2		
	Without depression	**With depression**	**P for interaction**	**Without depression**	**With depression**	**P for interaction**
Exposures						
Insomnia symptoms						
No	Ref.	Ref.	0.736	Ref.	Ref.	0.600
Yes	1.08 (1.02, 1.14)	1.11 (0.98, 1.26)		1.10 (0.97, 1.25)	0.96 (0.72, 1.26)	
Sleep duration						
Short	1.09 (1.03, 1.16)	1.05 (0.91, 1.22)		1.11 (0.92, 1.33)	1.07 (0.67, 1.69)	
Normal	Ref.	Ref.	0.847	Ref.	Ref.	0.841
Long	1.09 (0.99, 1.20)	1.13 (0.94, 1.35)		0.93 (0.84, 1.05)	0.97 (0.74, 1.26)	
Chronotype						
Morning	Ref.	Ref.	0.997	-	-	
Evening	1.06 (1.01, 1.12)	1.06 (0.94, 1.21)		-	-	
Insomnia symptoms (INS) and Sleep duration (SLD)						
No INS & Short SLD	1.09 (1.00, 1.17)	1.01 (0.81, 1.26)		1.10 (0.90, 1.36)	0.87 (0.44, 1.73)	
No INS & Normal SLD	Ref.	Ref.	0.932	Ref.	Ref.	0.947
No INS & Long SLD	1.06 (0.96, 1.18)	1.00 (0.80, 1.26)		0.93 (0.84, 1.04)	0.99 (0.72, 1.35)	
INS & Short SLD	1.13 (1.04, 1.23)	1.10 (0.92, 1.31)		1.18 (0.83, 1.68)	1.23 (0.66, 2.30)	
INS & Normal SLD	1.06 (0.98, 1.14)	1.04 (0.87, 1.25)		1.11 (0.95, 1.29)	0.94 (0.66, 1.35)	
INS & Long SLD	1.27 (1.05, 1.52)	1.45 (1.10, 1.90)		1.00 (0.80, 1.25)	0.87 (0.56, 1.33)	
Insomnia symptoms (INS) and Chronotype (CT)						
No INS & Morning CT	Ref.	Ref.	0.888	-	-	
No INS & Evening CT	1.07 (1.00, 1.14)	1.11 (0.94, 1.31)		-	-	
INS & Morning CT	1.08 (1.00, 1.16)	1.16 (0.98, 1.38)		-	-	
INS & Evening CT	1.15 (1.05, 1.25)	1.16 (0.97, 1.40)		-	-	
Chronotype (CT) and Sleep duration (SLD)						
Morning CT & Short SLD	1.10 (1.02, 1.18)	1.07 (0.88, 1.29)		-	-	
Morning CT & Normal SLD	Ref.	Ref.	0.991	-	-	
Morning CT & Long SLD	1.12 (0.99, 1.26)	1.11 (0.86, 1.45)		-	-	
Evening CT & Short SLD	1.17 (1.06, 1.29)	1.11 (0.90, 1.37)		-	-	
Evening CT & Normal SLD	1.07 (1.01, 1.15)	1.06 (0.90, 1.26)		-	-	
Evening CT & Long SLD	1.13 (0.98, 1.30)	1.20 (0.94, 1.53)		-	-	

CI indicates confidence interval; INS, insomnia symptoms; SLD, sleep duration; and CT, chronotype.
* Adjustments were performed as in Model 3, Table 2

Table S7: Hazard ratios (95% CIs) for acute myocardial infarction according to insomnia symptoms, sleep duration, chronotype and their joint association stratified by anxiety in UK Biobank (UKBB) and HUNT2.*

	UK Biobank			HUNT2		
	Without anxiety	**With anxiety**	**P for interaction**	**Without anxiety**	**With anxiety**	**P for interaction**
Exposures						
Insomnia symptoms						
No	Ref.	Ref.	0.991	Ref.	Ref.	0.992
Yes	1.08 (1.02, 1.14)	1.12 (0.95, 1.31)		1.07 (0.94, 1.23)	1.07 (0.85, 1.35)	
Sleep duration						
Short	1.09 (1.03, 1.16)	1.05 (0.88, 1.25)		1.15 (0.95, 1.38)	0.88 (0.58, 1.33)	
Normal	Ref.	Ref.	0.406	Ref.	Ref.	0.632
Long	1.11 (1.02, 1.21)	1.01 (0.79, 1.29)		0.94 (0.85, 1.04)	0.90 (0.70, 1.15)	
Chronotype						
Morning	Ref.	Ref.	0.881	-	-	
Evening	1.06 (1.01, 1.12)	1.08 (0.92, 1.26)		-	-	
Insomnia symptoms (INS) and Sleep duration (SLD)						
No INS & Short SLD	1.09 (1.00, 1.17)	0.97 (0.75, 1.27)		1.12 (0.91, 1.38)	0.71 (0.36, 1.41)	
No INS & Normal SLD	Ref.	Ref.	0.804	Ref.	Ref.	0.842
No INS & Long SLD	1.06 (0.96, 1.17)	0.97 (0.72, 1.32)		0.96 (0.86, 1.06)	0.86 (0.62, 1.18)	
INS & Short SLD	1.12 (1.04, 1.22)	1.13 (0.92, 1.41)		1.29 (0.89, 1.87)	1.02 (0.60, 1.73)	
INS & Normal SLD	1.05 (0.97, 1.13)	1.07 (0.86, 1.33)		1.09 (0.92, 1.29)	1.01 (0.76, 1.35)	
INS & Long SLD	1.35 (1.15, 1.59)	1.17 (0.78, 1.75)		0.94 (0.74, 1.19)	0.98 (0.68, 1.41)	
Insomnia symptoms (INS) and Chronotype (CT)						
No INS & Morning CT	Ref.	Ref.	0.997	-	-	
No INS & Evening CT	1.07 (1.01, 1.14)	1.07 (0.88, 1.31)		-	-	
INS & Morning CT	1.09 (1.01, 1.17)	1.11 (0.90, 1.37)		-	-	
INS & Evening CT	1.14 (1.04, 1.24)	1.21 (0.96, 1.52)		-	-	
Chronotype (CT) and Sleep duration (SLD)						
Morning CT & Short SLD	1.10 (1.02, 1.18)	1.06 (0.85, 1.33)		-	-	
Morning CT & Normal SLD	Ref.	Ref.	0.381	-	-	
Morning CT & Long SLD	1.10 (0.98, 1.23)	1.22 (0.88, 1.69)		-	-	
Evening CT & Short SLD	1.16 (1.06, 1.27)	1.19 (0.92, 1.54)		-	-	
Evening CT & Normal SLD	1.06 (1.00, 1.14)	1.14 (0.93, 1.40)		-	-	
Evening CT & Long SLD	1.19 (1.04, 1.35)	0.92 (0.64, 1.33)		-	-	

CI indicates confidence interval; INS, insomnia symptoms; SLD, sleep duration; and CT, chronotype.
* Adjustments were performed as in Model 3, Table 2.

Table S8: Hazard ratios (95% CIs) for acute myocardial infarction (AMI) according to self-reported insomnia, sleep duration and chronotype excluding the first two years of follow-up in UK Biobank (UKBB) and HUNT2.

		Insomnia		Sleep duration			Chronotype	
		No	Yes	Short	Normal	Long	Morning	Evening
UK Biobank (n = 300 385)	AMI events/ Person-years	4 277/ 2 581 934	1 812/ 964 084	1 592/ 843 405	3 906/ 2 444 472	591/ 258 141	3 734/ 2 228 511	2 355/ 1 317 507
	Model 1	Ref.	1.16 (1.10, 1.23)	1.20 (1.13, 1.27)	Ref.	1.24 (1.13, 1.35)	Ref.	1.16 (1.10, 1.22)
	Model 2	Ref.	1.09 (1.03, 1.16)	1.09 (1.03, 1.16)	Ref.	1.11 (1.02, 1.22)	Ref.	1.10 (1.05, 1.16)
	Model 3	Ref.	1.07 (1.01, 1.13)	1.08 (1.02, 1.15)	Ref.	1.07 (0.98, 1.17)	Ref.	1.09 (1.03, 1.14)
HUNT2 (n = 30 464)	AMI events/ Person-years	1 999/ 576 661	391/ 76 290	144/ 41 265	1 595/ 469 968	651/ 141 718	-	-
	Model 1	Ref.	1.15 (1.03, 1.29)	1.15 (0.97, 1.36)	Ref.	0.98 (0.89, 1.08)	-	-
	Model 2	Ref.	1.09 (0.97, 1.21)	1.06 (0.89, 1.26)	Ref.	0.92 (0.84, 1.02)	-	-
	Model 3	Ref.	1.08 (0.95, 1.21)	1.10 (0.93, 1.31)	Ref.	0.90 (0.82, 0.99)	-	-

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index (for UKBB), ethnicity (for UKBB), shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S9: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and sleep duration excluding the first two years of follow-up in UK Biobank (UKBB) and HUNT2.

		No insomnia symptoms			Insomnia symptoms		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 300 385)	AMI events/ Person-years	808/ 427 384	3 033/ 1 950 013	436/ 204 537	784/ 416 020	873/ 494 459	155/ 53 604
	Model 1	1.18 (1.09, 1.27)	Ref.	1.19 (1.07, 1.31)	1.29 (1.19, 1.39)	1.12 (1.04, 1.20)	1.57 (1.34, 1.85)
	Model 2	1.08 (1.00, 1.17)	Ref.	1.08 (0.98, 1.20)	1.14 (1.05, 1.23)	1.07 (0.99, 1.16)	1.32 (1.12, 1.55)
	Model 3	1.08 (1.00, 1.17)	Ref.	1.04 (0.94, 1.16)	1.10 (1.02, 1.20)	1.05 (0.98, 1.14)	1.23 (1.04, 1.45)
HUNT2 (n = 30 464)	AMI events/ Person-years	103/ 32 939	1 357/ 420 657	539/ 123 065	41/ 8 326	238/ 49 311	112/ 18 653
	Model 1	1.12 (0.92, 1.37)	Ref.	0.99 (0.90, 1.10)	1.32 (0.97, 1.81)	1.17 (1.02, 1.35)	1.08 (0.89, 1.31)
	Model 2	1.05 (0.86, 1.28)	Ref.	0.93 (0.84, 1.04)	1.15 (0.84, 1.57)	1.10 (0.96, 1.27)	0.96 (0.79, 1.17)
	Model 3	1.11 (0.90, 1.35)	Ref.	0.91 (0.82, 1.01)	1.15 (0.84, 1.58)	1.09 (0.95, 1.27)	0.92 (0.75, 1.13)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index (for UKBB), ethnicity (for UKBB), shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S10: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and chronotype excluding the first two years of follow-up in UK Biobank.

		No insomnia symptoms		Insomnia symptoms	
		Chronotype		Chronotype	
		Morning	Evening	Morning	Evening
UK Biobank (n = 300 385)	AMI events/ Person-years	2 634/ 1 624 446	1 643/ 957 489	1 100/ 604 066	712/ 360 018
	Model 1	Ref.	1.14 (1.07, 1.21)	1.14 (1.06, 1.22)	1.37 (1.26, 1.49)
	Model 2	Ref.	1.10 (1.03, 1.17)	1.09 (1.02, 1.17)	1.21 (1.11, 1.31)
	Model 3	Ref.	1.09 (1.02, 1.16)	1.07 (1.00, 1.15)	1.16 (1.06, 1.26)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, ethnicity, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S11: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported chronotype and sleep duration excluding the first two years of follow-up in UK Biobank.

		Morning chronotype			Evening chronotype		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 300 385)	AMI events/ Person-years	1 006/ 539 027	2 398/ 1 539 879	330/ 149 605	586/ 304 377	1 508/ 904 594	261/ 108 536
	Model 1	1.20 (1.11, 1.29)	Ref.	1.23 (1.09, 1.38)	1.41 (1.28, 1.54)	1.15 (1.08, 1.23)	1.41 (1.24, 1.60)
	Model 2	1.09 (1.02, 1.18)	Ref.	1.12 (1.00, 1.26)	1.21 (1.10, 1.32)	1.11 (1.04, 1.18)	1.21 (1.06, 1.37)
	Model 3	1.08 (1.01, 1.17)	Ref.	1.09 (0.97, 1.23)	1.18 (1.08, 1.30)	1.09 (1.02, 1.17)	1.13 (0.99, 1.29)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, ethnicity, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S12: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to self-reported insomnia symptoms, sleep duration and chronotype after adjusting for chronic disorders within UK Biobank (UKBB) and HUNT2.

		Insomnia symptoms		Sleep duration			Chronotype	
		No	Yes	Short	Normal	Long	Morning	Evening
UK Biobank (n = 297 170)	AMI events/ Person-years	4 702/ 2 543 217	2 006/ 943 592	1 758/ 825 662	4 286/ 2 407 383	664/ 253 764	4 130/ 2 193 113	2 578/ 1 293 696
	Model 1	Ref.	1.19 (1.13, 1.25)	1.21 (1.14, 1.28)	Ref.	1.30 (1.20, 1.41)	Ref.	1.14 (1.08, 1.19)
	Model 2	Ref.	1.06 (1.01, 1.12)	1.07 (1.01, 1.13)	Ref.	1.11 (1.02, 1.21)	Ref.	1.07 (1.02, 1.12)
	Model 3	Ref.	1.04 (0.99, 1.10)	1.07 (1.01, 1.13)	Ref.	1.08 (0.99, 1.17)	Ref.	1.05 (1.00, 1.11)
HUNT2 (n = 30 604)	AMI events/ Person-years	2 087/ 568 946	408/ 74 696	146/ 40 478	1 641/ 463 792	708/ 139 371	-	-
	Model 1	Ref.	1.16 (1.04, 1.29)	1.13 (0.96, 1.34)	Ref.	1.05 (0.96, 1.15)	-	-
	Model 2	Ref.	1.04 (0.93, 1.16)	1.03 (0.87, 1.22)	Ref.	0.95 (0.87, 1.05)	-	-
	Model 3	Ref.	1.04 (0.93, 1.18)	1.08 (0.91, 1.28)	Ref.	0.93 (0.84, 1.02)	-	-

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index (for UKBB), ethnicity (for UKBB), shift work, employment status, and chronic disorders.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S13: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and sleep duration after adjusting for chronic disorders within UK Biobank (UKBB) and HUNT2.

		No insomnia symptoms			Insomnia symptoms		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 297 170)	AMI events/ Person-years	883/ 419 868	3 338/ 1 922 214	481/ 201 134	875/ 405 794	948/ 485 168	183/ 52 629
	Model 1	1.16 (1.08, 1.25)	Ref.	1.22 (1.11, 1.35)	1.33 (1.23, 1.43)	1.12 (1.04, 1.20)	1.74 (1.50, 2.02)
	Model 2	1.06 (0.98, 1.14)	Ref.	1.06 (0.96, 1.17)	1.10 (1.02, 1.19)	1.03 (0.95, 1.10)	1.31 (1.13, 1.52)
	Model 3	1.07 (0.99, 1.15)	Ref.	1.03 (0.93, 1.14)	1.08 (1.00, 1.17)	1.02 (0.95, 1.09)	1.25 (1.07, 1.45)
HUNT2 (n = 30 604)	AMI events/ Person-years	104/ 32 417	1 399/ 415 376	584/ 121 153	42/ 8 061	242/ 48 416	124/ 18 219
	Model 1	1.10 (0.90, 1.34)	Ref.	1.06 (0.96, 1.17)	1.33 (0.98, 1.80)	1.17 (1.02, 1.34)	1.18 (0.98, 1.42)
	Model 2	1.02 (0.84, 1.25)	Ref.	0.96 (0.87, 1.06)	1.07 (0.79, 1.46)	1.05 (0.92, 1.21)	0.97 (0.80, 1.17)
	Model 3	1.08 (0.89, 1.32)	Ref.	0.93 (0.84, 1.03)	1.10 (0.81, 1.50)	1.06 (0.91, 1.22)	0.93 (0.77, 1.14)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index (for UKBB), ethnicity (for UKBB), shift work, employment status, and chronic disorders.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S14: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and chronotype after adjusting for chronic disorders within UK Biobank.

		No insomnia symptoms		Insomnia symptoms	
		Chronotype		Chronotype	
		Morning	Evening	Morning	Evening
UK Biobank (n = 297 170)	AMI events/ Person-years	2 907/ 1 601 216	1 795/ 942 000	1 223/ 591 896	783/ 351 695
	Model 1	Ref.	1.12 (1.06, 1.19)	1.17 (1.09, 1.25)	1.37 (1.27, 1.49)
	Model 2	Ref.	1.07 (1.01, 1.13)	1.06 (0.99, 1.14)	1.13 (1.04, 1.23)
	Model 3	Ref.	1.06 (1.00, 1.12)	1.05 (0.98, 1.12)	1.10 (1.01, 1.19)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, ethnicity, shift work, employment status, and chronic disorders.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S15: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported chronotype and sleep duration after adjusting for chronic disorders within UK Biobank.

		Morning chronotype			Evening chronotype		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 297 170)	AMI events/ Person-years	1 124/ 528 105	2 635/ 1 517 813	371/ 147 195	634/ 297 557	1 651/ 889 570	293/ 106 569
	Model 1	1.22 (1.14, 1.31)	Ref.	1.29 (1.16, 1.44)	1.37 (1.25, 1.49)	1.14 (1.07, 1.21)	1.47 (1.30, 1.66)
	Model 2	1.09 (1.02, 1.17)	Ref.	1.12 (1.01, 1.25)	1.13 (1.04, 1.24)	1.08 (1.02, 1.15)	1.17 (1.03, 1.32)
	Model 3	1.08 (1.01, 1.16)	Ref.	1.10 (0.99, 1.23)	1.12 (1.03, 1.22)	1.07 (1.01, 1.14)	1.11 (0.98, 1.26)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, ethnicity, shift work, employment status, and chronic disorders.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S16: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to self-reported insomnia symptoms, sleep duration and chronotype within White British population in UK Biobank.

		Insomnia symptoms		Sleep duration			Chronotype	
		No	Yes	Short	Normal	Long	Morning	Evening
UK Biobank (n = 269 375)	AMI events/ Person-years	4 290/ 2 292 585	1 866/ 872 425	1 585/ 733 803	3 974/ 2 200 379	597/ 230 829	3 802/ 1 999 431	2 354/ 1 165 579
	Model 1	Ref.	1.19 (1.13, 1.26)	1.20 (1.13, 1.27)	Ref.	1.27 (1.16, 1.38)	Ref.	1.14 (1.08, 1.20)
	Model 2	Ref.	1.11 (1.05, 1.17)	1.09 (1.03, 1.15)	Ref.	1.12 (1.02, 1.22)	Ref.	1.07 (1.02, 1.13)
	Model 3	Ref.	1.09 (1.03, 1.15)	1.08 (1.02, 1.15)	Ref.	1.08 (0.99, 1.18)	Ref.	1.06 (1.01, 1.12)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S17: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia and sleep duration within White British population in UK Biobank.

		No insomnia symptoms			Insomnia symptoms		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 269 375)	AMI events/ Person-years	765/ 361 048	3 089/ 1 749 267	436/ 182 270	820/ 372 754	885/ 451 111	161/ 48 559
	Model 1	1.13 (1.05, 1.23)	Ref.	1.21 (1.09, 1.34)	1.33 (1.23, 1.44)	1.11 (1.03, 1.20)	1.64 (1.40, 1.92)
	Model 2	1.04 (0.96, 1.13)	Ref.	1.08 (0.97, 1.19)	1.16 (1.08, 1.26)	1.06 (0.98, 1.14)	1.34 (1.14, 1.57)
	Model 3	1.05 (0.97, 1.13)	Ref.	1.05 (0.94, 1.16)	1.14 (1.05, 1.23)	1.05 (0.97, 1.13)	1.27 (1.09, 1.50)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S18: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and chronotype within White British population in UK Biobank.

		No insomnia symptoms		Insomnia symptoms	
		Chronotype		Chronotype	
		Morning	Evening	Morning	Evening
UK Biobank (n = 269 375)	AMI events/ Person-years	2 652/ 1 448 671	1 638/ 843 914	1 150/ 550 760	716/ 321 666
	Model 1	Ref.	1.13 (1.06, 1.20)	1.18 (1.10, 1.26)	1.37 (1.26, 1.49)
	Model 2	Ref.	1.08 (1.01, 1.15)	1.11 (1.04, 1.19)	1.18 (1.09, 1.29)
	Model 3	Ref.	1.07 (1.00, 1.14)	1.09 (1.02, 1.17)	1.15 (1.06, 1.25)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S19: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported chronotype and sleep duration within White British population in UK Biobank.

		Morning chronotype			Evening chronotype		
		Sleep duration			Sleep duration		
		Short	Normal	Long	Short	Normal	Long
UK Biobank (n = 269 375)	AMI events/ Person-years	1 008/ 472 561	2 468/ 1 392 551	326/ 134 319	577/ 261 242	1 506/ 807 827	271/ 96 510
	Model 1	1.19 (1.11, 1.28)	Ref.	1.22 (1.09, 1.37)	1.38 (1.26, 1.51)	1.12 (1.05, 1.19)	1.47 (1.29, 1.66)
	Model 2	1.09 (1.01, 1.17)	Ref.	1.10 (0.97, 1.23)	1.17 (1.07, 1.28)	1.07 (1.00, 1.14)	1.21 (1.07, 1.38)
	Model 3	1.08 (1.00, 1.16)	Ref.	1.07 (0.95, 1.20)	1.16 (1.05, 1.27)	1.06 (0.99, 1.13)	1.16 (1.02, 1.31)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, Townsend deprivation index, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S20: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to self-reported insomnia symptoms and sleep duration in HUNT2 restricting the end of follow-up until December 31, 2008.

		Insomnia symptoms		Sleep duration		
		No	**Yes**	**Short**	**Normal**	**Long**
HUNT2 (n = 31 091)	AMI events/ Person-years	939/ 317 050	205/ 43 809	62/ 22 521	691/ 256 386	391/ 81 952
	Model 1	Ref.	1.16 (0.99, 1.35)	1.19 (0.92, 1.54)	Ref.	1.03 (0.91, 1.18)
	Model 2	Ref.	1.08 (0.93, 1.26)	1.06 (0.82, 1.38)	Ref.	0.99 (0.87, 1.13)
	Model 3	Ref.	1.12 (0.94, 1.32)	1.13 (0.87, 1.47)	Ref.	0.97 (0.85, 1.11)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S21: Hazard ratios (95% confidence intervals) for acute myocardial infarction (AMI) according to the joint association of self-reported insomnia symptoms and sleep duration in HUNT2 restricting the end of follow-up until December 31, 2008.

		No insomnia symptoms			Insomnia symptoms		
		Sleep duration			Sleep duration		
		Short	**Normal**	**Long**	**Short**	**Normal**	**Long**
HUNT2 (n = 31 091)	AMI events/ Person-years	43/ 17 840	581/ 228 578	315/ 70 632	19/ 4 681	110/ 27 808	76/ 11 320
	Model 1	1.17 (0.86, 1.59)	Ref.	1.03 (0.89, 1.18)	1.32 (0.84, 2.09)	1.14 (0.93, 1.40)	1.20 (0.94, 1.54)
	Model 2	1.06 (0.78, 1.45)	Ref.	0.99 (0.85, 1.14)	1.09 (0.69, 1.73)	1.08 (0.88, 1.32)	1.08 (0.84, 1.39)
	Model 3	1.14 (0.84, 1.56)	Ref.	0.97 (0.84, 1.12)	1.18 (0.74, 1.87)	1.12 (0.90, 1.38)	1.09 (0.83, 1.42)

Model 1, adjusted for age and gender.
Model 2, adjusted for covariates in Model 1, along with marital status, alcohol intake frequency, smoking status, body mass index, physical activity, education, shift work, and employment status.
Model 3, adjusted for covariates in Model 2, along with systolic blood pressure, serum cholesterol level, blood glucose level, time since last meal, use of sleep medication(s), depression, and anxiety.

Table S22: List of medications used to define the sleep medications covariate in UKBB.

Sleep medication	**Treatment/medication code** (UKBB field ID: 20003)
Oxazepam	1140863442
Meprobamate	1140863378
Medazepam	1140863372
Bromazepam	1140863318
Lorazepam	1140863302
Clobazam	1140863268
Chlormezanone	1140863262, 1140868274
Temazepam	1140863202
Nitrazepam	1140863182, 1140863104
Lormetazepam	1140863176
Diazepam	1140863152, 1141157496
Zopiclone	1140863144
Triclofos sodium	1140863140
Methyprylone	1140856040
Prazepam	1140855944
Triazolam	1140855914
Ketazolam	1140855860
Dichloralphenazone	1140855824
Clomethiazole	1140909798
Zaleplon	1141171404
Butobarbital	1141180444
Clonazepam	1140872150
Flurazepam	1140863110
Loprazolam	1140863120
Alprazolam	1140863308
Butobarbitone	1140882090

Arora N, Bhatta L, Skarpsno ES, Dalen H, Åsvold BO, Brumpton BM, Richmond RC, Strand LB.

Investigating the causal interplay between sleep traits and risk of acute myocardial infarction: a Mendelian randomization study

BMC Medicine. 2023;21:385
doi: 10.1186/s12916-023-03078-0

Paper II

Paper II

Arora *et al. BMC Medicine* (2023) 21:385
https://doi.org/10.1186/s12916-023-03078-0

BMC Medicine

RESEARCH ARTICLE Open Access

Investigating the causal interplay between sleep traits and risk of acute myocardial infarction: a Mendelian randomization study

Nikhil Arora[1,2*], Laxmi Bhatta[1,3,4], Eivind Schjelderup Skarpsno[5,6], Håvard Dalen[7,8,9], Bjørn Olav Åsvold[1,10,11], Ben Michael Brumpton[1,10,12], Rebecca Claire Richmond[2,13] and Linn Beate Strand[5]

Abstract

Background Few studies have investigated the joint effects of sleep traits on the risk of acute myocardial infarction (AMI). No previous study has used factorial Mendelian randomization (MR) which may reduce confounding, reverse causation, and measurement error. Thus, it is prudent to study joint effects using robust methods to propose sleep-targeted interventions which lower the risk of AMI.

Methods The causal interplay between combinations of two sleep traits (including insomnia symptoms, sleep duration, or chronotype) on the risk of AMI was investigated using factorial MR. Genetic risk scores for each sleep trait were dichotomized at their median in UK Biobank (UKBB) and the second survey of the Trøndelag Health Study (HUNT2). A combination of two sleep traits constituting 4 groups were analyzed to estimate the risk of AMI in each group using a 2×2 factorial MR design.

Results In UKBB, participants with high genetic risk for both insomnia symptoms and short sleep had the highest risk of AMI (hazard ratio (HR) 1.10; 95% confidence interval (CI) 1.03, 1.18), although there was no evidence of interaction (relative excess risk due to interaction (RERI) 0.03; 95% CI −0.07, 0.12). These estimates were less precise in HUNT2 (HR 1.02; 95% CI 0.93, 1.13), possibly due to weak instruments and/or small sample size. Participants with high genetic risk for both a morning chronotype and insomnia symptoms (HR 1.09; 95% CI 1.03, 1.17) and a morning chronotype and short sleep (HR 1.11; 95% CI 1.04, 1.19) had the highest risk of AMI in UKBB, although there was no evidence of interaction (RERI 0.03; 95% CI −0.06, 0.12; and RERI 0.05; 95% CI −0.05, 0.14, respectively). Chronotype was not available in HUNT2.

Conclusions This study reveals no interaction effects between sleep traits on the risk of AMI, but all combinations of sleep traits increased the risk of AMI except those with long sleep. This indicates that the main effects of sleep traits on AMI are likely to be independent of each other.

Keywords Insomnia, Sleep duration, Chronotype, Myocardial infarction, Mendelian randomization

*Correspondence:
Nikhil Arora
nikhil.arora@ntnu.no
Full list of author information is available at the end of the article

Background

Poor sleep is a major public health problem that has emerged as being associated with several health conditions [1, 2], including those related to cardiovascular health such as hypertension [2, 3], obesity [2, 4], and dyslipidemia [5]. Cardiovascular diseases (CVDs) account for a large part of global morbidity and are the leading cause of death [6]. Since sleep problems can be managed through cognitive-behavioral therapy and medication [7], understanding how sleep impacts cardiovascular health can have important implications for interventions that aim to target sleep with an objective to lower the risk of CVDs.

Sleep is a complex and multifaceted biological phenomenon which comprises several traits [8]. Previous observational studies have mainly focused on individual sleep traits as separate risk factors for CVDs [9–13]. Insomnia symptoms, short or long sleep duration, and evening chronotype have been identified as individual risk factors for acute myocardial infarction (AMI) [9, 11, 13, 14]. Sleep traits are often correlated and can together assert their influence on the disease risk. Few observational studies have investigated the joint effects of sleep traits and have found evidence that sleep traits interact to increase the risk of cardiovascular outcomes [14–20]. For instance, insomnia with short sleep considered the most biologically severe sleep disorder phenotype [21], has been found to be associated with increased cardiometabolic risk [14, 16, 18–20]. In our recent study, we observed that those reporting two sleep traits (including insomnia symptoms, short sleep, long sleep, and evening chronotype) had a higher incidence of AMI than those reporting only one sleep trait. Any relative excess risk due to interaction (RERI) was only observed among those reporting insomnia symptoms and long sleep duration [14]. However, the available evidence on the joint effects of sleep traits on the risk of AMI is based on conventional observational studies that are prone to bias due to residual confounding, reverse causation, and measurement error [22].

Mendelian randomization (MR) uses genetic variants as instruments that are robustly associated with a modifiable risk factor to investigate the causal effect on an outcome [23]. MR exploits the fact that genetic variants are randomly assigned to individuals and fixed at conception, making it less susceptible to the bias observed in conventional observational studies. Recent MR studies have evaluated individual effects of sleep traits on CVDs, providing evidence of an adverse effect of insomnia symptoms on prevalent coronary artery disease (CAD) [24–27] and AMI [28], and a protective effect per hour increase in sleep duration and an adverse effect of short sleep on CAD and AMI [11, 29] (see a summary in Additional file 1: Table S1) [11, 24–33]. MR investigation of chronotype is scarce and lacks compelling evidence [28]; thus, it remains unclear whether chronotype itself is causally associated with an increased risk of AMI or if the adverse effect of circadian preference can be explained by insomnia symptoms or sleep duration. More importantly, MR investigations exploring the joint causal effects of sleep traits on risk of AMI remain largely untapped, which could provide robust evidence on the risk of AMI from experiencing two sleep traits simultaneously.

In this study, we therefore used one-sample and factorial MR to investigate the causal effects of individual sleep traits (insomnia symptoms, sleep duration and chronotype) and their joint effects on incident AMI, in two large longitudinal studies (UK Biobank (UKBB) and the second survey of the Trøndelag Health Study (HUNT2)).

Methods

Study participants

UK Biobank

Out of 9.2 million eligible adults (ranging between 40 and 70 years) in the UK who were invited to participate, more than 500 000 participated in the study during March 2006–July 2010 (5.5% response rate). The participants visited one of the 22 study assessment centers located throughout England, Scotland, and Wales, where they signed an electronic consent and completed a touchscreen questionnaire along with a brief computer-assisted interview. They provided detailed information about their lifestyle and physical measures and had blood, urine, and saliva samples collected and stored for future analysis, as described elsewhere [34]. The UKBB received approval from the National Health Service (NHS) Research Ethics Service (reference number 11/NW/0382), and the database was created in compliance with the Declaration of Helsinki.

HUNT study

All inhabitants aged 20 years or older in the Nord-Trøndelag region of Norway were invited to participate in a four-phase population-based health survey (the HUNT study), first in 1984–1986 (HUNT1), then in 1995–1997 (HUNT2) and 2006–2008 (HUNT3), and last in 2017–2019 (HUNT4). This study is based on data from HUNT2, where 93 898 individuals were invited and 65 228 (69.5%) participated [35]. The invitation letter was sent by mail along with a self-administered questionnaire. The participants attended examination stations where clinical examination was performed, and blood samples were drawn by trained personnel. Detailed information regarding HUNT2 study has been published elsewhere [36]. The HUNT Study was approved by the Data Inspectorate of Norway and recommended by the Regional

Committee for Ethics in Medical Research (REK; reference number 152/95/AH/JGE). Additionally, the ethical clearance for conducting this study was obtained from the Regional Committee for Ethics in Medical Research (REK nord; reference number 2020/47206).

Sleep traits

Insomnia symptoms

In both UKBB and HUNT2, insomnia symptoms were defined as two night-time insomnia symptoms (i.e., difficulty falling asleep, difficulty maintaining sleep or waking up too early) without information about daytime impairment. Thus, our definition for insomnia symptoms did not include all components used in the frameworks for diagnosing insomnia [37].

In UKBB, participants were asked: "Do you have trouble falling asleep at night or do you wake up in the middle of the night?" (Field ID: 1200) with response options "Never/rarely", "Sometimes", "Usually" or "Prefer not to answer". Participants were classified as having insomnia symptoms if they answered "Usually"; and not having insomnia symptoms if they answered "Never/rarely" or "Sometimes". Other responses were coded as missing.

In HUNT2, insomnia symptoms were assessed by the following two questions: "Have you had difficulty falling asleep in the last month?", and "During the last month, have you woken too early and not been able to get back to sleep?" with response options "Never", "Sometimes", "Often" or "Almost every night". Participants who responded "Often" or "Almost every night" to at least one of these questions were classified as having insomnia symptoms. For participants who answered only one of these insomnia symptom questions, we did the following: (1) if they answered "Often" or "Almost every night" to one of the questions, but did not answer the other, they were classified as having insomnia symptoms, and (2) if they answered "Never" or "Sometimes" to one of the questions, but did not answer the other, they were excluded to avoid possible misclassification. The remaining participants were classified as not having insomnia symptoms.

Sleep duration

Sleep duration was assessed by the questions: "About how many hours sleep do you get in every 24 hours? (please include naps)" (Field ID: 1160) and "How many hours do you usually spend lying down (i.e., sleeping and/or napping) during a 24-hour period?" in UKBB and HUNT2, respectively. The answers could only contain integer values. Any influence of poor health on implausible short or long sleep durations was avoided by excluding extreme responses of less than 3 hours or more than 18 hours. Binary variables for short sleep ($\leq$6 hours vs. 7–8 hours) and long sleep ($\geq$9 hours vs. 7–8 hours) were also constructed.

Chronotype

Chronotype (morning or evening chronotype) in UKBB was assessed by the question: "Do you consider yourself to be?" (Field ID: 1180) with response options "Definitely a 'morning' person", "More a 'morning' than 'evening' person", "More an 'evening' than a 'morning' person", "Definitely an 'evening' person", "Do not know" or "Prefer not to answer". Participants were classified as having a morning chronotype if they reported "Definitely a 'morning' person" or "More a 'morning' than 'evening' person" and as having an evening chronotype if they reported "More an 'evening' than a 'morning' person" or "Definitely an 'evening' person". Other responses were coded as missing. Chronotype was not reported in any survey of the HUNT Study.

Acute myocardial infarction (AMI)

In UKBB, participants were followed through record linkage to the Hospital Episode Statistics (HES) for England, Scottish Morbidity Record (SMR), and Patient Episode Database for Wales (PEDW) where health-related outcomes had been defined by International Classification of Diseases (ICD)-9 and ICD-10 codes (Field IDs: 41270, 41271, 41280 and 41281). Also, mortality records were obtained from the NHS Digital for participants in England and Wales, and from the NHS Central Register (part of the National Records of Scotland) for participants in Scotland where cause of death had been defined by ICD-10 codes (Field IDs: 40001 and 40000).

In HUNT2, participants were followed via linkage to the medical records from the three hospitals (St. Olavs Hospital, Levanger Hospital and Namsos Hospital) of the Nord-Trøndelag region where health-related outcomes had been defined by ICD-9 and ICD-10 codes. Mortality records were identified by a linkage to the National Cause of Death Registry where cause of death had been defined by ICD-10 codes.

Any hospitalization or death due to AMI were identified using ICD-9 code 410 and ICD-10 codes I21 and I22. Each participant was followed until either first diagnosis/death due to AMI, death due to other cause, loss to follow-up, or end of follow-up (March 23, 2021 for UKBB and December 31, 2020 for HUNT2). Incident cases were defined as the first occurrence of either hospitalization or death due to AMI during follow-up. Participants with any previous AMI episode(s) before their date of

participation in the study regarded as prevalent cases, were excluded in the study.

Covariates

Several factors to be potential confounders of the exposure-outcome relation were considered. The covariates selected a priori were age, gender, marital status (married, unmarried, or separated/divorced/widowed), frequency of alcohol intake (never, monthly, weekly, or daily), smoking history (never, ex-smoker, or current smoker), body mass index (BMI), level of physical activity (inactive/low, moderate, or high), Townsend deprivation index (TDI; for UKBB only), education attainment ($\leq$10 years, 11–13 years, or $\geq$14 years), shift work (yes or no), employment status (employed or not employed), systolic blood pressure (SBP), blood cholesterol levels, blood glucose levels, depression (yes or no in UKBB; and Hospital Anxiety and Depression Scale (HADS) – Depression scores in HUNT2), anxiety (yes or no in UKBB; and HADS – Anxiety scores in HUNT2), use of sleep medication (yes or no), and chronic illness (yes or no). The details on how covariates were handled are described in the supplementary material (see Additional file 1) [36, 38–47].

Genetic variants

In UKBB, participants were genotyped using either one of the UK BiLEVE or the UK Biobank Axiom genotyping chips. The genetic variants used were extracted genotypes from the UK Biobank imputation dataset (imputed to the UK10K plus 1000 Genomes phase 3 and Haplotype Reference Consortium reference panels), that were quality controlled using a standard protocol [48, 49]. In HUNT, participants were genotyped with one of three different Illumina HumanCoreExome genotyping chips (HumanCoreExome 12 v.1.0, HumanCoreExome 12 v.1.1, and UM HUNT Biobank v.1.0), where genotypes from different chips were quality controlled separately and reduced to a common set of variants. The quality control measures used were similar to UKBB [50]. All genotyped samples included were of European decent.

A total of 248 single nucleotide polymorphisms (SNPs) were identified as robustly associated with insomnia symptoms [30], 78 SNPs associated with 24-hour sleep duration [31], and 351 SNPs associated with morning preference chronotype [32], at a genome-wide significance level ($P<5\times10^{-8}$) from three large genome-wide association studies (GWASs). In addition, 27 and 8 SNPs were identified to associate with short and long sleep duration, respectively [31]. The detailed information about discovery GWASs from where genetic instruments were identified were listed in Table 1.

Statistical analysis

Genetic risk score (GRS) for each sleep trait were created as an instrument that could overcome the weak effect of most SNPs on their corresponding sleep trait [51]. Weighted GRS (wGRS) were calculated as the sum of the participants' sleep trait increasing alleles (morning preference alleles for chronotype; thus evening chronotype as reference), weighted by the variant effect sizes from the external GWAS. wGRS were incorporated for our main analysis in HUNT2 only, whereas in UKBB, we used unweighted GRS (uwGRS) calculated as sum of the sleep trait increasing alleles. Since all included discovery GWASs used the UKBB cohort, the use of internal weights to calculate wGRS is not recommended [51].

Instrument strength was assessed by regressing each sleep trait on their respective GRS and reporting R^2 and *F*-statistics. The causal effects of individual sleep traits (insomnia symptoms, 24-hour sleep duration, short sleep, long sleep and chronotype) on the risk of incident

Table 1 Summary of genome-wide significant genetic instruments of sleep traits in the discovery genome-wide association studies

Sleep traits	Discovery GWAS	PMID	*N*	Cohorts used by the discovery GWAS		No. of SNPs identified
				UKBB	23andMe	
Insomnia symptoms	Jansen et al., 2019 [30]	30804565	1 331 010	109 402 cases and 277 131 controls	288 557 cases and 655 920 controls	248
24-hour sleep duration (h)	Dashti et al., 2019 [31]	30846698	446 118	446 118 samples	Not included	78
Short sleep ($\leq$6 h vs. 7–8 h)	Dashti et al., 2019 [31]	30846698	411 934	106 192 cases and 305 742 controls	Not included	27
Long sleep ($\geq$9 h vs. 7–8 h)	Dashti et al., 2019 [31]	30846698	339 926	34 184 cases and 305 742 controls	Not included	8
Chronotype (morning preference)*	Jones et al., 2019 [32]	30696823	651 295	252 287 cases and 150 908 controls	120 478 cases and 127 622 controls	351

GWAS indicated genome-wide association studies, *N* sample size, *SNPs* single nucleotide polymorphisms, *UKBB* UK Biobank

* In the discovery GWAS of chronotype, the chronotype increasing allele is morning preference

AMI were tested using a one-sample MR analysis. A factorial MR analysis was used to investigate the joint causal effects of any two sleep traits (i.e., insomnia symptoms and short sleep, or insomnia symptoms and long sleep, or insomnia symptoms and chronotype, or short sleep and chronotype, or long sleep and chronotype) on the risk of incident AMI. All analyses were conducted using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

One-sample MR analysis

One-sample MR analysis was performed for each sleep trait using individual-level data separately in UKBB and HUNT2. A two-stage predictor substitution (TSPS) regression estimator method was used to calculate average causal hazard ratios (HRs). The first stage involved regression of each sleep trait (linear regression for 24-hour sleep duration, and logistic regression for other sleep traits) on their GRS, and the second stage consisted of a Cox regression of AMI status on the fitted values from the first stage regression, with adjustment for age at recruitment, gender, assessment center (in UKBB), genetic principal components (40 in UKBB and 20 in HUNT2), and genotyping chip in both stages. As recommended for MR analysis with a binary outcome [52], the first stage regression was restricted to participants who did not experience AMI. To obtain corrected standard errors, a bootstrapping method was applied with 2000 iterations in UKBB and 5000 iterations in HUNT2 [52]. The causal estimates for insomnia symptoms, short sleep, long sleep, and chronotype were scaled to represent the risk increase in AMI per doubling in the odds of these exposures, by multiplying the obtained β values by 0.693 as previously described [53]. The causal estimate for 24-hour sleep duration represents the risk increase in AMI per additional hour of sleep.

Factorial MR analysis

A 2×2 factorial MR was applied where each of the sleep traits (except 24-hour sleep duration) was dichotomized at their median GRS (uwGRS for UKBB and wGRS for HUNT2), with values equal to or below the median represented low genetic risk for the sleep trait, and values above the median represented high genetic risk for the sleep trait. Thus, for any combination of two sleep traits, participants were categorized into 4 groups according to their genetic predisposition. For instance, when combining insomnia symptoms and short sleep, participants were categorized into: "*Both GRS* ≤ *median*" (reference; representing low genetic risk for both insomnia symptoms and short sleep), "*Insomnia GRS* > *median*" (representing high genetic risk for insomnia symptoms only), "*Short sleep GRS* > *median*" (representing high genetic risk for short sleep only), and "*Both GRS* > *median*" (representing high genetic risk for both insomnia symptoms and short sleep). Cox regression was then used to investigate the association between these groups and incident AMI, with adjustment for age at recruitment, gender, assessment center (in UKBB), genetic principal components (40 in UKBB and 20 in HUNT2), and genotyping chip. Furthermore, interaction between any two sleep traits on risk of AMI was assessed by calculating relative excess risk due to interaction (RERI) using the risk estimates obtained for each sleep trait combination when none of the HRs were less than 1 (i.e., preventive) [54, 55]. RERI equals 0 implies exact additivity (no interaction), RERI > 0 implies more than additivity (positive interaction or synergism), and RERI < 0 implies less than additivity (negative interaction or antagonism).

Sensitivity analyses

To check the proportionality of hazards, the Pearson's correlations were used to test Schoenfeld residuals from one-sample MR and 2×2 factorial MR Cox regression models for an association with follow-up time.

To check the robustness of the findings, the one-sample MR and 2×2 factorial MR analyses were repeated using uwGRS in HUNT2.

To assess the second MR assumption that the genetic instruments used are independent of confounders, associations of the GRS and potential confounders were investigated in UKBB and HUNT2. Furthermore, one-sample MR analysis adjusted for any potential confounders found strongly associated with the sleep trait GRS in two cohorts (beyond a Bonferroni significance threshold of $P < 5.88\times10^{-4}$ in UKBB and $P < 7.81\times10^{-4}$ in HUNT2) were performed.

To investigate potential directional pleiotropy, the estimates of the SNP-exposure and SNP-outcome associations from the same participants were obtained, and two-sample MR methods, such as MR-Egger, weighted median, and weighted mode-based methods, were applied. Each of these methods makes different assumptions about the genetic instruments used, where the MR-Egger regression method gives a valid causal estimate under the InSIDE (instrument strength independent of direct effect) assumption and its intercept allows the size of any unbalanced pleiotropic effect to be determined [56], weighted median method assumes at least 50% of genetic variants are valid [57], and weighted mode-based estimation method assumes a plurality of genetic variants are valid [58]. These methods can be applied in a one-sample setting [59], and consistent estimates across these methods strengthens causal evidence. To further investigate pleiotropy due to insomnia symptoms' instruments, 57 SNPs found robustly associated with insomnia

symptoms by Lane et al. [24] in another GWAS on UKBB ($n=345\ 022$ cases and 108 357 controls) representing crucial variants with effect sizes for any insomnia symptoms ("sometimes"/ "usually" as cases versus "never/ rarely" as controls), were used in a post hoc one-sample MR Cox regression analysis using different methods.

To evaluate potential impact of winner's curse, one-sample MR analysis was repeated using genetic variants that replicated at a genome-wide significance level ($P<5\times10^{-8}$) in a large independent dataset for insomnia symptoms (23andMe, $n=944\ 477$; see Additional file 2: Table G1) [30] and chronotype (23andMe, $n=240\,098$; see Additional file 2: Table G5) [32].

As an additional analysis, continuous factorial MR analysis using two GRS (for any combination of two sleep traits) as quantitative traits and their product term was applied, to avoid potential bias due to arbitrary dichotomization and to maximize power [60]. Furthermore, RERI was calculated as test of interaction using the risk estimates for the quantitative GRS and their product term for each sleep trait combination when none of the HRs were less than 1 (i.e., preventive) for AMI [54, 61].

As use of sleep medication has been associated with CVDs [62], one-sample MR (without applying bootstrap method) and 2×2 factorial MR analyses were repeated excluding participants who reported use of sleep medication(s).

Results

Among the 336 262 participants in UKBB who passed the genetic quality control and had information available on the sleep traits, 11 399 (3.4%) had ever received the diagnosis of AMI. Of these, 3 586 (1.1%) prevalent cases with AMI diagnosis were excluded, and 7 813 (2.3%) had their first AMI diagnosis during a mean (standard deviation (SD)) follow-up of 11.7 (1.9) years (see Additional file 1: Figure S1). Among the 45 602 participants in HUNT2 who passed the genetic quality control and had information available for sleep traits of interest, 5 362 (11.7%) had ever received diagnosis of AMI. Of these, 874 (1.9%) prevalent cases with AMI diagnosis were excluded, and 4 488 (10.0%) had their first AMI diagnosis during a mean (SD) follow-up of 20.4 (6.9) years (see Additional file 1: Figure S1).

Table 2 represents the baseline characteristics of the study participants stratified by their AMI status in UKBB and HUNT2. Participants with an incidence of AMI during follow-up in the UKBB and HUNT2 were older and more likely to be males and current smokers. They were more likely to have used sleep medication(s), have a higher BMI, have higher systolic blood pressure, higher blood glucose levels, and were suffering more from depression and chronic illness. They were also less likely to consume alcohol, be physically active, have a tertiary education, and be employed compared to participants with no episodes of AMI. The HUNT2 participants with an AMI incidence during follow-up were more likely to have higher serum cholesterol levels, but less likely to be suffering from anxiety and working shifts in contrast to UKBB participants when compared to participants with no episode of AMI.

Among UKBB participants, the variance explained by the uwGRS in insomnia symptoms, 24-hour sleep duration (h), short sleep (≤6 h vs. 7–8 h), long sleep (≥9 h vs. 7–8 h), and morning chronotype were 0.41%, 0.59%, 0.18%, 0.11%, and 1.54%, respectively, and corresponding F-statistics were 1370.92, 1962.0, 558.68, 285.42, and 5202.20 (see Additional file 1: Table S2). The variance explained by the wGRS among HUNT2 participants in insomnia symptoms, 24-hour sleep duration, short sleep, and long sleep were 0.16%, 0.09%, 0.01%, and 0.01%, respectively, and the corresponding F-statistics were 71.17, 38.94, 4.97, and 4.07 (see Additional file 1: Table S2).

Causal effects of individual sleep traits on the risk of AMI

There was evidence for an adverse causal effect on AMI risk per doubling in odds of insomnia symptoms in UKBB (HR 1.18; 95% CI 1.07, 1.31) and HUNT2 (HR 1.23; 95% CI 1.00, 1.55) (Fig. 1). The estimates for 24-hour sleep duration suggested no causal effect on AMI per hour increase in sleep duration in UKBB (HR 0.97; 95% CI 0.75, 1.29) and HUNT2 (HR 0.76; 95% CI 0.31, 1.79). The sleep duration findings were further investigated using genetic variants specifically associated with short and long sleep duration. There was weak evidence for an adverse causal effect on AMI per doubling in odds of short sleep in UKBB (HR 1.14; 95% CI 0.97, 1.32) but not in HUNT2 (HR 0.87; 95% CI 0.15, 3.24). However, there was evidence for a protective causal effect on AMI per doubling in odds of long sleep in UKBB (HR 0.83; 95% CI 0.67, 0.99), which was underpowered in HUNT2 (HR 0.53; 95% CI 0.01, 8.28). Also, there was some evidence for an adverse causal effect on AMI per doubling in odds of morning chronotype in UKBB (HR 1.06; 95% CI 0.99, 1.11) (Fig. 1).

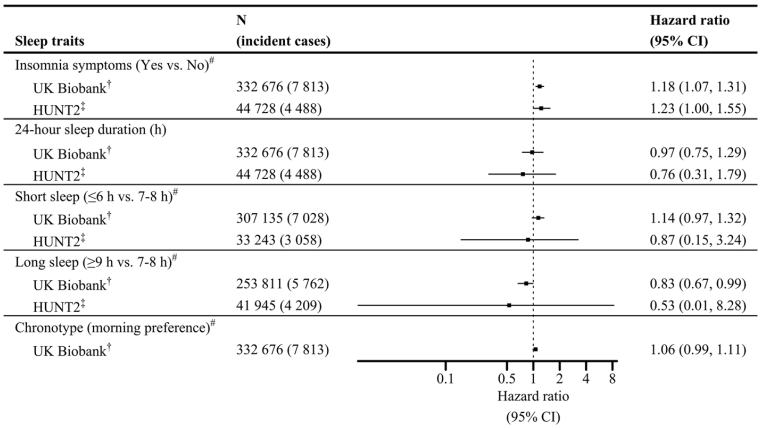
Joint causal effects of sleep traits on the risk of AMI

The distribution of the baseline characteristics across the factorial groups for any combinations of two sleep traits were equal (see Additional file 1: Tables S3 – S9), which indicates random allocation of the study participants into

Table 2 Baseline characteristics of study participants who had an episode of acute myocardial infarction (AMI) and not had AMI during follow-up in UK Biobank and HUNT2

	UK Biobank (*N* = 332 676)		HUNT2 (*N* = 44 728)	
	No AMI diagnosis	**AMI diagnosis**	**No AMI diagnosis**	**AMI diagnosis**
Total, % (*n*)	97.65 (324 863)	2.35 (7 813)	89.97 (40 240)	10.03 (4 488)
Variables, % (*n*)				
Male	44.03 (143 029)	70.24 (5 488)	43.87 (17 654)	61.27 (2 750)
Missing, % (*n*)	-	-	-	-
Married	74.19 (241 001)	72.60 (5 672)	61.67 (24 814)	69.43 (3 116)
Missing, % (*n*)	0.48 (1 561)	0.93 (73)	0.23 (91)	0.07 (3)
Weekly alcohol intake	50.55 (164 215)	47.43 (3 706)	22.59 (9 089)	18.72 (840)
Missing, % (*n*)	0.05 (161)	0.08 (6)	7.31 (2 943)	9.54 (428)
Current smokers	9.97 (32 392)	18.17 (1 420)	27.31 (10 991)	32.53 (1 460)
Missing, % (*n*)	0.29 (956)	0.37 (29)	1.52 (610)	1.96 (88)
Highly physically active	33.39 (108 475)	31.95 (2 496)	33.41 (13 443)	22.59 (1 041)
Missing, % (*n*)	17.69 (57 463)	19.12 (1 494)	7.33 (2 950)	14.84 (666)
Tertiary education	43.24 (140 458)	37.71 (2 946)	21.74 (8 747)	11.85 (532)
Missing, % (*n*)	0.74 (2 418)	1.15 (90)	3.16 (1 273)	7.20 (323)
Shift workers	5.17 (16 797)	5.30 (414)	15.91 (6 403)	9.05 (406)
Missing, % (*n*)	0.27 (888)	0.22 (17)	7.31 (2 940)	6.66 (299)
Employed	57.28 (186 073)	43.45 (3 395)	68.66 (27 627)	45.94 (2 062)
Missing, % (*n*)	0.24 (773)	0.18 (14)	0.95 (381)	0.62 (28)
Use of sleep medication(s)	0.97 (3 135)	1.37 (107)	6.10 (2 454)	10.90 (489)
Missing, % (*n*)	-	-	9.39 (3 778)	10.90 (489)
Suffering from depression	12.06 (39 190)	15.10 (1 180)	-	-
Missing, % (*n*)	-	-	-	-
Suffering from anxiety	6.63 (21 543)	10.11 (790)	-	-
Missing, % (*n*)	-	-	-	-
Suffering from chronic illness	30.84 (100 189)	47.13 (3 682)	30.27 (12 182)	48.84 (2 192)
Missing, % (*n*)	2.03 (6 600)	2.20 (172)	3.02 (1 215)	4.48 (201)
Variables, mean (SD)				
Age, *years*	56.82 (7.95)	60.36 (6.82)	47.71 (16.07)	61.12 (13.23)
Missing, % (*n*)	-	-	-	-
TDI	−1.60 (2.91)	−1.22 (3.12)	-	-
Missing, % (*n*)	0.12 (386)	0.12 (9)	-	-
BMI, kg/m^2	27.37 (4.75)	28.74 (4.80)	26.18 (4.04)	27.41 (4.03)
Missing, % (*n*)	0.31 (993)	0.41 (32)	0.46 (184)	0.87 (39)
SBP, *mmHg*	138.20 (18.59)	145.80 (19.18)	135.50 (20.44)	148.80 (22.56)
Missing, % (*n*)	0.09 (297)	0.06 (6)	0.11 (43)	0.13 (6)
Serum cholesterol, *mmol/L*	5.74 (1.13)	5.70 (1.28)	5.80 (1.23)	6.55 (1.21)
Missing, % (*n*)	4.56 (14 819)	4.62 (361)	0.11 (44)	0.11 (5)
Blood glucose, *mmol/L*	5.11 (1.17)	5.41 (1.86)	5.36 (1.36)	5.90 (1.96)
Missing, % (*n*)	12.73 (41 362)	12.39 (968)	0.15 (61)	0.20 (9)
HADS – D scores	-	-	3.31 (2.97)	4.01 (3.15)
Missing, % (*n*)	-	-	6.38 (2 569)	11.25 (505)
HADS – A scores	-	-	4.18 (3.25)	4.06 (3.37)
Missing, % (*n*)	-	-	13.01 (5 237)	22.08 (991)

AMI indicates acute myocardial infarction, *SD* standard deviation, *TDI* Townsend deprivation index, *BMI* body mass index, *SBP* systolic blood pressure, *HADS – D scores* Hospital Anxiety and Depression Scale – Depression scores, *HADS – A scores* Hospital Anxiety and Depression Scale – Anxiety scores



CI indicates confidence interval.
† Derived using unweighted genetic risk score for each sleep trait, with adjustment for age, gender, assessment centre, 40 genetic principal components, and genotyping chip.
‡ Derived using weighted genetic risk score for each sleep trait, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.
# Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait. Chronotype was missing in HUNT2.

Fig. 1 One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with sleep traits in UK Biobank and HUNT2

approximately equal-sized groups based on their genetic risk for any combinations of two sleep traits.

In UKBB, participants with high genetic risk for insomnia symptoms and high genetic risk for short sleep had slightly higher risk of AMI (HR 1.03; 95% CI 0.96, 1.10 and HR 1.05; 95% CI 0.98, 1.12, respectively), whereas participants with high genetic risk for both traits had the highest risk (HR 1.10; 95% CI 1.03, 1.12) (Fig. 2), but there was no evidence of interaction (RERI 0.03; 95% CI −0.07, 0.12). This pattern was however not consistent in HUNT2, with imprecise estimates and a lack of evidence of interaction (RERI −0.05; 95% CI −0.20, 0.09) (Fig. 2). The joint effects of insomnia symptoms and long sleep on risk of AMI were inconclusive in both UKBB and HUNT2 (Fig. 2).

In addition, UKBB participants with high genetic risk for insomnia symptoms and high genetic risk for a morning chronotype had slightly higher risk of AMI (HR 1.03; 95% CI 0.97, 1.10 and HR 1.03; 95% CI 0.97, 1.10, respectively) whereas participants with high genetic risk for both sleep traits had the highest risk (HR 1.09; 95% CI 1.03, 1.17) (Fig. 2). There was no evidence of interaction (RERI 0.03; 95% CI −0.06, 0.12). Similarly, the UKBB participants with high genetic risk for short sleep and high genetic risk for a morning chronotype had slightly higher risk of AMI (HR 1.04; 95% CI 0.98, 1.12 and HR 1.02; 95% CI 0.96, 1.10, respectively) whereas participants with high genetic risk for both had the highest risk (HR 1.11; 95% CI 1.04, 1.19) (Fig. 2), with no strong statistical evidence of interaction (RERI 0.05; 95% CI −0.05, 0.14). The joint effects of long sleep and morning chronotype were imprecise and not conclusive (Fig. 2).

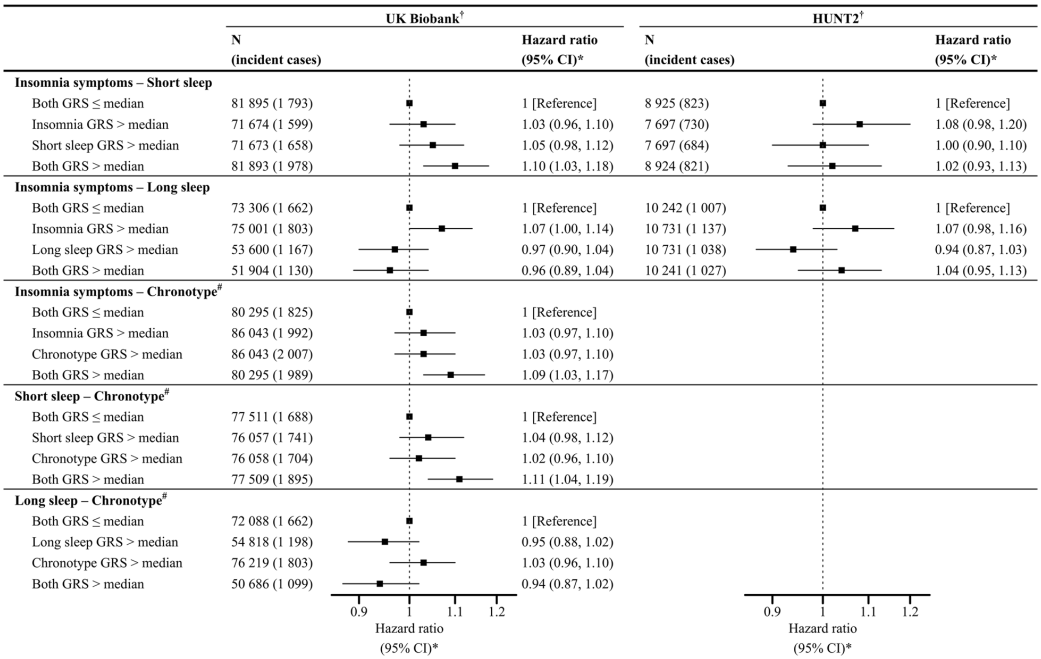
Sensitivity analysis

The proportionality of hazards assumption was met for the one-sample and the 2×2 factorial MR Cox regression analyses (see Additional file 1: Tables S10 and S11).

The one-sample MR and 2×2 factorial MR estimates in HUNT2 using the uwGRS for the sleep traits remained unchanged (see Additional file 1: Table S12 and Figure S2).

After adjusting for multiple testing, several confounding factors were associated with the sleep trait uwGRS in UKBB, whereas only a few were associated with the sleep trait wGRS in HUNT2 (see Additional file 1: Tables S13 and S14). When the one-sample MR analysis adjusting for these potential confounding factors was carried out, evidence of adverse causal effects of insomnia symptoms was slightly weaker and less precise in UKBB (HR 1.04; 95% CI 0.92, 1.17) and HUNT2 (HR 1.13; 95% CI 0.87, 1.47) (see Additional file 1: Table S15).

The causal estimates obtained using MR-Egger, weighted median- and weighted mode-based methods attenuated slightly and were less precise (see Additional file 1: Figures S3-S7, Tables S16 and S17). The MR-Egger regression for insomnia symptoms in UKBB showed evidence of directional pleiotropy (HR 0.77; 95% CI 0.62,



CI indicates confidence interval; and GRS, genetic risk score.
For each sleep trait combination, both GRS ≤ median represents low genetic risk for both sleep traits in combination, sleep trait 1 GRS > median represents high genetic risk for sleep trait 1 only, sleep trait 2 GRS > median represents high genetic risk for sleep trait 2 only and both GRS > median represents high genetic risk for both sleep traits.
† Derived using unweighted genetic risk score for each sleep trait in UK Biobank, whereas using weighted genetic risk score for each sleep trait in HUNT2.
* Adjusted for age, gender, assessment centre (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2), and genotyping chip.
# Chronotype genetic risk score calculated using alleles for morning preference.

Fig. 2 2×2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in UK Biobank and HUNT2

0.95; and intercept 0.007; 95% CI 0.003, 0.012). Furthermore, the post hoc one-sample MR analysis using insomnia symptoms variants from Lane et al. [24] gave similar estimates (see Additional file 1: Figure S8 and Table S18), where the MR-Egger regression showed evidence of directional pleiotropy in UKBB (HR 0.69; 95% CI 0.50, 0.96; and intercept 0.013; 95% CI 0.005, 0.022) and in HUNT2 (HR 0.97; 95% CI 0.78, 1.19; and intercept 0.006; 95% CI 0.001, 0.012).

The causal estimates were consistent when using GRS comprising 116 insomnia SNPs (one missing in HUNT imputed dataset) and 72 chronotype SNPs which replicated at genome-wide significance level ($P<5\times10^{-8}$) in the independent 23andMe dataset (see Additional file 1: Tables S19 and S20).

The estimates from the continuous factorial MR analysis using sleep trait GRS as quantitative traits (per SD increase) and their product term inferred similar effects (see Additional file 1: Figure S9). In UKBB, the GRS for insomnia symptoms and short sleep were independently linked to an increased risk of AMI (HR 1.03; 95% CI 1.01, 1.06 and HR 1.02; 95% CI 0.99, 1.04, respectively), with no evidence of interaction (RERI 0.02; 95% CI −0.01, 0.04). Similarly, the GRS for insomnia symptoms and morning chronotype were independently associated with an increased risk of AMI (HR 1.04; 95% CI 1.02, 1.06 and HR 1.03; 95% CI 1.00, 1.05, respectively), though there was no evidence of interaction (RERI 0.02; 95% CI −0.01, 0.04). Also, the GRS for short sleep and morning chronotype were both independently linked to an increased risk of AMI (HR 1.02; 95% CI 1.00, 1.04 and HR 1.03; 95% CI 1.00, 1.05, respectively) but suggested no evidence of interaction (RERI 0.01; 95% CI −0.02, 0.03).

On excluding the participants who self-reported the use of sleep medication(s), our one-sample and 2×2

factorial MR estimates remain unchanged (see Additional file 1: Figures S10 and S11).

Discussion

Using individual-level data from the UKBB and HUNT2 cohorts, we performed one-sample and factorial MR analyses to investigate the causal effects of individual sleep traits (insomnia symptoms, sleep duration and morning chronotype) and their joint effects on the risk of AMI. We found evidence of an adverse causal effect of insomnia symptoms and a weak causal effect of short sleep on the risk of incident AMI, while long sleep had a protective effect in UKBB. We found no statistical evidence of interaction effects between sleep traits on the risk of AMI, but those with a high genetic risk for two sleep traits in combination (including insomnia symptoms, short sleep, and a morning chronotype) had the highest risk of AMI in UKBB. Moreover, our results showed a protective effect of genetically predisposed long sleep that was not affected by additionally being genetically predisposed to insomnia symptoms or a morning chronotype on incident AMI in UKBB. However, these results were not replicated in HUNT2, where the estimates were imprecise. These findings indicate that the main effects of sleep traits on the risk of AMI are likely to be independent of each other.

Comparison with other studies

Direct comparison of MR results with observational findings is limited given that inherited genetic variation influences sleep behaviors over the life course, whereas observational estimates represent sleep behaviors measured at one time-point. Additionally, caution should be made when comparing our findings with other studies, due to variation in the definitions used for sleep traits.

Causal effects of individual sleep traits on the risk of AMI

Nonetheless, our finding showing evidence of an adverse causal effect of insomnia symptoms and a weak adverse causal effect of short sleep on the risk of AMI is consistent with prior observational [9, 11, 14] and MR research [11, 28, 29]. Our causal estimate of short sleep on the risk of AMI in UKBB was weaker compared to Daghlas et al. [11] (odds ratio (OR) 1.21; 95% CI 1.08, 1.37) and Ai et al. [29] (OR 1.21; 95% CI 1.09, 1.34), which might be due to different methodological approaches. Our analyses relied on survival data and reported HR considering incident cases of AMI on follow-up after recruitment in the cohorts, rather than OR. Our finding suggests a protective causal effect of long sleep on the risk of AMI contradicts with prior observational studies [11, 14] but aligns with a weak concordant effect shown by another MR study [29]. Long sleep may be an indicator of poor health status, being closely associated with depression, poor sleep quality, sedentary lifestyles, and underlying comorbid conditions [63, 64], and so residual confounding or reverse causation may have biased previous observational findings. Moreover, our finding suggesting a weak causal effect of morning chronotype on the risk of AMI is inconsistent with our prior study that identified evening chronotype as detrimental [14]. It is likely that the previously reported protective association of morning chronotype is confounded.

Joint causal effects of sleep traits on the risk of AMI

Our finding that UKBB participants with high genetic risk for both insomnia symptoms and short sleep had the highest risk of AMI is consistent with evidence from our previous observational study where we found that insomnia symptoms and short sleep together increased the risk of AMI in UKBB more than the risk attributed to either insomnia symptoms or short sleep alone [14], and is supported by finding from another prospective study [16]. Moreover, our finding suggesting no interaction between insomnia symptoms and short sleep on risk of AMI is also in line with prior research [14, 16]. However, our finding of no positive interaction between insomnia symptoms and long sleep on the risk of AMI in UKBB contrasts with our previous observational study [14], where insomnia symptoms and long sleep together were found to increase the risk of AMI beyond their mere additive effects. This observed interaction could be due to confounding apparent in conventional observational studies, where poor health could be a confounder that would lead to false indications of harmful consequences of prolonged sleep. As previously mentioned, our finding in UKBB suggests a protective effect of genetic predisposition to long sleep on incident AMI, which was not affected by additionally being genetically predisposed to insomnia symptoms.

Our findings that UKBB participants with high genetic risk for both insomnia symptoms and a morning chronotype; and those with high genetic risk for both short sleep and a morning chronotype had the highest risk of AMI are in contrast with our observational study where we found evening chronotype to be more deleterious than morning chronotype in combination with insomnia symptoms or short sleep [14]. Although there was no interaction, these findings may suggest that the weak adverse effect of morning chronotype on AMI might partly be explained by concomitant genetic predisposition to insomnia symptoms or short sleep. Our finding that UKBB participants with high genetic risk for both long sleep and a morning chronotype likely decreased the risk of AMI is incongruous to our previous observational

study, where long sleep together with morning chronotype was associated with an increased risk [14]. Again, there was no interaction and — if anything — our finding suggests a protective effect of genetic predisposition to long sleep on incident AMI, which was not affected by additionally being genetically predisposed to morning chronotype.

Potential mechanisms

The underlying mechanisms by which insomnia symptoms or short sleep increase in the risk of AMI are multifactorial [65]. Insomnia and short sleep independently increase the risk of autonomic dysfunction, by increasing sympathetic tone (stress response) consequently accompanied by increased metabolic rate, increased heart rate, and decreased heart rate variability [66–69]. Furthermore, experimentally induced sleep restriction has been shown to cause hormonal imbalance which stimulate proinflammatory pathways [70], increase appetite [71, 72], and increase insulin resistance [73]. These autonomic and hormonal disturbances lead to hypertension [74, 75], diabetes [73], dyslipidemia, and obesity [71, 72], thus constituting a set of interrelated metabolic disorders that are pathophysiological in the development of cardiac dysfunction by accelerating endothelial dysfunction and atherosclerosis [76].

Our findings and these potential mechanisms might raise a concern that insomnia symptoms and short sleep could be regarded as similar traits. However, insomnia symptoms and sleep duration were found only moderately phenotypically ($r = -0.25$; $P < 0.001$) and genetically ($r_g = -0.50$; $P < 6 \times 10^{-17}$) correlated to each other [77]. It is also important to highlight that our findings on the joint causal effects of insomnia symptoms and short sleep on the risk of AMI do not employ that concomitant presence of insomnia symptoms and short sleep causes higher increase in risk of AMI through overstimulation of the suggested underlying mechanisms, or involve any supplementary mechanisms yet to be determined.

The underlying mechanism by which chronotype may influence AMI is not yet established. Studies have found evening chronotypes have more susceptibility for cardiometabolic risk behaviors and risk factors [12, 78, 79]. On the contrary, our causal findings suggesting that having a morning chronotype may be detrimental for incident AMI compared to having an evening chronotype might be explained by the concomitant genetic predisposition to insomnia symptoms or short sleep.

Strengths and limitations

This MR study leverages genetic information to assess the causal relationships between sleep traits and AMI, reducing the potential bias due to residual confounding, reverse causation, and measurement error in conventional observational studies [22]. The novelty of this study is our application of factorial MR to explore the causal interplay between sleep traits on the risk of AMI, where participants were grouped based on their genetic predisposition for multiple sleep traits [60]. We are not aware of another study that has investigated the joint effects of sleep traits in the MR context. Another major novelty is that the study benefitted from the use of results from three large GWASs for insomnia symptoms [30], sleep duration [31], and chronotype [32] and used two large cohorts (UKBB and HUNT2) to replicate the findings. Moreover, this study draws on the principle of triangulation [80], where findings were compared from different methodological approaches, which further strengthened evidence supporting causation.

Nonetheless, there are a number of limitations of this study. Factorial MR analysis is usually underpowered to detect interaction which may raise the concerns of false negative results [60]. However, this study included the UKBB cohort with 332 676 participants constituting the largest factorial MR study on sleep traits to date. The strong instrument strength observed in UKBB cohort partially overcomes concerns due to underpowered factorial MR findings [81]. Another limitation is that although factorial MR can identify whether two independent exposures interact and have a joint effect of public health importance [81], it assumes exposures remain stable throughout the life course. Thus, the magnitude of effects should be cautiously interpreted.

Also, the validity of MR findings can be weakened by pleiotropy [82]. We used several sensitivity analyses to investigate possible sources of bias in MR. We found that the genetic risk for insomnia symptoms was strongly associated with BMI, smoking status, depression, and education among other covariates [30], which may be indicative of confounding, mediation, or horizontal pleiotropy. Further to this, our results remained consistent across various MR methods, except for insomnia symptoms which showed evidence of an unbalanced pleiotropy in MR-Egger analysis. Additionally, previous studies have shown only mild attenuation of causal effects of insomnia symptoms on CAD risk when adjusted for BMI, smoking, depression, and education using multivariable Mendelian randomization (MVMR) [25, 26]. Moreover, simulations have shown that MR-Egger may be unreliable when applied to a single dataset [59], and this is a limitation of our study.

The sleep traits were based on self-report. It remains unclear if self-reported sleep duration represents time in bed or actual sleep time. Also, the insomnia questions in UKBB or HUNT2 did not cover all aspects of insomnia (difficulty falling asleep, night awakenings, waking

up early and daytime impairments) [83]. Chronotype in this study was assessed from a single question in UKBB, whereas validated instruments such as the Morningness-Eveningness Questionnaire and the Munich Chronotype Questionnaire use diverse questions to better estimate chronotype [84, 85]. Other sleep traits (e.g., sleep apnea, snoring, daytime napping) were not included, and we do not know whether these interact with insomnia symptoms or sleep duration. Moreover, the sleep traits we used are binary exposures (except for 24-hour sleep duration), which are likely coarsened approximations of the true latent exposure [86]. This opens up alternate pathways from the genetic instruments to the outcome, which may violate the exclusion restriction assumption, resulting in biased effect estimates [86]. In addition, causal estimates from MR of binary exposures on a binary outcome are difficult to interpret [87].

Due to the small sample size in HUNT2, we might have missed weak causal effects due to insufficient power. In addition, the genetic instrument explained little variance in short sleep and long sleep within HUNT2, implying possible weak instrument bias [88] and leading to wide CIs as shown in the bootstrap simulations [89]. Furthermore, SNPs for short and long sleep were not replicated in other independent cohorts [31], meaning that the GRS used is not validated in any other population.

The inclusion of UKBB in all exposure GWASs could lead to winner's curse that might bias the causal estimates in UKBB [90]. We therefore used unweighted GRS for our exposures in UKBB as recommended [51]. Also, we derived GRS for insomnia symptoms and chronotype composed of SNPs that replicated in an independent study (23andMe) [30, 32], which showed similar estimates, indicating winner's curse is unlikely to have substantially biased effect estimates. However, we could not apply the same approach to explore the impact of winner's curse on the sleep duration due to the limited sample size of the replication datasets in those studies [31], meaning that genetic associations might be imprecise.

The variation in the occurrence of AMI between UKBB (2.35%) and HUNT2 (10.03%) may be attributed to several factors related to the composition of the cohorts: (a) the HUNT2 cohort followed up relative older participants, aged 20 years or above, with a mean baseline age of 48 years, while UKBB consisted of participants aged 40 to 69 years, with a mean baseline age of 56 years; (b) the duration of follow-up was longer in HUNT2, spanning 20.4 years, compared to UKBB's follow-up period of 11.7 years; (c) UKBB (5.5% response rate) may represent a healthier sample [91], whereas HUNT2 (69.5% response rate) may be a more representative sample [36]; and (d) baseline differences in the two underlying populations or differences due to time trend (for example, more current smokers in HUNT2 which was conducted about a decade earlier than UKBB). Moreover, competing risk from death among participants would potentially hinder the occurrence of AMI, that might overestimate the risks [92]. This is another limitation of our study.

Finally, our findings rely on analyses in UKBB due to its large sample. However, the generalizability of these findings may be limited due to a selected sample (5.5% response rate) in the UKBB cohort, which can bias both observational and MR estimates [93, 94]. Selection bias may artificially induce associations between genetic variants and confounders leading to the instrumental variable becoming invalid [95]. This might partly explain differences in UKBB and HUNT2 estimates observed in this study, where HUNT2 sample (69.5% response rate) more closely represents target population. The difference in demographics of the two cohorts might also cause inconsistent estimates. Moreover, the inclusion of cohorts from the European ancestry may further restrict generalizability of our findings.

Conclusions

This study reveals no interaction effects between sleep traits on the risk of AMI, but found that two sleep traits in combination (including insomnia symptoms, short sleep, and a morning chronotype) had the highest risk of AMI. The role of chronotype in AMI risk remains uncertain, as the adverse causal effect of morning chronotype could partly be explained by genetic predisposition to insomnia symptoms or short sleep. This indicates that the main effects of insomnia symptoms and short sleep are likely to be independent of each other, i.e., the magnitude of the effect of insomnia symptoms on AMI does not depend on whether there is accompanying genetic predisposition to short sleep, and vice-versa. Thus, interventions targeting both insomnia symptoms and short sleep could be relevant for preventive initiatives to reduce the risk of AMI. Moreover, this study also suggests a potential protective effect of genetically predisposed long sleep that was not affected by additionally being genetically predisposed to insomnia symptoms and a morning chronotype.

Abbreviations

AMI	Acute myocardial infarction
BMI	Body mass index
CAD	Coronary artery disease
CI	Confidence interval
GRS	Genetic risk score
GWAS	Genome-wide association study
HADS	Hospital Anxiety and Depression Scale
HES	Hospital Episode Statistics
HR	Hazard ratio
HUNT	The Trøndelag Health Study
ICD	International Classification of Diseases

MR	Mendelian randomization
MVMR	Multivariable Mendelian randomization
NHS	National Health Service
OR	Odds ratio
PEDW	Patient Episode Database for Wales
RERI	Relative excess risk due to interaction
SBP	Systolic blood pressure
SD	Standard deviation
SMR	Scottish Morbidity Record
SNP	Single nucleotide polymorphism
TDI	Townsend deprivation index
TSPS	Two-stage predictor substitution
UKBB	UK Biobank

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-023-03078-0.

Additional file 1. Information on covariates; Supplementary figures. **Figure S1.** Flow chart of the participant selection process. **Figure S2.** 2×2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in HUNT2 using weighted and unweighted genetic risk scores for sleep traits. **Figure S3.** Association of insomnia SNPs from Jansen et al., 2019 and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively. **Figure S4.** Association of 24-hour sleep duration SNPs from Dashti et al., 2019 and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively. **Figure S5.** Association of short sleep duration SNPs from Dashti et al., 2019 and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively. **Figure S6.** Association of long sleep duration SNPs from Dashti et al., 2019 and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively. **Figure S7.** Association of chronotype (morning preference) SNPs from Jones et al., 2019 and acute myocardial infarction (AMI) within UK Biobank. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively. **Figure S8.** Association of insomnia SNPs from Lane et al., 2019 and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively. **Figure S9.** Continuous factorial Mendelian randomization analysis using genetic risk score as quantitative traits with their product term assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in UK Biobank and HUNT2. **Figure S10.** One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with sleep traits in UK Biobank and HUNT2 after excluding participants who reported self-reported use of sleep medication. **Figure S11.** 2×2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in UK Biobank and HUNT2 after excluding participants who reported self-reported use of sleep medication; and Supplementary tables. **Table S1.** Detailed summary of Mendelian randomization (MR) studies previously conducted on sleep traits and risk of coronary artery disease (CAD) or acute myocardial infarction (AMI). **Table S2.** Summary of genetic instruments showing their strength applying to UK Biobank and HUNT2. **Table S3.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and short sleep in UK Biobank. **Table S4.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and short sleep in HUNT2. **Table S5.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and long sleep in UK Biobank. **Table S6.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and long sleep in HUNT2. **Table S7.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and chronotype (morning preference) in UK Biobank. **Table S8.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for short sleep and chronotype (morning preference) in UK Biobank. **Table S9.** Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for long sleep and chronotype (morning preference) in UK Biobank. **Table S10.** Statistical test of the proportional hazard assumption for one-sample Mendelian randomization (MR) Cox regression models. **Table S11.** Statistical test of the proportional hazard assumption for 2×2 factorial Mendelian randomization (MR) Cox regression models. **Table S12.** One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with sleep traits in HUNT2 using weighted and unweighted genetic risk scores for sleep traits. **Table S13.** Associations between genetic risk scores and potential confounders in UK Biobank. **Table S14.** Associations between genetic risk scores and potential confounders in HUNT2. **Table S15.** One-sample Mendelian randomization analysis for risk of incident acute myocardial infarction associated with sleep traits with and without adjustment for potential confounders in UK Biobank and HUNT2. **Table S16.** Sensitivity analysis for risk of incident acute myocardial infarction associated with sleep traits in UK Biobank. **Table S17.** Sensitivity analysis for risk of incident acute myocardial infarction associated with sleep traits in HUNT2. **Table S18.** One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with insomnia symptoms using instruments from Lane et al., 2019 in UK Biobank and HUNT2. **Table S19.** Sensitivity analysis for risk of incident acute myocardial infarction associated with insomnia symptoms and chronotype in UK Biobank using genetic variants genome-wide significant in 23andMe. **Table S20.** Sensitivity analysis for risk of incident acute myocardial infarction associated with insomnia symptoms in HUNT2 using genetic variants genome-wide significant in 23andMe. **Table S21.** List of medications used to define the sleep medication covariate in UK Biobank.

Additional file 2. Genetic variants. **Table G1.** Summary information of genetic variants identified for insomnia symptoms. **Table G2.** Summary information of genetic variants identified for sleep duration. **Table G3.** Summary information of genetic variants identified for short sleep. **Table G4.** Summary information of genetic variants identified for long sleep. **Table G5.** Summary information of genetic variants identified for chronotype.

Acknowledgements
This research has been conducted using data from UK Biobank, a major biomedical database (www.ukbiobank.ac.uk), under application number 40135. The Trøndelag Health Study (HUNT) is a collaboration between HUNT Research Centre (Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU)), Trøndelag County Council, Central Norway Regional Health Authority, and the Norwegian Institute of Public Health. We want to thank clinicians and other employees at Nord-Trøndelag Hospital Trust for their support and for contributing to data collection in this research project.

Authors' contributions
N.A. interpreted and analyzed the data, interpreted the findings, and wrote the paper; L.B.S. and R.C.R. had the original idea for this study, interpreted the data, and critically revised the paper; E.S.S., B.M.B., and B.O.Å. had the original idea for this study and critically revised the paper; L.B. assisted with analysis and critically revised the paper; and H.D. assisted with interpreting data on acute myocardial infarction from medical records assessed through hospitals in the Trøndelag County and critically revised the paper. All authors read and approved the final manuscript.

Authors' Twitter handles
Nikhil Arora: @dr_nikhil_arora
Laxmi Bhatta: @laxmibhatta001

Eivind Schjelderup Skarpsno: @E_Skarpsno
Bjørn Olav Åsvold: @BAsvold
Ben Michael Brumpton: @bmbrumpton
Rebecca Claire Richmond: @BeckyRichmond90
Linn Beate Strand: @strandlb

Funding
Open access funding provided by Norwegian University of Science and Technology This study was made possible with the financial support from the National Association for Public Health in Norway (Nasjonalforeningen for folkehelsen; project number 19479), mobility grant funds from the Liaison Committee for Education, Research and Innovation in Central Norway (Helse Midt-Norge; project number 2023/34249), and top-up financing from the Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials
The data supporting the findings are available in the supplementary material and upon request. The UK Biobank data is available to researchers, subject to successful registration and application process via their website (https://www.ukbiobank.ac.uk/). The data from the HUNT Study are available from the HUNT Research Centre, but restrictions apply to the availability of these data, which were used under license for the current study and so are not publicly available. However, the data are available for export given approval of application to the HUNT Research Centre (http://www.ntnu.edu/hunt/data). The data on hospital records linkages to the HUNT Study participants are available from Nord-Trøndelag Hospital Trust and require permission. All other data used are publicly available and referenced according in the main text.

Declarations

Ethics approval and consent to participate
UK Biobank received ethical approval from the National Health Service (NHS) Research Ethics Service (reference number 11/NW/0382). The HUNT Study was approved by the Data Inspectorate of Norway and recommended by the Regional Committee for Ethics in Medical Research (REK; reference number 152/95/AH/JGE). The ethical approval for conducting this study was also obtained from the Regional Committee for Ethics in Medical Research (REK nord; reference number 2020/47206).
Informed consent was obtained from all individual participants of both the cohorts included in this study.

Competing interests
The authors declare no competing interests.

Author details
[1]K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway. [2]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK. [3]Division of Mental Health Care, St. Olavs Hospital, Trondheim, Norway. [4]Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia. [5]Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway. [6]Department of Neurology and Clinical Neurophysiology, St. Olavs Hospital, Trondheim, Norway. [7]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway. [8]Department of Medicine, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, Norway. [9]Clinic of Cardiology, St. Olavs Hospital, Trondheim, Norway. [10]HUNT Research Centre, Department of Public Health and Nursing, Norwegian University of Science and Technology, Levanger, Norway. [11]Department of Endocrinology, Clinic of Medicine, St. Olavs Hospital, Trondheim, Norway. [12]Department of Medicine, St. Olavs Hospital, Trondheim, Norway. [13]MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK.

Received: 21 April 2023 Accepted: 11 September 2023
Published online: 05 October 2023

References

1. Roth T. Insomnia: definition, prevalence, etiology, and consequences. J Clin Sleep Med. 2007;3(5 Suppl):S7-10.
2. St-Onge M-P, Grandner MA, Brown D, Conroy MB, Jean-Louis G, Coons M, et al. Sleep duration and quality: impact on lifestyle behaviors and cardiometabolic health: a scientific statement from the American Heart Association. Circulation. 2016;134:e367–86.
3. Vgontzas AN, Liao D, Bixler EO, Chrousos GP, Vela-Bueno A. Insomnia with objective short sleep duration is associated with a high risk for hypertension. Sleep. 2009;32:491–7.
4. Cappuccio FP, Taggart FM, Kandala NB, Currie A, Peile E, Stranges S, et al. Meta-analysis of short sleep duration and obesity in children and adults. Sleep. 2008;31:619–26.
5. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag, Chronotype, and cardiometabolic risk. J Clin Endocrinol Metab. 2015;100:4612–20.
6. Cardiovascular diseases (CVDs). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Accessed 27 Mar 2023.
7. Qaseem A, Kansagara D, Forciea MA, Cooke M, Denberg TD. Management of chronic insomnia disorder in adults: a clinical practice guideline from the American College of Physicians. Ann Intern Med. 2016;165:125–33.
8. Buysse DJ. Sleep health: can we define it? Does it matter? Sleep. 2014;37:9–17.
9. Laugsand LE, Vatten LJ, Platou C, Janszky I. Insomnia and the risk of acute myocardial infarction. Circulation. 2011;124:2073–81.
10. Laugsand LE, Strand LB, Platou C, Vatten LJ, Janszky I. Insomnia and the risk of incident heart failure: a population study. Eur Heart J. 2014;35:1382–93.
11. Daghlas I, Dashti HS, Lane J, Aragam KG, Rutter MK, Saxena R, et al. Sleep duration and myocardial infarction. J Am Coll Cardiol. 2019;74:1304–14.
12. Merikanto I, Lahti T, Puolijoki H, Vanhala M, Peltonen M, Laatikainen T, et al. Associations of chronotype and sleep with cardiovascular diseases and type 2 diabetes. Chronobiol Int. 2013;30:470–7.
13. Fan Y, Wu Y, Peng Y, Zhao B, Yang J, Bai L, et al. Sleeping late increases the risk of myocardial infarction in the middle-aged and older populations. Front Cardiovasc Med. 2021;8:709468.
14. Arora N, Richmond RC, Brumpton BM, Åsvold BO, Dalen H, Skarpsno ES, et al. Self-reported insomnia symptoms, sleep duration, chronotype and the risk of acute myocardial infarction (AMI): a prospective study in the UK Biobank and the HUNT Study. Eur J Epidemiol. 2023;38(6):643–56.
15. Fan M, Sun D, Zhou T, Heianza Y, Lv J, Li L, et al. Sleep patterns, genetic susceptibility, and incident cardiovascular disease: a prospective study of 385 292 UK biobank participants. Eur Heart J. 2020;41:1182–9.
16. Bertisch SM, Pollock BD, Mittleman MA, Buysse DJ, Bazzano LA, Gottlieb DJ, et al. Insomnia with objective short sleep duration and risk of incident cardiovascular disease and all-cause mortality: Sleep Heart Health Stud. Sleep. 2018;41(6):zsy047.
17. Sands-Lincoln M, Loucks EB, Lu B, Carskadon MA, Sharkey K, Stefanick ML, et al. Sleep duration, insomnia, and coronary heart disease among postmenopausal women in the women's health initiative. J Womens Health (Larchmt). 2013;22:477–86.
18. Westerlund A, Bellocco R, Sundström J, Adami H-O, Åkerstedt T, Trolle LY. Sleep characteristics and cardiovascular events in a large Swedish cohort. Eur J Epidemiol. 2013;28:463–73.
19. Chien K-L, Chen P-C, Hsu H-C, Su T-C, Sung F-C, Chen M-F, et al. Habitual sleep duration and insomnia and the risk of cardiovascular events and all-cause death: report from a community-based cohort. Sleep. 2010;33:177–84.
20. Chandola T, Ferrie JE, Perski A, Akbaraly T, Marmot MG. The effect of short sleep duration on coronary heart disease risk is greatest among those with sleep disturbance: a prospective study from the Whitehall II cohort. Sleep. 2010;33:739–44.
21. Vgontzas AN, Fernandez-Mendoza J, Liao D, Bixler EO. Insomnia with objective short sleep duration: the most biologically severe phenotype of the disorder. Sleep Med Rev. 2013;17:241–54.
22. Davies NM, Holmes MV, Smith GD. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362: k601.

23. Burgess S, Thompson SG. Mendelian randomization: methods for causal inference using genetic variants. 2nd ed. New York: Chapman and Hall/CRC; 2021.
24. Lane JM, Jones SE, Dashti HS, Wood AR, Aragam KG, van Hees VT, et al. Biological and clinical insights from genetics of insomnia symptoms. Nat Genet. 2019;51:387–93. https://www.nature.com/articles/s41588-019-0361-7.
25. Larsson SC, Markus HS. Genetic liability to insomnia and cardiovascular disease risk. Circulation. 2019;140:796–8.
26. Yuan S, Mason AM, Burgess S, Larsson SC. Genetic liability to insomnia in relation to cardiovascular diseases: a Mendelian randomisation study. Eur J Epidemiol. 2021;36:393–400.
27. Liu X, Li C, Sun X, Yu Y, Si S, Hou L, et al. Genetically predicted insomnia in relation to 14 cardiovascular conditions and 17 cardiometabolic risk factors: a Mendelian randomization study. J Am Heart Assoc. 2021;10:e020187.
28. Yang Y, Fan J, Shi X, Wang Y, Yang C, Lian J, et al. Causal associations between sleep traits and four cardiac diseases: a Mendelian randomization study. ESC Heart Failure. 2022;9:3160–6.
29. Ai S, Zhang J, Zhao G, Wang N, Li G, So H-C, et al. Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK Biobank. Eur Heart J. 2021;42:3349–57.
30. Jansen PR, Watanabe K, Stringer S, Skene N, Bryois J, Hammerschlag AR, et al. Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways. Nat Genet. 2019;51:394–403. https://www.nature.com/articles/s41588-018-0333-3.
31. Dashti HS, Jones SE, Wood AR, Lane JM, van Hees VT, Wang H, et al. Genome-wide association study identifies genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates. Nat Commun. 2019;10:1100. https://www.nature.com/articles/s41467-019-08917-4.
32. Jones SE, Lane JM, Wood AR, van Hees VT, Tyrrell J, Beaumont RN, et al. Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms. Nat Commun. 2019;10:343. https://www.nature.com/articles/s41467-018-08259-7.
33. Nikpay M, Goel A, Won H-H, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet. 2015;47:1121–30.
34. UK Biobank: Protocol for a large-scale prospective epidemiological resource. 2006. https://www.ukbiobank.ac.uk/learn-more-about-uk-biobank/about-us. Accessed 25 Oct 2021.
35. Åsvold BO, Langhammer A, Rehn TA, Kjelvik G, Grøntvedt TV, Sørgjerd EP, et al. Cohort Profile Update: The HUNT Study, Norway. Int J Epidemiol. 2023;52:e80-91.
36. Holmen J, Midthjell K, Krüger Ø, Langhammer A, Holmen TL, Bratberg GH, et al. The Nord-Trøndelag Health Study 1995–97 (HUNT 2): objectives, contents, methods and participation. Norsk epidemiologi. 2003;13:19–32.
37. Riemann D, Baglioni C, Bassetti C, Bjorvatn B, Dolenc Groselj L, Ellis JG, et al. European guideline for the diagnosis and treatment of insomnia. J Sleep Res. 2017;26:675–700.
38. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381–95.
39. International Physical Activity Questionnaire (IPAQ): Guidelines for data processing analysis of the International Physical Activity Questionnaire (IPAQ) - Short and long forms. 2005. https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=540. Accessed 25 Oct 2021.
40. International Physical Activity Questionnaire (IPAQ): IPAQ scoring protocol. 2005. https://sites.google.com/site/theipaq/scoring-protocol. Accessed 25 Oct 2021.
41. Brumpton BM, Langhammer A, Ferreira MAR, Chen Y, Mai X-M. Physical activity and incident asthma in adults: the HUNT Study Norway. BMJ Open. 2016;6: e013856.
42. Kurtze N, Rangul V, Hustvedt BE, Flanders WD. Reliability and validity of self-reported physical activity in the Nord-Trøndelag Health Study (HUNT 2). Eur J Epidemiol. 2007;22:379–87.
43. Townsend P. Poverty in the United Kingdom: a survey of household resources and standards of living. London: Allen Lane and Penguin Books; 1979.
44. Elliott P, Peakman TC, UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. Int J Epidemiol. 2008;37:234–44.
45. Skapinakis P. Hospital Anxiety and Depression Scale (HADS). In: Michalos AC, editor. Encyclopedia of quality of life and well-being research. Dordrecht: Springer, Netherlands; 2014. p. 2930–3.
46 Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res. 2002;52:69–77.
47. Mykletun A, Stordal E, Dahl AA. Hospital Anxiety and Depression (HAD) scale: factor structure, item analyses and internal consistency in a large population. Br J Psychiatry. 2001;179:540–4.
48. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018;562:203–9.
49. Mitchell RE, Hemani G, Dudding T, Corbin L, Harrison S, Paternoster L. UK Biobank Genetic Data: MRC-IEU Quality Control, version 2. 2019. https://data.bris.ac.uk/data/dataset/1ovaau5sxunp2cv8rcy88688v.
50. Brumpton BM, Graham S, Surakka I, Skogholt AH, Løset M, Fritsche LG, et al. The HUNT study: a population-based cohort for genetic research. Cell Genomics. 2022;2: 100193.
51. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian randomization. Int J Epidemiol. 2013;42:1134–44.
52. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. Stat Methods Med Res. 2017;26:2333–55.
53. Burgess S, Labrecque JA. Mendelian randomization with a binary exposure variable: interpretation and presentation of causal estimates. Eur J Epidemiol. 2018;33:947–52.
54. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology. 1992;3:452.
55. Knol MJ, VanderWeele TJ, Groenwold RHH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. Eur J Epidemiol. 2011;26:433–8.
56. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44:512–25.
57. 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;40:304–14.
58. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol. 2017;46:1985–98.
59. Minelli C, Del Greco MF, van der Plaat DA, Bowden J, Sheehan NA, Thompson J. The use of two-sample methods for Mendelian randomization analyses on single large datasets. Int J Epidemiol. 2021;50:1651–9.
60. Rees JMB, Foley CN, Burgess S. Factorial Mendelian randomization: using genetic variants to assess interactions. Int J Epidemiol. 2020;49:1147–58.
61. Knol MJ, van der Tweel I, Grobbee DE, Numans ME, Geerlings MI. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. Int J Epidemiol. 2007;36:1111–8.
62. Kim Y-H, Kim H-B, Kim D-H, Kim J-Y, Shin H-Y. Use of hypnotics and the risk of or mortality from heart disease: a meta-analysis of observational studies. Korean J Intern Med. 2017;33:727–36.
63. Grandner MA, Drummond SPA. Who are the long sleepers? Towards an understanding of the mortality relationship. Sleep Med Rev. 2007;11:341–60.
64. Stamatakis KA, Punjabi NM. Long sleep duration: a risk to health or a marker of risk? Sleep Med Rev. 2007;11:337.
65. Hsieh CG, Martin JL. Short sleep, insomnia, and cardiovascular disease. Curr Sleep Med Rep. 2019;5:234–42.
66. D'Aurea C, Poyares D, Piovezan RD, Passos GS, Tufik S, de Mello MT. Objective short sleep duration is associated with the activity of the hypothalamic-pituitary-adrenal axis in insomnia. Arq Neuro-Psiquiatr. 2015;73:516–9.
67. Grimaldi D, Carter JR, Van Cauter E, Leproult R. Adverse impact of sleep restriction and circadian misalignment on autonomic function in healthy young adults. Hypertension. 2016;68:243–50.
68. Castro-Diehl C, Diez Roux AV, Redline S, Seeman T, McKinley P, Sloan R, et al. Sleep duration and quality in relation to autonomic nervous system measures: the multi-ethnic study of atherosclerosis (MESA). Sleep. 2016;39:1927–40.

69. Jarrin DC, Ivers H, Lamy M, Chen IY, Harvey AG, Morin CM. Cardiovascular autonomic dysfunction in insomnia patients with objective short sleep duration. J Sleep Res. 2018;27: e12663.
70. Vgontzas AN, Zoumakis E, Bixler EO, Lin H-M, Follett H, Kales A, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J Clin Endocrinol Metab. 2004;89:2119–26.
71. Kjeldsen JS, Hjorth MF, Andersen R, Michaelsen KF, Tetens I, Astrup A, et al. Short sleep duration and large variability in sleep duration are independently associated with dietary risk factors for obesity in Danish school children. Int J Obes (Lond). 2014;38:32–9.
72. Broussard JL, Kilkus JM, Delebecque F, Abraham V, Day A, Whitmore HR, et al. Elevated ghrelin predicts food intake during experimental sleep restriction. Obesity (Silver Spring). 2016;24:132–8.
73. Broussard JL, Ehrmann DA, Van Cauter E, Tasali E, Brady MJ. Impaired insulin signaling in human adipocytes after experimental sleep restriction: a randomized, crossover study. Ann Intern Med. 2012;157:549–57.
74. Lanfranchi PA, Pennestri M-H, Fradette L, Dumont M, Morin CM, Montplaisir J. Nighttime blood pressure in normotensive subjects with chronic insomnia: implications for cardiovascular risk. Sleep. 2009;32:760–6.
75. Fernandez-Mendoza J, He F, Vgontzas AN, Liao D, Bixler EO. Objective short sleep duration modifies the relationship between hypertension and all-cause mortality. J Hypertens. 2017;35:830–6.
76. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: pathophysiologic mechanisms and emerging clinical indications. Vascul Pharmacol. 2016;77:1–7.
77. Lane JM, Liang J, Vlasac I, Anderson SG, Bechtold DA, Bowden J, et al. Genome-wide association analyses of sleep disturbance traits identify new loci and highlight shared genetics with neuropsychiatric and metabolic traits. Nat Genet. 2017;49:274–81.
78. Makarem N, Paul J, Giardina E-GV, Liao M, Aggarwal B. Evening chronotype is associated with poor cardiovascular health and adverse health behaviors in a diverse population of women. Chronobiol Int. 2020;37:673–85.
79. Maukonen M, Kanerva N, Partonen T, Kronholm E, Konttinen H, Wennman H, et al. The associations between chronotype, a healthy diet and obesity. Chronobiol Int. 2016;33:972–81.
80. Lawlor DA, Tilling K, Davey SG. Triangulation in aetiological epidemiology. Int J Epidemiol. 2016;45:1866–86.
81. North T-L, Davies NM, Harrison S, Carter AR, Hemani G, Sanderson E, et al. Using genetic instruments to estimate interactions in Mendelian randomization studies. Epidemiology. 2019;30:e33–5.
82. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23:R89-98.
83. Littner M, Hirshkowitz M, Kramer M, Kapen S, Anderson WM, Bailey D, et al. Practice parameters for using polysomnography to evaluate insomnia: an update. Sleep. 2003;26:754–60.
84. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4:97–110.
85. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003;18:80–90.
86. Tudball MJ, Bowden J, Hughes RA, Ly A, Munafò MR, Tilling K, et al. Mendelian randomisation with coarsened exposures. Genet Epidemiol. 2021;45:338–50.
87. Palmer TM, Sterne JAC, Harbord RM, Lawlor DA, Sheehan NA, Meng S, et al. Instrumental Variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. Am J Epidemiol. 2011;173:1392–403.
88. Burgess S, Thompson SG, CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol. 2011;40:755–64.
89. Moreira MJ, Porter JR, Suarez GA. Bootstrap validity for the score test when instruments may be weak. J Econometr. 2009;149:52–64.
90. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. Genet Epidemiol. 2016;40:597–608.
91. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank Participants with those of the general population. Am J Epidemiol. 2017;186:1026–34.
92. Puddu PE, Amaduzzi PL, Ricci B. Coronary heart disease incidence and competing risks: an important issue. J Geriatr Cardiol. 2017;14:425–9.
93. Hernán MA, Hernández-Díaz S, Robins JM. A Structural approach to selection bias. Epidemiology. 2004;15:615.
94. Canan C, Lesko C, Lau B. Instrumental variable analyses and selection bias. Epidemiology. 2017;28:396.
95. Gkatzionis A, Burgess S. Contextualizing selection bias in Mendelian randomization: how bad is it likely to be? Int J Epidemiol. 2019;48:691–701.

Publisher's Note

Supplementary material, Paper II

The supplementary material for Paper II can also be found from the following links, as part of the published paper:

https://static-content.springer.com/esm/art%3A10.1186%2Fs12916-023-03078-0/MediaObjects/12916_2023_3078_MOESM1_ESM.docx (Additional file 1)

https://static-content.springer.com/esm/art%3A10.1186%2Fs12916-023-03078-0/MediaObjects/12916_2023_3078_MOESM2_ESM.docx (Additional file 2; Included under appendices)

Information on covariates

A self-administered questionnaire was used to gather information on various covariates including gender, age at recruitment, marital status, alcohol intake, smoking status, physical activity, Townsend Deprivation Index (in UK Biobank only), education, shift work, and use of sleep medication(s). Additionally, participants attended examination stations where clinical examination was performed, and blood samples were drawn by trained staff.

In UKBB, participants were categorized as “Married” if they live with their husband/wife/partner, and as “Unmarried” if they don’t. Also, the information about the number of people living in a household was used to categorize individuals living alone as “Unmarried” in cases where the marital status information was missing. In HUNT2, marital status was categorized into “Unmarried”, “Married”, and “Separated/Divorced/Widowed”.

In UKBB and HUNT2, participants were asked about their alcohol intake frequency and were categorized as follows: “Never/rarely” for non-drinkers or those who only drink on special occasions, “Monthly” for those who drink 1 - 3 times a month, “Weekly” for those who drink 1- 4 times a week or “Daily/almost daily” for those who drink more frequently. Additionally, in HUNT2, the information about participants who had never consumed alcohol was used to categorize them as “Never/rarely” for observations having missing information on alcohol intake frequency. Thus, this information will be categorized as “Never/rarely”, “Monthly”, “Weekly” or “Daily/almost daily” alcohol intake.

The information on smoking status was categorized as “Never”, “Previous” or “Current” smoker for UKBB and HUNT2.

In UKBB, physical activity (PA) was evaluated using adapted questions from the validated short International Physical Activity Questionnaire (IPAQ) [1], following guidelines published for data processing by IPAQ [2]. IPAQ assessed total physical activity, including walking, moderate, and vigorous PA performed over the last 7 days. Participants were categorized into three mutually exclusive PA categories: “High” (≥ 1h of moderate PA or ≥ ½ h of vigorous PA above basal level of activity on most days), “Moderate” (≥ ½ h of moderate PA above basal level of activity on most days) or “Low/inactive” (anything else) based on a standard scoring criteria [3], where approximately 5000 steps per day was considered as basal activity. In HUNT2, PA was classified based on self-reported leisure time light and hard PA during the past year. Light PA was defined as activity that did not cause sweating or shortness of breath, while hard PA was defined as activity that resulted in sweating or shortness of breath. Participants were instructed to include the commute to work as leisure time. The study participants were grouped into three mutually exclusive categories: “High” (defined by ≥ 1h of hard PA regardless of light PA or ≥ 3h of light PA with < 1h of hard PA), “Moderate” (defined by ≥ 3h of light PA with no hard PA or < 3h of light PA with < 1h of hard PA), or “Low/inactive” (for anything else). This categorization strategy for PA was previously used by Brumpton *et al.* [4]. The reliability and validity of the questions on PA from HUNT2 have been reported to be acceptable for hard PA and poor for light PA [5].

In UKBB and HUNT2, education level was classified into three categories: “10 years or less” (for primary and lower secondary school education), “11-13 years” (for upper secondary school education), or “14 years or more” (for university/college education).

In UKBB, the Townsend Deprivation Index (TDI) was used as a measure for socioeconomic status to account for socioeconomic disparities and urban-rural mix within the UK. The index was created from census data on housing, employment, car availability and social class based on postal codes of participants, with higher values indicating a higher level of deprivation. The TDI has been validated for use in a UK-based population [6]. The HUNT2 sample was based on the population of the northern region of Trøndelag County in Norway, which is fairly representative of Norway regarding socio-economic characteristics [7]. Thus, any potential socioeconomic differences would be largely captured by education attainment.

The UKBB participants were asked about working shift work or working night shifts separately. These responses were then combined to create a proxy variable, with the highest response category being used as the final value. This proxy variable was then dichotomized, with "Usually" or "Always" being classified as "Yes", and all other responses as "No". In HUNT2, working shifts/at night/on call was also dichotomized as "Yes" or "No". Additionally, the information on current employment/work status from both UKBB and HUNT2 was used to categorize those without paid employment or who were self-employed as "No" for observations with missing information on working shifts/at night/on call.

In UKBB, the use of sleep medication(s) was ascertained by the self-reported use of medications from the list of sleep medications as used by Daghlas *et al*. [8], along with five other commonly used anxiolytics or sleep medications (list included in Table S21). These responses were then dichotomized as "Yes" or "No" for the use of sleep medication(s). In the HUNT2 study, participants were asked about their use of anxiolytics or sleep medications in the last month and categorized as "Yes" if they reported daily or weekly intake, and "No" otherwise.

Clinical examination

Within UKBB, weight was measured using the Tanita BC-418MA body composition analyzer to the nearest 0.1kg and height was measured using a Seca 202 height measure. Within HUNT2, weight was measured to the nearest 0.5kg and height was measured to the nearest 1cm. Participants in both UKBB and HUNT2 wore light clothes and no shoes during these measurements. The body mass index (BMI) was then calculated by dividing weight (in kg) by the square of height (in meter).

In UKBB, systolic and diastolic measurements of blood pressure were recorded automatedly (using Omron HEM-705 IT electronic blood pressure monitor) and/or manually (using manual sphygmomanometer). Two sets of measurements were taken with a one-minute interval and the average of these two were used in our analyses. In cases where automated readings were not available, the manual readings were used. In HUNT2, systolic and diastolic measurements of blood pressure were recorded automatedly (using a Dinamap 845XT (Critikon) sphygmomanometer based on oscillometry). Three sets of measurements were taken with a one-minute interval, and the average of second and third measurements were used in the analysis.

Laboratory measurements

For UKBB, a random (non-fasting) blood sample was collected from each participant in accordance with standard operating procedures for the UKBB. The samples were stored in refrigerators between 2 to 8 °C. The fasting time was recorded as the interval between last consumption of food or drink and the blood sample being taken. The samples were transferred to a central laboratory for storage and analyses on a daily basis. The serum samples were centrifuged for 10 minutes at 2000 RCF and the serum

concentrations of glucose, total cholesterol, HDL-cholesterol, and triglycerides were analyzed using a Beckman Coulter AU5800 automated analyzer. Glucose was measured using hexokinase analysis, while total cholesterol, HDL-cholesterol and triglycerides were measured by CHO-POD analysis, enzyme immunoinhibition analysis and GPO-POD analysis, respectively [9].

For HUNT2, a random (non-fasting) blood sample was collected from each participant. The samples were then analyzed at the Central Laboratory, Levanger Hospital, using a Hitachi 911 Autoanalyzer (Hitachi, Mito, Japan). The serum was separated from the blood by centrifugation within 2 hours of collection and stored in a refrigerator (4 °C). Time between the last meal and venipuncture was recorded. The samples were sent to the laboratory on the same day or within two to three days (for example on weekends). The serum concentrations of glucose, total cholesterol, HDL-cholesterol, and triglycerides were analyzed applying reagents from Boehringer Mannheim (Mannheim, Germany). The day-to-day coefficients of variation were 1.3-2.0%, 1.3-1.9%, 2.4%, and 0.7-1.3%, respectively. The glucose was measured using an enzymatic hexokinase method, total cholesterol and HDL-cholesterol were measured by an enzymatic colorimetric cholesterol esterase method, and triglycerides were measured with an enzymatic colorimetric method [7].

Depression and anxiety

For UKBB, hospitals recorded ICD-10 codes - F40 and F41 for anxiety; and F32, F33, F34, F38 and F39 for depression were used to identify participants with anxiety or depression episodes. From this information, two binary proxy variables were created for anxiety and depression, each categorized as “Yes” or “No”.

For HUNT2, the Hospital Anxiety and Depression Scale (HADS) was used to assess the symptoms of anxiety and depression. The questionnaire consisted of 14 Likert-scaled items (7 each for anxiety and depression) having a four-point scale ranging from 0 (not at all) to 3 (very often). Responses were summed to provide scores each for anxiety and depression ranging from 0 to 21. A higher score indicates an increased likelihood of anxiety and depression [10]. The HADS does not include somatic items or items about sleep difficulties. This tool is useful for assessing the symptom severity due to anxiety and depression in both primary care and hospital settings [11], and its psychometric properties have been validated as part of the HUNT study [12].

Supplementary figures

Figure S1: Flow chart of the participant selection process.

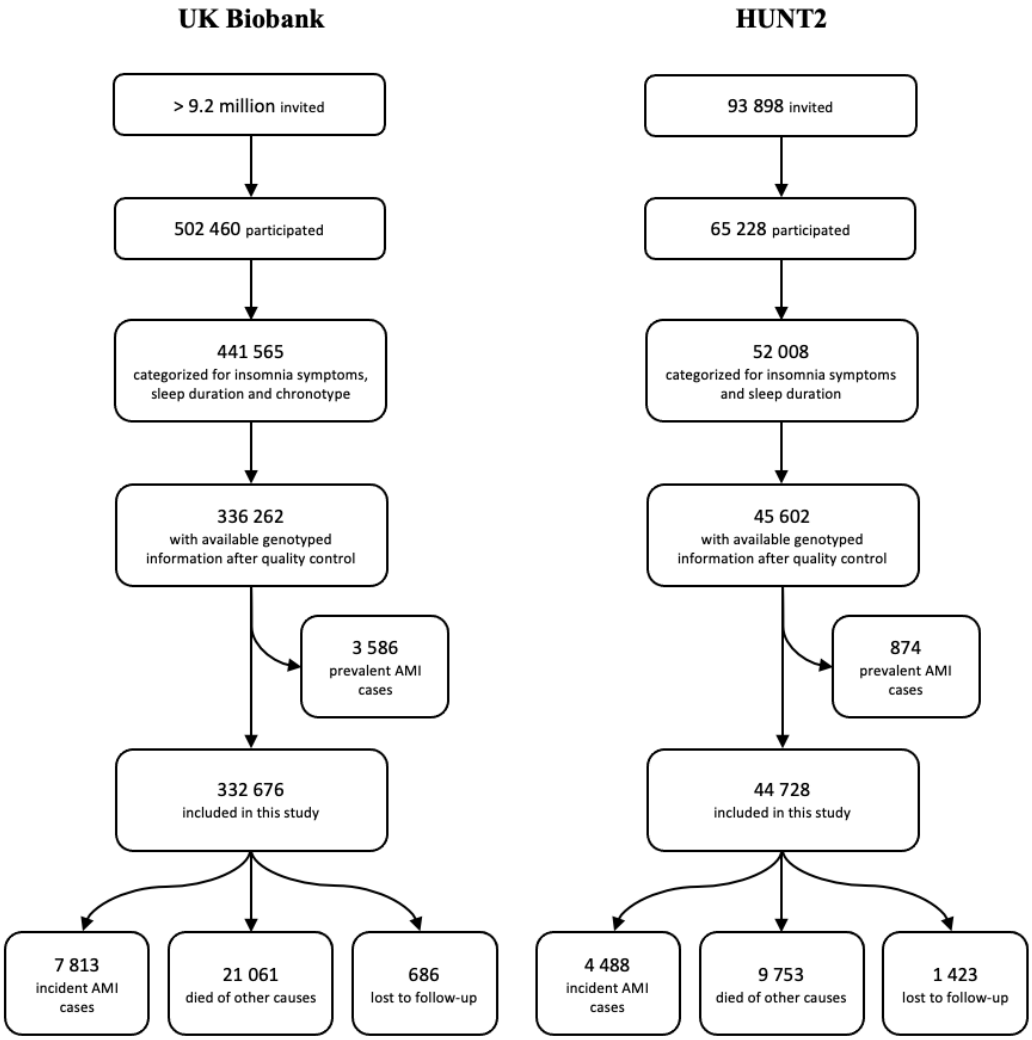
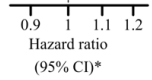
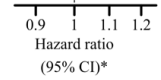


Figure S2: 2x2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in HUNT2 using weighted and unweighted genetic risk scores for sleep traits.

	Weighted genetic risk score		Unweighted genetic risk score	
	N (incident cases)	**Hazard ratio (95% CI)***	**N (incident cases)**	**Hazard ratio (95% CI)***
Insomnia symptoms – Short sleep				
Both GRS ≤ median	8 925 (823)	1 [Reference]	8 797 (803)	1 [Reference]
Insomnia GRS > median	7 697 (730)	1.08 (0.98, 1.20)	7 830 (750)	1.10 (1.00, 1.22)
Short sleep GRS > median	7 697 (684)	1.00 (0.90, 1.10)	7 825 (708)	1.04 (0.94, 1.14)
Both GRS > median	8 924 (821)	1.02 (0.93, 1.13)	8 791 (797)	1.03 (0.93, 1.13)
Insomnia symptoms – Long sleep				
Both GRS ≤ median	10 242 (1 007)	1 [Reference]	10 594 (1 029)	1 [Reference]
Insomnia GRS > median	10 731 (1 137)	1.07 (0.98, 1.16)	10 810 (1 137)	1.10 (1.01, 1.20)
Long sleep GRS > median	10 731 (1 038)	0.94 (0.87, 1.03)	10 380 (1 012)	1.01 (0.92, 1.10)
Both GRS > median	10 241 (1 027)	1.04 (0.95, 1.13)	10 161 (1 031)	1.07 (0.98, 1.16)





CI indicates confidence interval; and GRS, genetic risk score.
For each sleep trait combination, both GRS ≤ median represents low genetic risk for both sleep traits in combination, sleep trait 1 GRS > median represents high genetic risk for sleep trait 1 only, sleep trait 2 GRS > median represents high genetic risk for sleep trait 2 only and both GRS > median represents high genetic risk for both sleep traits.
* Adjusted for age, gender, 20 genetic principal components, and genotyping chip.

Figure S3: Association of insomnia SNPs from Jansen et al., 2019 [13] and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively.

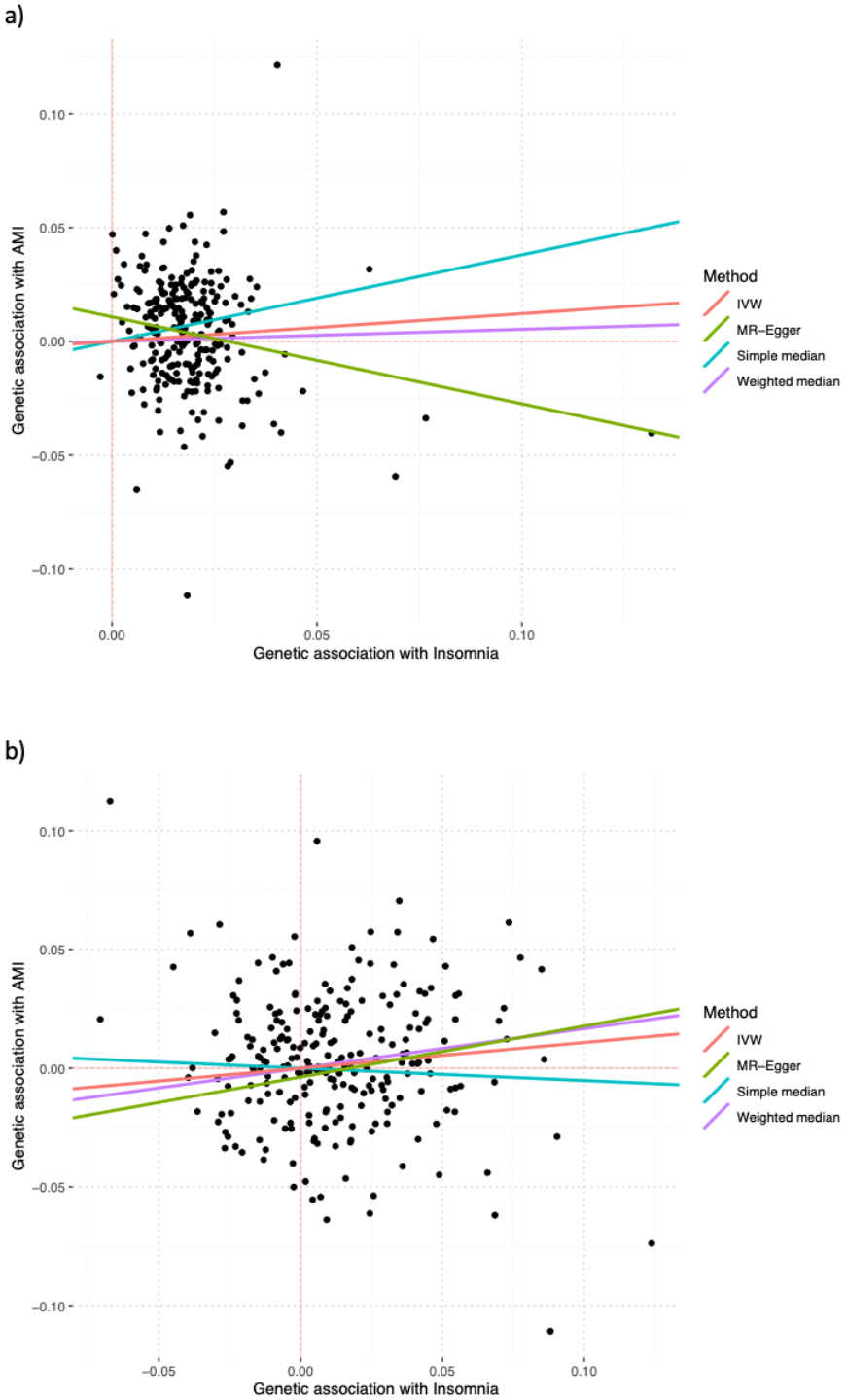


Figure S4: Association of 24-hour sleep duration SNPs from Dashti et al., 2019 [14] and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively.

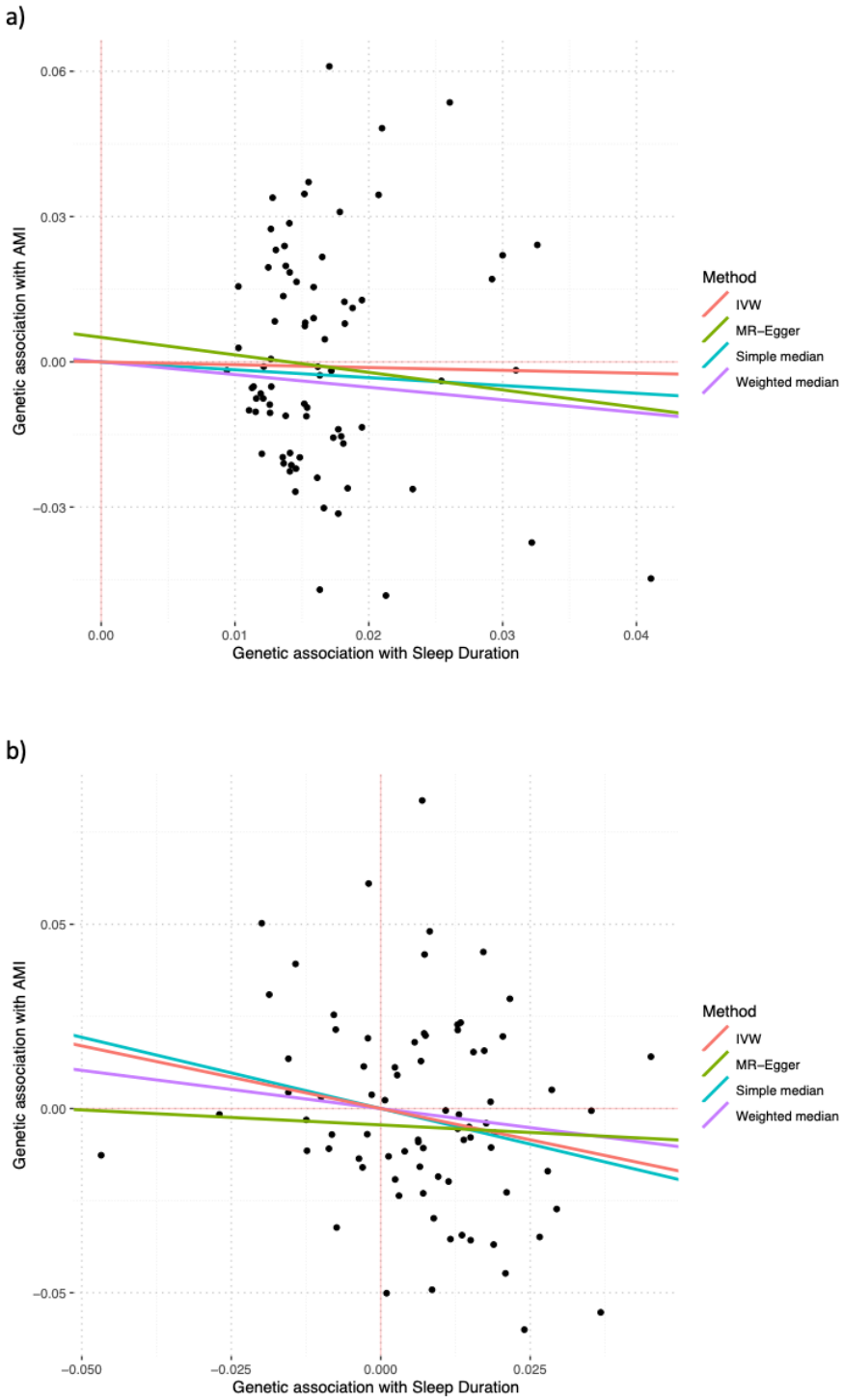


Figure S5: Association of short sleep duration SNPs from Dashti et al., 2019 [14] and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively.

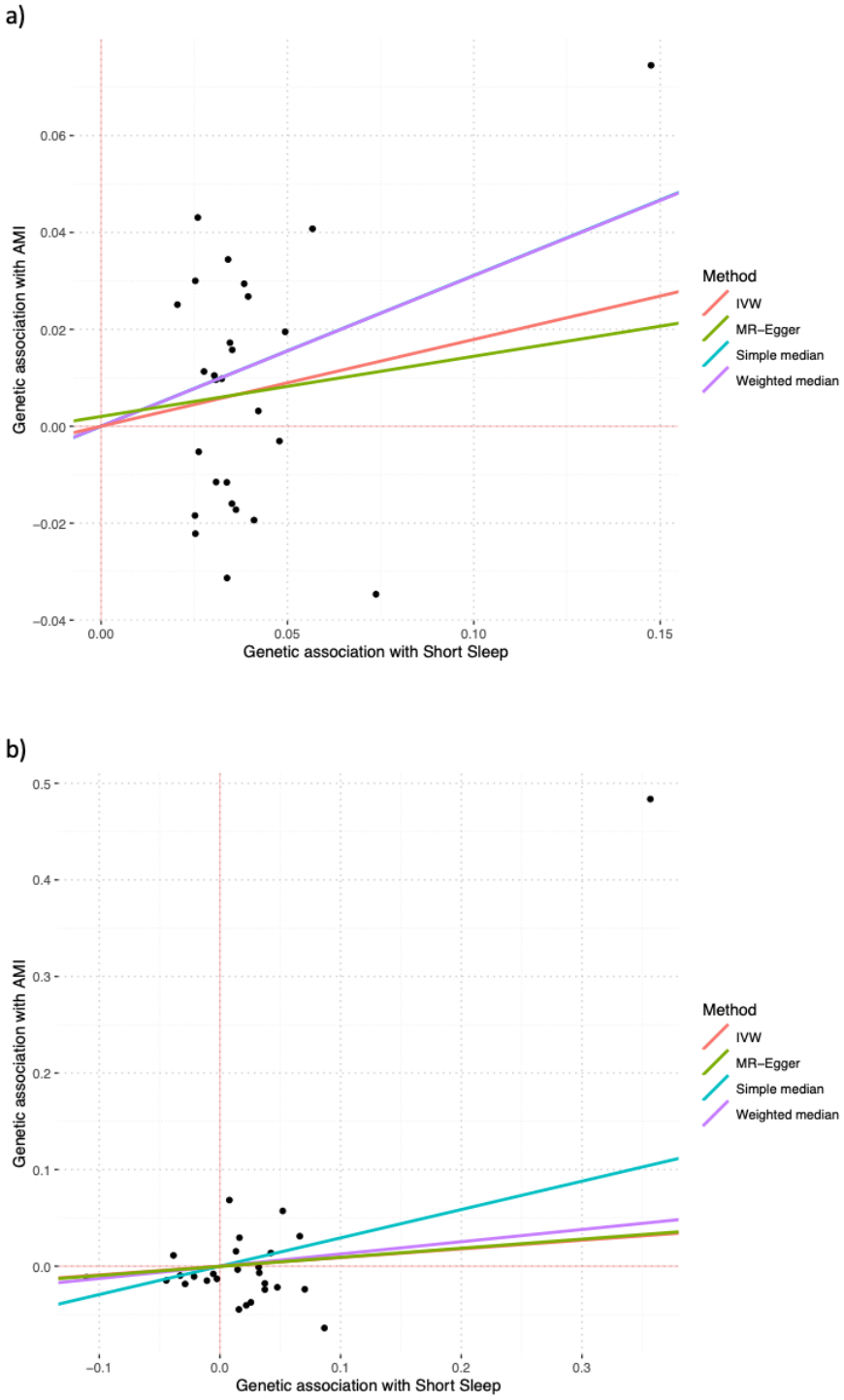


Figure S6: Association of long sleep duration SNPs from Dashti et al., 2019 [14] and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively.

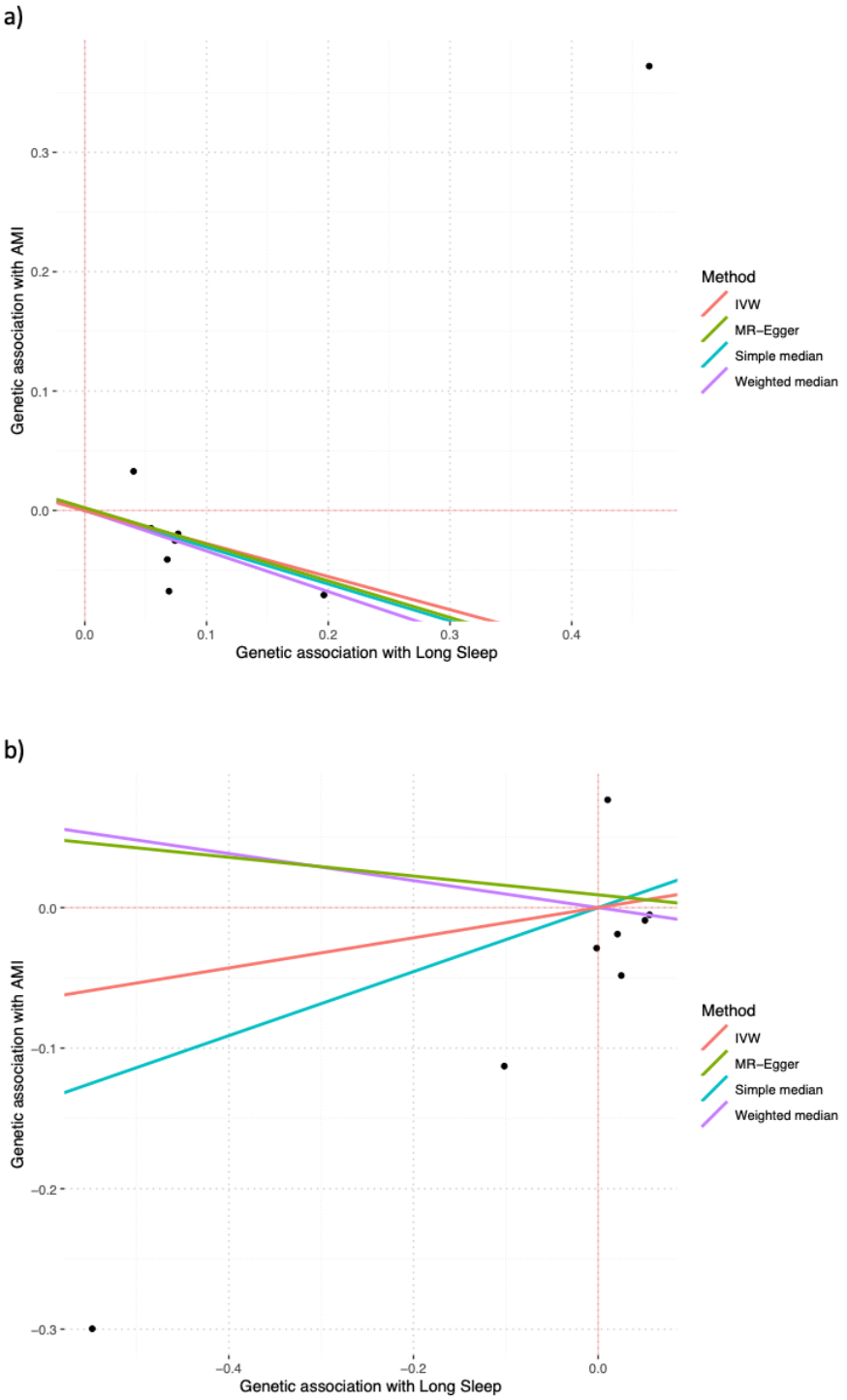


Figure S7: Association of chronotype (morning preference) SNPs from Jones et al., 2019 [15] and acute myocardial infarction (AMI) within UK Biobank. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively.

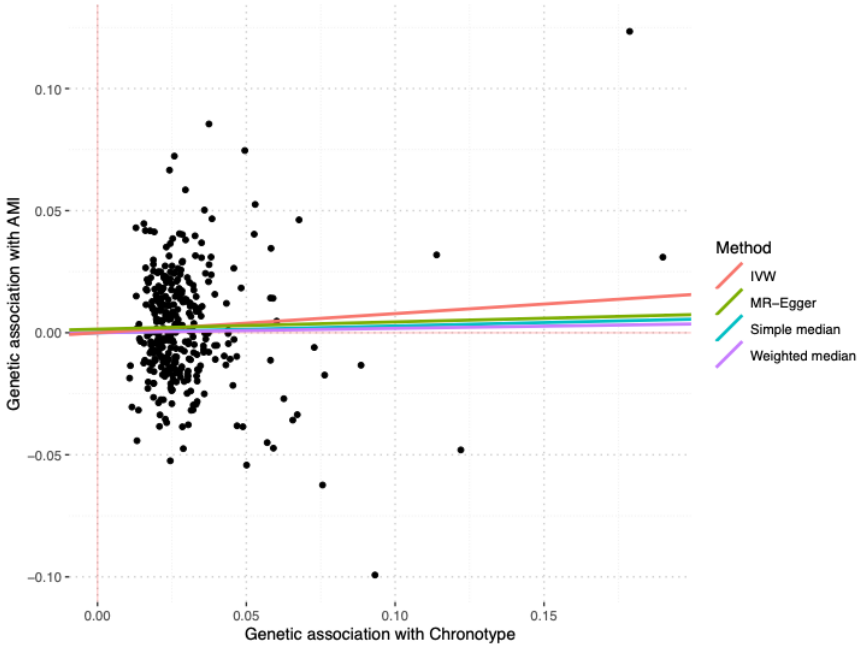


Figure S8: Association of insomnia SNPs from Lane et al., 2019 [16] and acute myocardial infarction (AMI) within a) UK Biobank b) HUNT2. IVW, MR-Egger, simple median and weighted median estimates are indicated by the red, green, blue and purple lines respectively.

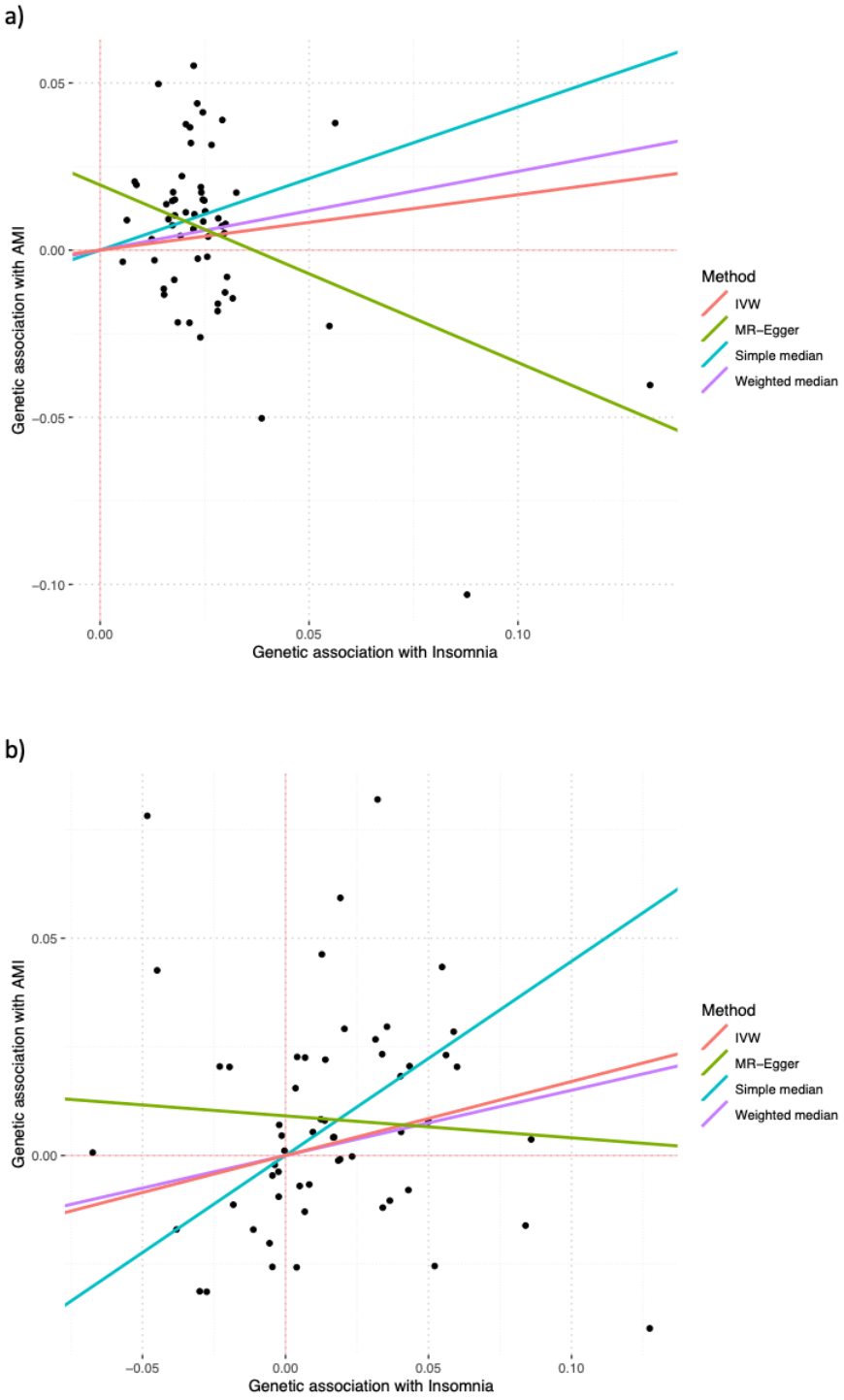
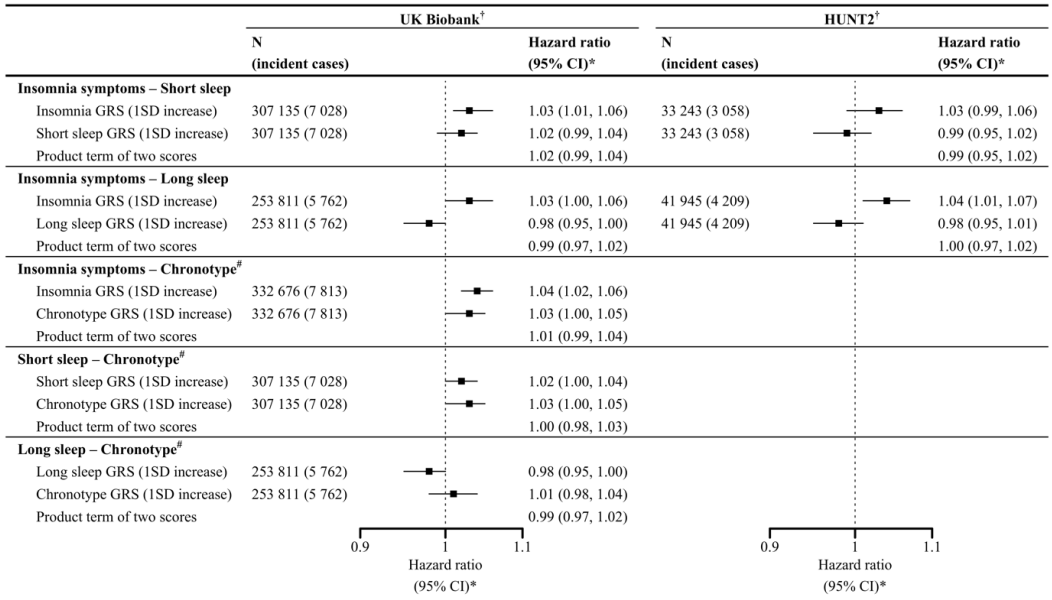


Figure S9: Continuous factorial Mendelian randomization analysis using genetic risk score as quantitative traits with their product term assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in UK Biobank and HUNT2.



CI indicates confidence interval; GRS, genetic risk score; and SD, standard deviation

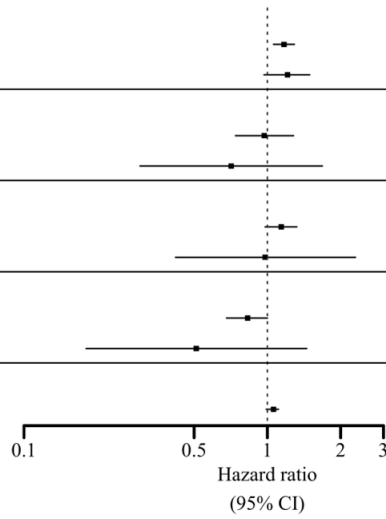
† Derived using unweighted genetic risk score for each sleep trait in UK Biobank, whereas using weighted genetic risk score for each sleep trait in HUNT2.

* Adjusted for age, gender, assessment center (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2), and genotyping chip.

Chronotype genetic risk score calculated using alleles for morning preference.

Figure S10: One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with sleep traits in UK Biobank and HUNT2 after excluding participants who reported self-reported use of sleep medication.

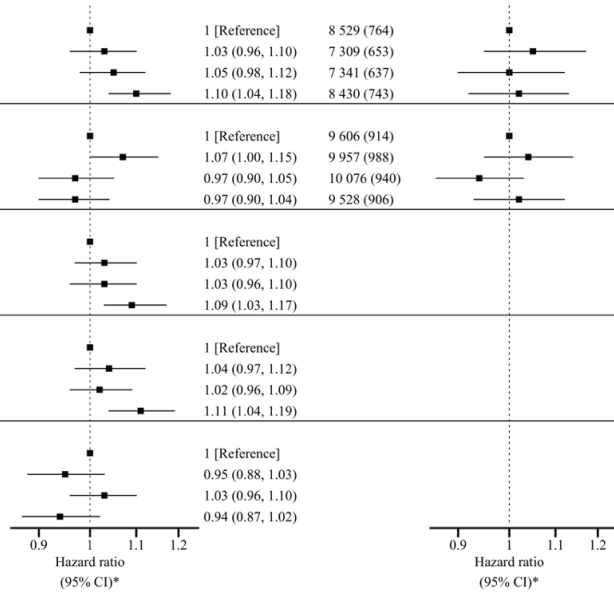
Sleep traits	N (incident cases)	Hazard ratio (95% CI)
Insomnia symptoms (Yes vs. No)[#]		
UK Biobank[†]	329 434 (7 706)	1.17 (1.06, 1.29)
HUNT2[‡]	41 785 (3 999)	1.21 (0.97, 1.49)
24-hour sleep duration (h)		
UK Biobank[†]	329 434 (7 706)	0.97 (0.74, 1.28)
HUNT2[‡]	41 785 (3 999)	0.71 (0.30, 1.68)
Short sleep (≤6 h vs. 7-8 h)[#]		
UK Biobank[†]	304 345 (6 933)	1.14 (0.98, 1.32)
HUNT2[‡]	31 609 (2 797)	0.98 (0.42, 2.30)
Long sleep (≥9 h vs. 7-8 h)[#]		
UK Biobank[†]	251 860 (5 707)	0.83 (0.68, 1.00)
HUNT2[‡]	39 167 (3 748)	0.51 (0.18, 1.45)
Chronotype (morning preference)[#]		
UK Biobank[†]	329 434 (7 706)	1.06 (0.99, 1.11)



CI indicates confidence interval.
No bootstrapping method applied for the confidence interval.
[†] Derived using unweighted genetic risk score for each sleep trait, with adjustment for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
[‡] Derived using weighted genetic risk score for each sleep trait, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.
[#] Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait. Chronotype was missing in HUNT2.

Figure S11: 2x2 factorial Mendelian randomization Cox regression analysis assessing the joint effects of two sleep traits with risk of incident acute myocardial infarction in UK Biobank and HUNT2 after excluding participants who reported self-reported use of sleep medication.

	UK Biobank†		HUNT2†	
	N (incident cases)	**Hazard ratio (95% CI)***	**N (incident cases)**	**Hazard ratio (95% CI)***
Insomnia symptoms – Short sleep				
Both GRS ≤ median	81 251 (1 769)	1 [Reference]	8 529 (764)	1 [Reference]
Insomnia GRS > median	70 993 (1 577)	1.03 (0.96, 1.10)	7 309 (653)	1.05 (0.95, 1.17)
Short sleep GRS > median	71 040 (1 637)	1.05 (0.98, 1.12)	7 341 (637)	1.00 (0.90, 1.12)
Both GRS > median	81 061 (1 950)	1.10 (1.04, 1.18)	8 430 (743)	1.02 (0.92, 1.13)
Insomnia symptoms – Long sleep				
Both GRS ≤ median	72 818 (1 644)	1 [Reference]	9 606 (914)	1 [Reference]
Insomnia GRS > median	74 393 (1 788)	1.07 (1.00, 1.15)	9 957 (988)	1.04 (0.95, 1.14)
Long sleep GRS > median	53 186 (1 154)	0.97 (0.90, 1.05)	10 076 (940)	0.94 (0.86, 1.03)
Both GRS > median	51 463 (1 121)	0.97 (0.90, 1.04)	9 528 (906)	1.02 (0.93, 1.12)
Insomnia symptoms – Chronotype#				
Both GRS ≤ median	79 595 (1 803)	1 [Reference]		
Insomnia GRS > median	85 133 (1 963)	1.03 (0.97, 1.10)		
Chronotype GRS > median	85 269 (1 978)	1.03 (0.96, 1.10)		
Both GRS > median	79 437 (1 962)	1.09 (1.03, 1.17)		
Short sleep – Chronotype#				
Both GRS ≤ median	76 862 (1 667)	1 [Reference]		
Short sleep GRS > median	75 326 (1 717)	1.04 (0.97, 1.12)		
Chronotype GRS > median	75 382 (1 679)	1.02 (0.96, 1.09)		
Both GRS > median	76 775 (1 870)	1.11 (1.04, 1.19)		
Long sleep – Chronotype#				
Both GRS ≤ median	71 549 (1 644)	1 [Reference]		
Long sleep GRS > median	54 380 (1 187)	0.95 (0.88, 1.03)		
Chronotype GRS > median	75 662 (1 788)	1.03 (0.96, 1.10)		
Both GRS > median	50 269 (1 088)	0.94 (0.87, 1.02)		



CI indicates confidence interval; and GRS, genetic risk score.

For each sleep trait combination, both GRS ≤ median represents low genetic risk for both sleep traits in combination, sleep trait 1 GRS > median represents high genetic risk for sleep trait 1 only, sleep trait 2 GRS > median represents high genetic risk for sleep trait 2 only and both GRS > median represents high genetic risk for both sleep traits.

† Derived using unweighted genetic risk score for each sleep trait in UK Biobank, whereas using weighted genetic risk score for each sleep trait in HUNT2.

* Adjusted for age, gender, assessment center (in UK Biobank), genetic principal components (40 in UK Biobank and 20 in HUNT2), and genotyping chip.

Chronotype genetic risk score calculated using alleles for morning preference.

Supplementary tables

Table S1: Detailed summary of Mendelian randomization (MR) studies previously conducted on sleep traits and risk of coronary artery disease (CAD) or acute myocardial infarction (AMI).

Outcomes	Study	Design	Exposures	Results	Summary
Coronary artery disease (CAD)*	Daghlas et al. [8]	**Two-sample MR:** Sleep duration SNPs from GWAS by Dashti et al. [14], and CAD summary GWAS data from the CARDIoGRAMplusC4D Consortium (60 801 cases and 123 504 controls). **One-sample MR:** Unweighted genetic instruments derived from sleep duration SNPs from Dashti et al. [14], and CAD from UK Biobank (17 157 cases and 320 375 controls)	Sleep duration and short sleep. (Did not test long sleep in MR given the limited number of SNPs).	**Two-sample MR** Sleep duration (per additional hour of sleep) IVW OR 0.79 (95% CI 0.68, 0.92); and Short sleep IVW OR 1.24 (95% CI 1.11, 1.38) **One-sample MR** Sleep duration (per additional hour of sleep) OR 0.81 (95% CI 0.68, 0.97); and Short sleep OR 1.14 (95% CI 1.03, 1.26)	Genetically predicted per additional hour of sleep was associated with significantly lower odds of CAD. Short sleep a causal risk factor for CAD.
	Ai et al. [17]	**One-sample MR:** Unweighted genetic risk scores derived from sleep duration SNPs from Dashti et al. [14], and CAD from UK Biobank (17 655 cases/404 044 UK Biobank samples)	Sleep duration, short sleep and long sleep.	Sleep duration (per additional hour of sleep) TSPS OR 0.80 (95% CI 0.66, 0.97); Short sleep (15 594 cases) TSPS OR 1.24 (95% CI 1.12, 1.37); and Long sleep (12 788 cases) TSPS OR 0.88 (95% CI 0.69, 1.34)	Genetically predicted per additional hour of sleep was associated with a lower odds of CAD. Short sleep was associated with significantly higher odds of CAD.
	Larsson et al. [18]	**Two-sample MR:** Insomnia SNPs from GWAS by Jansen et al. [13], and CAD summary GWAS data from the CARDIoGRAMplusC4D Consortium (n = 184 305 individuals of primarily (77%) European ancestry)) [19].	Insomnia	IVW OR 1.12 (95% CI 1.08, 1.17)	Genetically predicted insomnia was associated with significantly higher odds of CAD.
	Liu et al. [20]	**Two-sample MR:** Insomnia SNPs from GWAS by Jansen et al. [13], and CAD summary data derived based in imputed genotype data from the UK Biobank (32 463 cases/278 757 UK Biobank samples). Replication using CAD summary GWAS data from the CARDIoGRAMplusC4D Consortium [19].	Insomnia	IVW OR 1.22 (95% CI 1.17, 1.27); and Replication IVW OR 1.13 (95% CI 1.08, 1.18)	Genetically predicted insomnia was significantly positively associated with CAD.

	Yuan et al. [21]	**Two-sample MR:** Insomnia SNPs from GWAS by Jansen et al. [13], and CAD summary data derived based in imputed genotype data from the UK Biobank (29 278 cases and 338 308 controls).	Insomnia	IVW OR 1.19 (95% CI 1.14, 1.25)	Genetic liability to insomnia was associated with higher odds of CAD.
	Lane et al. [16]	**Two-sample MR:** Insomnia SNPs from GWAS by Lane et al. [16], and CAD summary GWAS data from the CARDIoGRAMplusC4D Consortium [19]. **One-sample MR:** Insomnia SNPs from GWAS by Lane et al. [16], and CAD summary data derived based in imputed genotype data from the UK Biobank (23 980 cases and 361 706 controls).	Insomnia (any insomnia symptoms i.e., "sometimes"/"usually" as cases versus "never/rarely" as controls)	**Two-sample MR** IVW OR 2.15 (95% CI 1.38, 3.35) **One-sample MR** OR 2.95 (95% CI 2.18, 3.99)	Genetically predicted insomnia was significantly positively associated with CAD.
Acute myocardial infarction (AMI)	Daghlas et al. [8]	**Two-sample MR:** Sleep duration SNPs from GWAS by Dashti et al. [14], and AMI summary GWAS data from the CARDIoGRAMplusC4D Consortium with no participant overlap with UK Biobank (43 878 cases and 128 199 controls). **One-sample MR:** Weighted genetic instruments derived from sleep duration SNPs from Dashti et al. [14], and AMI from UK Biobank (12 111 cases and 325 421 controls)	Sleep duration and short sleep. (Did not test long sleep in MR given the limited number of SNPs).	**Two-sample MR** Sleep duration (per additional hour of sleep) IVW OR 0.80 (95% CI 0.67, 0.95) Short sleep IVW OR 1.19 (95% CI 1.09, 1.29) **One-sample MR** Sleep duration (per additional hour of sleep) OR 0.86 (95% CI 0.70, 1.06) Short sleep OR 1.21 (95% CI 1.08, 1.37)	Genetically predicted per additional hour of sleep was associated with lower odds of AMI. Short sleep a causal risk factor for AMI.
	Ai et al. [17]	**One-sample MR:** Unweighted genetic risk scores derived from sleep duration SNPs from Dashti et al.[14], and AMI from UK Biobank (16 845 cases/404 044 UK Biobank samples)	Sleep duration, short sleep and long sleep.	Sleep duration (per additional hour of sleep) TSPS OR 0.90 (95% CI 0.74, 1.09); Short sleep (14 871 cases) TSPS OR 1.21 (95% CI 1.09, 1.34); and Long sleep (12 206 cases) TSPS OR 0.94 (0.73, 1.22)	A weak evidence of protective effect of sleep duration on AMI. Short sleep was associated with significantly higher odds of AMI.
	Yang et al. [22]	**Two-sample MR:** Insomnia SNPs from Lane et al. [16], sleep duration SNPs from Dashti et al. [14], chronotype SNPs (111 SNPs only significant in UK Biobank) from Jones et al. [15]; and GWAS data for AMI	Insomnia, sleep duration, short sleep, long sleep and chronotype	Insomnia IVW OR 1.0049 (95% CI 1.0019, 1.0079); Sleep duration (per additional hour of sleep) IVW OR 0.9999 (95% CI 0.9998, 1.0000);	Genetically predicted insomnia was significantly positively associated with AMI.

		released by UK Biobank (7 018 cases and 354 176 controls).		Short sleep IVW OR 1.0040 (95% CI 0.9989, 1.0091); Long sleep IVW OR 0.9971 (95% CI 0.9910, 1.0031); and Chronotype IVW OR 0.9992 (95% CI 0.9957, 1.0026).	A suggestive evidence that genetically predicted per additional hour of sleep was negatively associated with AMI.

SNPs indicates single nucleotide polymorphisms; TSPS, two-stage predictor substitution; IVW, inverse variance weighted; OR, odds ratio; CI, confidence interval; GWAS, genome-wide association study; CAD, coronary artery disease; and AMI, acute myocardial infarction.

* CAD diagnosis was broadly defined as myocardial infarction, acute coronary syndrome, chronic stable angina or coronary stenosis of more than 50% [19].

Table S2: Summary of genetic instruments showing their strength applying to UK Biobank and HUNT2.

UK Biobank

Sleep traits	N	No. of SNPs to generate the uwGRS	Mean (SD) no. of increasing allele	Association of uwGRS with sleep trait †		
				Coefficient (SE)	R^2 §	F-statistics §§
Insomnia symptoms	332 676	248	245.17 (10.27)	0.1560 (0.0039)	0.41%	1370.92
24-hour sleep duration (h)	332 676	78	76.26 (5.43)	0.0825 (0.0019)	0.59%	1962.0
Short sleep (≤6 h vs. 7-8 h)	307 135	27	26.34 (3.15)	0.1044 (0.0041)	0.18%	558.68
Long sleep (≥9 h vs. 7-8 h)	253 811	8	4.11 (1.42)	0.0900 (0.0066)	0.11%	285.42
Chronotype (morning preference)	332 676	341 #	334.12 (11.64)	0.2998 (0.0037)	1.54%	5202.20

HUNT2

Sleep traits	N	No. of SNPs to generate the wGRS	Mean (SD) no. of increasing allele	Association of wGRS with sleep trait ‡		
				Coefficient (SE)	R^2 §	F-statistics §§
Insomnia symptoms	44 728	244 #	240.41 (10.16)	0.1036 (0.0137)	0.16%	71.17
24-hour sleep duration (h)	44 728	78	77.25 (5.35)	0.0361 (0.0058)	0.09%	38.94
Short sleep (≤6 h vs. 7-8 h)	33 243	27	26.12 (3.13)	0.0335 (0.0198)	0.01%	4.97
Long sleep (≥9 h vs. 7-8 h)	41 945	8	4.18 (1.40)	0.0239 (0.0109)	0.01%	4.07

SNPs indicates single nucleotide polymorphisms; SD, standard deviation; uwGRS, unweighted genetic risk score; wGRS, weighted genetic risk score; and SE, standard error

† Adjusted for age, gender, assessment center, 40 principal components, and genotyping chip.

‡ Adjusted for age, gender, 20 principal components, and genotyping chip.

§ McFadden R^2 statistics for sleep traits – insomnia symptoms, short sleep, long sleep and chronotype.

§§ F-statistics was calculated using $F = (R^2/K) / ((1 – R^2) / (N-K-1))$; where R^2 = McFadden R^2 statistics, K = 1, and N = sample size.

rs146820337, rs112201801, rs10610420, rs9991917, rs67169439, rs34125199, rs60521023, rs3747463, rs213462, and rs7060620 were absent in the imputed UK Biobank genetic data; and rs1264419, rs138678612, rs238869 and rs3131638 were absent in the imputed HUNT genetic data.

Table S3: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and short sleep in UK Biobank.

	UK Biobank (N = 307 135)			
	Both GRS ≤ median	**Insomnia GRS > median**	**Short sleep GRS > median**	**Both GRS > median**
Total, % (n)	26.66 (81 895)	23.34 (71 674)	23.34 (71 673)	26.66 (81 893)
Variables, % (n)				
Male	44.78 (36 673)	44.51 (31 900)	44.91 (32 187)	45.04 (36 885)
Missing, % (n)	-	-	-	-
Married	74.53 (61 035)	74.04 (53 069)	74.36 (53 294)	73.92 (60 538)
Missing, % (n)	0.45 (368)	0.50 (361)	0.45 (322)	0.50 (410)
Weekly alcohol intake	51.43 (42 121)	50.70 (36 341)	51.25 (36 735)	50.53 (41 379)
Missing, % (n)	0.04 (35)	0.06 (42)	0.04 (26)	0.05 (43)
Current smokers	9.46 (7 751)	10.40 (7 456)	9.59 (6 876)	10.67 (8 737)
Missing, % (n)	0.28 (232)	0.28 (200)	0.31 (219)	0.30 (248)
Highly physically active	33.98 (27 824)	33.39 (23 934)	33.68 (24 140)	33.41 (27 363)
Missing, % (n)	17.20 (14 085)	17.75 (12 723)	17.21 (12 336)	18.09 (14 814)
Tertiary education	45.19 (37 005)	43.28 (31 020)	43.84 (31 420)	42.34 (34 671)
Missing, % (n)	0.69 (568)	0.75 (541)	0.71 (508)	0.77 (631)
Shift workers	5.15 (4 214)	5.35 (3 831)	5.26 (3 770)	5.42 (4 442)
Missing, % (n)	0.25 (208)	0.24 (171)	0.28 (204)	0.29 (236)
Employed	59.37 (48 625)	59.00 (42 286)	58.71 (42 082)	58.65 (48 034)
Missing, % (n)	0.22 (184)	0.21 (153)	0.24 (175)	0.24 (200)
Use of sleep medication(s)	0.79 (644)	0.95 (681)	0.88 (633)	1.02 (832)
Missing, % (n)	-	-	-	-
Suffering from depression	10.56 (8 648)	12.14 (8 704)	10.82 (7 756)	12.34 (10 107)
Missing, % (n)	-	-	-	-
Suffering from anxiety	6.12 (5 016)	6.79 (4 866)	6.23 (4 463)	6.80 (5 572)
Missing, % (n)	-	-	-	-
Suffering from chronic illness	28.48 (23 325)	31.18 (22 346)	29.06 (20 831)	31.98 (26 189)
Missing, % (n)	1.92 (1 571)	2.13 (1 528)	1.95 (1 399)	2.15 (1 759)
Variables, mean (SD)				
Age, *years*	56.75 (7.92)	56.68 (7.94)	56.83 (7.90)	56.69 (7.95)
Missing, % (n)	-	-	-	-
TDI	-1.67 (2.87)	-1.57 (2.92)	-1.65 (2.89)	-1.54 (2.94)
Missing, % (n)	0.11 (92)	0.10 (74)	0.13 (93)	0.13 (104)
BMI, kg/m^2	27.16 (4.63)	27.43 (4.77)	27.24 (4.67)	27.53 (4.81)
Missing, % (n)	0.27 (220)	0.31 (219)	0.31 (220)	0.30 (245)
SBP, *mmHg*	138.10 (18.65)	138.30 (18.61)	138.20 (18.55)	138.40 (18.57)
Missing, % (n)	0.08 (69)	0.10 (72)	0.08 (55)	0.08 (65)
Serum cholesterol, *mmol/L*	5.76 (1.13)	5.74 (1.13)	5.75 (1.13)	5.72 (1.13)
Missing, % (n)	4.54 (3 722)	4.60 (3 299)	4.58 (3 282)	4.44 (3 635)
Blood glucose, *mmol/L*	5.09 (1.12)	5.11 (1.21)	5.10 (1.15)	5.11 (1.19)
Missing, % (n)	12.70 (10 397)	12.76 (9 144)	12.73 (9 124)	12.70 (10 400)

GRS indicates genetic risk score; SD, standard deviation; TDI, Townsend deprivation index; BMI, body mass index; and SBP, systolic blood pressure.

Table S4: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and short sleep in HUNT2.

	HUNT2 (N = 33 243)			
	Both GRS ≤ median	**Insomnia GRS > median**	**Short sleep GRS > median**	**Both GRS > median**
Total, % (n)	26.85 (8 925)	23.15 (7 697)	23.15 (7 697)	26.84 (8 924)
Variables, % (n)				
Male	48.07 (4 290)	47.46 (3 653)	47.65 (3 668)	46.62 (4 160)
Missing, % (n)	-	-	-	-
Married	64.17 (5 727)	64.09 (4 933)	62.69 (4 825)	62.82 (5 606)
Missing, % (n)	-	-	-	-
Weekly alcohol intake	23.70 (2 115)	23.97 (1 845)	23.48 (1 807)	22.98 (2 051)
Missing, % (n)	6.89 (615)	7.03 (541)	7.03 (541)	7.33 (654)
Current smokers	27.59 (2 462)	29.96 (2 306)	27.22 (2 095)	29.71 (2 651)
Missing, % (n)	1.30 (116)	1.51 (116)	1.33 (102)	1.42 (127)
Highly physically active	35.87 (3 201)	34.42 (2 649)	35.07 (2 699)	33.54 (2 993)
Missing, % (n)	6.42 (573)	6.31 (486)	6.66 (513)	7.26 (648)
Tertiary education	23.10 (2 062)	22.96 (1 767)	22.35 (1 720)	21.91 (1 955)
Missing, % (n)	2.78 (248)	2.64 (203)	2.96 (228)	3.08 (275)
Shift workers	16.01 (1 429)	17.02 (1 310)	16.72 (1 287)	16.62 (1 483)
Missing, % (n)	7.70 (687)	7.73 (595)	7.96 (613)	7.66 (684)
Employed	74.26 (6 628)	74.54 (5 737)	73.43 (5 652)	72.93 (6 508)
Missing, % (n)	0.86 (77)	0.95 (73)	0.77 (59)	0.92 (82)
Use of sleep medication(s)	4.44 (396)	5.04 (388)	4.63 (356)	5.54 (494)
Missing, % (n)	9.30 (830)	9.37 (721)	9.80 (754)	9.04 (807)
Suffering from chronic illness	27.51 (2 455)	28.18 (2 169)	26.21 (2 017)	29.66 (2 657)
Missing, % (n)	2.80 (250)	2.86 (220)	2.91 (224)	3.09 (276)
Variables, mean (SD)				
Age, *years*	47.49 (14.94)	46.97 (14.81)	47.04 (15.06)	47.26 (15.04)
Missing, % (n)	-	-	-	-
BMI, kg/m^2	26.12 (3.92)	26.23 (3.91)	26.14 (3.95)	26.31 (4.08)
Missing, % (n)	0.19 (17)	0.30 (23)	0.29 (22)	0.28 (25)
SBP, *mmHg*	135.40 (20.17)	135.00 (19.94)	135.50 (19.88)	135.20 (20.08)
Missing, % (n)	0.06 (5)	0.09 (7)	0.16 (12)	0.12 (11)
Serum cholesterol, *mmol/L*	5.82 (1.21)	5.81 (1.24)	5.80 (1.21)	5.79 (1.22)
Missing, % (n)	0.04 (4)	0.16 (12)	0.16 (12)	0.10 (9)
Blood glucose, *mmol/L*	5.33 (1.24)	5.36 (1.28)	5.34 (1.31)	5.37 (1.32)
Missing, % (n)	0.08 (7)	0.18 (14)	0.17 (13)	0.16 (14)
HADS - D scores	3.25 (2.87)	3.27 (2.94)	3.19 (2.88)	3.28 (2.94)
Missing, % (n)	6.04 (539)	5.43 (418)	6.09 (469)	6.36 (568)
HADS - A scores	4.12 (3.19)	4.20 (3.27)	4.03 (3.11)	4.21 (3.26)
Missing, % (n)	12.09 (1 079)	12.08 (930)	12.08 (930)	12.72 (1 135)

GRS indicates genetic risk score; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; HADS – D scores, Hospital Anxiety and Depression Scale – Depression scores; and HADS – A scores, Hospital Anxiety and Depression Scale – Anxiety scores.

Table S5: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and long sleep in UK Biobank.

	UK Biobank (N = 253 811)			
	Both GRS ≤ median	**Insomnia GRS > median**	**Long sleep GRS > median**	**Both GRS > median**
Total, % (n)	28.88 (73 306)	29.55 (75 001)	21.12 (53 600)	20.45 (51 904)
Variables, % (n)				
Male	44.60 (32 692)	44.62 (33 468)	43.84 (23 497)	44.37 (23 030)
Missing, % (n)	-	-	-	-
Married	76.11 (55 790)	75.70 (56 777)	75.70 (40 573)	75.57 (39 226)
Missing, % (n)	0.42 (305)	0.43 (321)	0.39 (208)	0.47 (245)
Weekly alcohol intake	51.53 (37 772)	50.84 (38 131)	51.26 (27 477)	50.80 (26 367)
Missing, % (n)	0.03 (24)	0.04 (30)	0.04 (23)	0.06 (32)
Current smokers	8.89 (6 520)	9.87 (7 399)	9.22 (4 940)	9.96 (5 169)
Missing, % (n)	0.30 (219)	0.27 (206)	0.26 (138)	0.25 (129)
Highly physically active	33.80 (24 774)	33.48 (25 113)	33.59 (18 004)	32.96 (17 105)
Missing, % (n)	16.78 (12 300)	17.37 (13 026)	16.92 (9070)	17.59 (9 130)
Tertiary education	45.22 (33 151)	43.68 (32 759)	44.90 (24 065)	43.43 (22 540)
Missing, % (n)	0.67 (494)	0.75 (559)	0.64 (342)	0.69 (359)
Shift workers	4.52 (3 315)	4.58 (3 432)	4.36 (2 338)	4.53 (2 351)
Missing, % (n)	0.28 (205)	0.26 (193)	0.26 (140)	0.26 (134)
Employed	56.18 (41 184)	56.23 (42 174)	56.08 (30 058)	56.01 (29 070)
Missing, % (n)	0.24 (177)	0.22 (167)	0.22 (120)	0.22 (115)
Use of sleep medication(s)	0.67 (488)	0.81 (608)	0.77 (414)	0.85 (435)
Missing, % (n)	-	-	-	-
Suffering from depression	10.90 (7 993)	12.22 (9 163)	10.64 (5 702)	12.04 (6 248)
Missing, % (n)	-	-	-	-
Suffering from anxiety	6.00 (4 402)	6.60 (4 949)	6.12 (3 282)	6.71 (3 482)
Missing, % (n)	-	-	-	-
Suffering from chronic illness	28.43 (20 843)	31.19 (23 390)	28.29 (15 166)	31.00 (16 092)
Missing, % (n)	1.74 (1 278)	1.92 (1 443)	1.88 (1 007)	2.04 (1 059)
Variables, mean (SD)				
Age, *years*	57.00 (7.97)	56.88 (8.02)	56.96 (8.02)	56.87 (8.05)
Missing, % (n)	-	-	-	-
TDI	-1.74 (2.83)	-1.66 (2.85)	-1.74 (2.84)	-1.66 (2.88)
Missing, % (n)	0.13 (97)	0.10 (78)	0.10 (56)	0.13 (69)
BMI, kg/m^2	27.17 (4.62)	27.44 (4.76)	27.01 (4.50)	27.27 (4.65)
Missing, % (n)	0.28 (207)	0.31 (232)	0.31 (168)	0.26 (137)
SBP, *mmHg*	138.30 (18.69)	138.50 (18.73)	138.20 (18.78)	138.50 (18.73)
Missing, % (n)	0.07 (50)	0.09 (69)	0.09 (49)	0.09 (47)
Serum cholesterol, *mmol/L*	5.75 (1.14)	5.72 (1.14)	5.76 (1.14)	5.73 (1.14)
Missing, % (n)	4.54 (3 330)	4.53 (3 394)	4.67 (2 504)	4.60 (2 387)
Blood glucose, *mmol/L*	5.10 (1.17)	5.12 (1.20)	5.09 (1.13)	5.11 (1.18)
Missing, % (n)	12.61 (9 245)	12.64 (9 483)	12.96 (6 947)	12.89 (6 692)

GRS indicates genetic risk score; SD, standard deviation; TDI, Townsend deprivation index; BMI, body mass index; and SBP, systolic blood pressure.

Table S6: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and long sleep in HUNT2.

	HUNT2 (N = 41 945)			
	Both GRS ≤ median	**Insomnia GRS > median**	**Long sleep GRS > median**	**Both GRS > median**
Total, % (n)	24.42 (10 242)	25.58 (10 731)	25.58 (10 731)	24.42 (10 241)
Variables, % (n)				
Male	45.19 (4 628)	45.01 (4 830)	45.34 (4 865)	44.26 (4 533)
Missing, % (n)	-	-	-	-
Married	62.93 (6 445)	63.42 (6 806)	63.03 (6 764)	61.83 (6 332)
Missing, % (n)	-	-	-	-
Weekly alcohol intake	22.13 (2 267)	21.98 (2 359)	22.24 (2 387)	22.46 (2 300)
Missing, % (n)	7.21 (738)	7.48 (803)	7.64 (820)	7.79 (798)
Current smokers	26.29 (2 693)	27.90 (2 994)	26.14 (2 805)	28.85 (2 955)
Missing, % (n)	1.59 (163)	1.53 (164)	1.61 (173)	1.50 (154)
Highly physically active	33.26 (3 407)	31.55 (3 386)	32.36 (3 473)	31.83 (3 260)
Missing, % (n)	7.90 (809)	8.09 (868)	8.12 (871)	8.25 (845)
Tertiary education	20.92 (2 143)	20.63 (2 214)	21.59 (2 317)	21.18 (2 169)
Missing, % (n)	3.63 (372)	3.41 (366)	3.56 (382)	3.65 (374)
Shift workers	15.26 (1 563)	14.84 (1 593)	14.24 (1 528)	15.39 (1 576)
Missing, % (n)	7.08 (725)	6.98 (749)	7.43 (797)	7.28 (746)
Employed	66.71 (6 832)	65.47 (7 026)	65.35 (7 013)	66.46 (6 806)
Missing, % (n)	0.84 (86)	0.94 (101)	0.90 (97)	0.98 (100)
Use of sleep medication(s)	6.21 (636)	7.21 (774)	6.10 (655)	6.96 (713)
Missing, % (n)	9.78 (1 002)	9.32 (1 000)	9.69 (1 040)	9.26 (948)
Suffering from chronic illness	30.47 (3 121)	33.35 (3 579)	31.91 (3 424)	32.55 (3 333)
Missing, % (n)	3.12 (320)	3.08 (330)	3.04 (326)	3.22 (330)
Variables, mean (SD)				
Age, *years*	49.17 (16.37)	49.26 (16.27)	49.57 (16.43)	48.84 (16.33)
Missing, % (n)	-	-	-	-
BMI, kg/m^2	26.27 (4.06)	26.39 (4.10)	26.18 (3.98)	26.32 (4.08)
Missing, % (n)	0.51 (52)	0.56 (60)	0.51 (55)	0.47 (48)
SBP, *mmHg*	137.00 (21.24)	136.70 (21.01)	137.30 (21.27)	136.70 (21.00)
Missing, % (n)	0.09 (9)	0.12 (13)	0.12 (13)	0.12 (12)
Serum cholesterol, *mmol/L*	5.88 (1.25)	5.88 (1.26)	5.90 (1.25)	5.86 (1.25)
Missing, % (n)	0.12 (12)	0.15 (16)	0.09 (10)	0.08 (8)
Blood glucose, *mmol/L*	5.41 (1.46)	5.44 (1.54)	5.42 (1.41)	5.41 (1.37)
Missing, % (n)	0.17 (17)	0.19 (20)	0.13 (14)	0.13 (13)
HADS - D scores	3.31 (2.97)	3.40 (3.01)	3.34 (2.96)	3.35 (2.96)
Missing, % (n)	6.88 (705)	6.64 (713)	7.09 (761)	6.76 (692)
HADS - A scores	4.09 (3.18)	4.18 (3.29)	4.06 (3.18)	4.18 (3.26)
Missing, % (n)	13.70 (1 403)	14.16 (1 519)	13.91 (1 493)	13.81 (1 414)

GRS indicates genetic risk score; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; HADS – D scores, Hospital Anxiety and Depression Scale – Depression scores; and HADS – A scores, Hospital Anxiety and Depression Scale – Anxiety scores.

Table S7: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for insomnia symptoms and chronotype (morning preference) in UK Biobank.

	UK Biobank (N = 332 676)			
	Both GRS ≤ median	**Insomnia GRS > median**	**Chronotype GRS > median**	**Both GRS > median**
Total, % (n)	24.14 (80 295)	25.86 (86 043)	25.86 (86 043)	24.14 (80 295)
Variables, % (n)				
Male	44.66 (35 857)	44.39 (38 195)	44.68 (38 440)	44.87 (36 025)
Missing, % (n)	-	-	-	-
Married	74.06 (59 465)	73.55 (63 288)	74.70 (64 270)	74.29 (59 650)
Missing, % (n)	0.47 (375)	0.53 (459)	0.48 (409)	0.49 (391)
Weekly alcohol intake	50.78 (40 777)	49.92 (42 951)	50.91 (43 804)	50.30 (40 389)
Missing, % (n)	0.04 (34)	0.07 (60)	0.05 (42)	0.04 (31)
Current smokers	9.74 (7 821)	10.88 (9 363)	9.55 (8 219)	10.47 (8 409)
Missing, % (n)	0.29 (236)	0.29 (251)	0.30 (260)	0.30 (238)
Highly physically active	33.14 (26 611)	32.67 (28 110)	33.98 (29 240)	33.64 (27 010)
Missing, % (n)	17.69 (14 206)	18.17 (15 633)	17.06 (14 676)	17.99 (14 442)
Tertiary education	44.21 (35 499)	42.20 (36 309)	43.77 (37 660)	42.26 (33 936)
Missing, % (n)	0.69 (553)	0.74 (639)	0.76 (650)	0.83 (666)
Shift workers	5.13 (4 119)	5.36 (4 611)	5.05 (4 343)	5.15 (4 138)
Missing, % (n)	0.28 (228)	0.26 (227)	0.27 (232)	0.27 (218)
Employed	57.10 (45 846)	56.95 (49 003)	56.96 (49 013)	56.80 (45 606)
Missing, % (n)	0.24 (194)	0.22 (192)	0.24 (207)	0.24 (194)
Use of sleep medication(s)	0.87 (700)	1.06 (910)	0.90 (774)	1.07 (858)
Missing, % (n)	-	-	-	-
Suffering from depression	11.38 (9 134)	12.99 (11 180)	11.34 (9 756)	12.83 (10 300)
Missing, % (n)	-	-	-	-
Suffering from anxiety	6.40 (5 138)	7.02 (6 039)	6.41 (5 516)	7.02 (5 640)
Missing, % (n)	-	-	-	-
Suffering from chronic illness	30.00 (24 086)	32.94 (28 344)	29.53 (25 406)	32.42 (26 035)
Missing, % (n)	1.98 (1 591)	2.17 (1 865)	1.90 (1 638)	2.09 (1 678)
Variables, mean (SD)				
Age, *years*	56.93 (7.94)	56.82 (7.97)	56.98 (7.91)	56.87 (7.94)
Missing, % (n)	-	-	-	-
TDI	-1.60 (2.92)	-1.51 (2.96)	-1.68 (2.87)	-1.56 (2.92)
Missing, % (n)	0.10 (82)	0.13 (108)	0.13 (114)	0.11 (91)
BMI, kg/m^2	27.23 (4.66)	27.54 (4.83)	27.27 (4.70)	27.55 (4.83)
Missing, % (n)	0.31 (246)	0.30 (262)	0.30 (257)	0.32 (260)
SBP, *mmHg*	138.30 (18.72)	138.50 (18.73)	138.30 (18.57)	138.40 (18.53)
Missing, % (n)	0.08 (68)	0.10 (85)	0.09 (76)	0.09 (73)
Serum cholesterol, *mmol/L*	5.75 (1.14)	5.73 (1.14)	5.75 (1.13)	5.73 (1.13)
Missing, % (n)	4.55 (3 651)	4.48 (3 853)	4.61 (3 966)	4.62 (3 710)
Blood glucose, *mmol/L*	5.11 (1.19)	5.13 (1.22)	5.10 (1.13)	5.12 (1.23)
Missing, % (n)	12.75 (10 236)	12.58 (10 823)	12.71 (10 934)	12.87 (10 337)

GRS indicates genetic risk score; SD, standard deviation; TDI, Townsend deprivation index; BMI, body mass index; and SBP, systolic blood pressure.

Table S8: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for short sleep and chronotype (morning preference) in UK Biobank.

	UK Biobank (N = 307 135)			
	Both GRS ≤ median	**Short sleep GRS > median**	**Chronotype GRS > median**	**Both GRS > median**
Total, % (n)	25.24 (77 511)	24.76 (76 057)	24.76 (76 058)	25.24 (77 509)
Variables, % (n)				
Male	44.52 (34 506)	44.87 (34 128)	44.79 (34 067)	45.08 (34 944)
Missing, % (n)	-	-	-	-
Married	74.07 (57 414)	73.70 (56 055)	74.54 (56 690)	74.54 (57 777)
Missing, % (n)	0.49 (376)	0.48 (368)	0.46 (353)	0.47 (364)
Weekly alcohol intake	50.96 (39 500)	50.77 (38 611)	51.23 (38 962)	50.97 (39 503)
Missing, % (n)	0.06 (48)	0.05 (36)	0.04 (29)	0.04 (33)
Current smokers	10.02 (7 766)	10.37 (7 888)	9.78 (7 441)	9.97 (7 725)
Missing, % (n)	0.28 (216)	0.31 (235)	0.28 (216)	0.30 (232)
Highly physically active	33.25 (25 770)	33.14 (25 202)	34.17 (25 988)	33.93 (26 301)
Missing, % (n)	17.70 (13 718)	17.87 (13 592)	17.21 (13 090)	17.49 (13 558)
Tertiary education	44.24 (34 289)	43.23 (32 883)	44.36 (33 736)	42.84 (33 208)
Missing, % (n)	0.68 (527)	0.71 (543)	0.77 (582)	0.77 (596)
Shift workers	5.28 (4 096)	5.42 (4 123)	5.19 (3 949)	5.28 (4 089)
Missing, % (n)	0.24 (183)	0.30 (226)	0.26 (196)	0.28 (214)
Employed	59.31 (45 968)	58.80 (44 722)	59.09 (44 943)	58.57 (45 394)
Missing, % (n)	0.20 (157)	0.24 (186)	0.24 (180)	0.24 (189)
Use of sleep medication(s)	0.84 (649)	0.96 (731)	0.89 (676)	0.95 (734)
Missing, % (n)	-	-	-	-
Suffering from depression	11.35 (8 797)	11.76 (8 943)	11.25 (8 555)	11.51 (8 920)
Missing, % (n)	-	-	-	-
Suffering from anxiety	6.50 (5 042)	6.47 (4 919)	6.36 (4 840)	6.60 (5 116)
Missing, % (n)	-	-	-	-
Suffering from chronic illness	30.02 (23 266)	30.84 (23 453)	29.46 (22 405)	30.41 (23 567)
Missing, % (n)	2.06 (1 600)	2.09 (1 588)	1.97 (1 499)	2.03 (1 570)
Variables, mean (SD)				
Age, *years*	56.67 (7.96)	56.74 (7.93)	56.76 (7.91)	56.77 (7.92)
Missing, % (n)	-	-	-	-
TDI	-1.59 (2.91)	-1.55 (2.94)	-1.66 (2.88)	-1.62 (2.89)
Missing, % (n)	0.10 (81)	0.12 (95)	0.11 (85)	0.13 (102)
BMI, kg/m^2	27.30 (4.69)	27.38 (4.74)	27.28 (4.70)	27.40 (4.75)
Missing, % (n)	0.30 (232)	0.29 (222)	0.27 (207)	0.31 (243)
SBP, *mmHg*	138.20 (18.73)	138.40 (18.66)	138.20 (18.53)	138.30 (18.47)
Missing, % (n)	0.09 (71)	0.08 (61)	0.09 (70)	0.08 (59)
Serum cholesterol, *mmol/L*	5.75 (1.13)	5.74 (1.14)	5.75 (1.13)	5.74 (1.13)
Missing, % (n)	4.52 (3 500)	4.49 (3 412)	4.63 (3 521)	4.52 (3 505)
Blood glucose, *mmol/L*	5.10 (1.18)	5.11 (1.18)	5.10 (1.14)	5.10 (1.17)
Missing, % (n)	12.73 (9 865)	12.62 (9 600)	12.72 (9 676)	12.80 (9 924)

GRS indicates genetic risk score; SD, standard deviation; TDI, Townsend deprivation index; BMI, body mass index; and SBP, systolic blood pressure.

Table S9: Baseline characteristics of participants across groups categorized by dichotomizing to the median genetic risk scores for long sleep and chronotype (morning preference) in UK Biobank.

	UK Biobank (N = 253 811)			
	Both GRS ≤ median	**Long sleep GRS > median**	**Chronotype GRS > median**	**Both GRS > median**
Total, % (n)	28.40 (72 088)	21.60 (54 818)	30.03 (76 219)	19.97 (50 686)
Variables, % (n)				
Male	44.52 (32 092)	43.95 (24 093)	44.70 (34 068)	44.26 (22 434)
Missing, % (n)	-	-	-	-
Married	75.49 (54 418)	75.46 (41 363)	76.29 (58 149)	75.83 (38 436)
Missing, % (n)	0.42 (305)	0.45 (245)	0.42 (321)	0.41 (208)
Weekly alcohol intake	51.00 (36 762)	50.94 (27 923)	51.35 (39 141)	51.14 (25 921)
Missing, % (n)	0.04 (29)	0.06 (33)	0.03 (25)	0.04 (22)
Current smokers	9.52 (6 862)	9.75 (5 345)	9.26 (7 057)	9.40 (4 764)
Missing, % (n)	0.28 (205)	0.26 (143)	0.29 (220)	0.24 (124)
Highly physically active	33.15 (23 896)	33.01 (18 093)	34.10 (25 991)	33.57 (17 016)
Missing, % (n)	17.30 (12 471)	17.52 (9 605)	16.87 (12 855)	16.96 (8 595)
Tertiary education	44.55 (32 116)	44.14 (24 194)	44.34 (33 794)	44.22 (22 411)
Missing, % (n)	0.70 (504)	0.63 (347)	0.72 (549)	0.70 (354)
Shift workers	4.65 (3 354)	4.50 (2 466)	4.45 (3 393)	4.39 (2 223)
Missing, % (n)	0.28 (199)	0.27 (146)	0.26 (199)	0.25 (128)
Employed	56.31 (40 594)	55.96 (30 674)	56.11 (42 764)	56.14 (28 454)
Missing, % (n)	0.23 (164)	0.23 (125)	0.24 (180)	0.22 (110)
Use of sleep medication(s)	0.75 (539)	0.80 (438)	0.73 (557)	0.82 (417)
Missing, % (n)	-	-	-	-
Suffering from depression	11.67 (8 411)	11.42 (6 262)	11.47 (8 745)	11.22 (5 688)
Missing, % (n)	-	-	-	-
Suffering from anxiety	6.42 (4 629)	6.33 (3 472)	6.20 (4 722)	6.49 (3 292)
Missing, % (n)	-	-	-	-
Suffering from chronic illness	30.21 (21 777)	29.86 (16 369)	29.46 (22 456)	29.37 (14 889)
Missing, % (n)	1.90 (1 369)	2.03 (1 114)	1.77 (1 352)	1.88 (952)
Variables, mean (SD)				
Age, *years*	56.94 (8.00)	56.90 (8.06)	56.94 (8.00)	56.92 (8.00)
Missing, % (n)	-	-	-	-
TDI	-1.67 (2.87)	-1.66 (2.88)	-1.73 (2.82)	-1.74 (2.83)
Missing, % (n)	0.12 (90)	0.10 (54)	0.11 (85)	0.14 (71)
BMI, kg/m^2	27.31 (4.69)	27.13 (4.56)	27.31 (4.70)	27.15 (4.60)
Missing, % (n)	0.28 (203)	0.30 (162)	0.31 (236)	0.28 (143)
SBP, *mmHg*	138.40 (18.84)	138.40 (18.79)	138.30 (18.60)	138.20 (18.73)
Missing, % (n)	0.09 (63)	0.08 (46)	0.07 (56)	0.10 (50)
Serum cholesterol, *mmol/L*	5.74 (1.14)	5.75 (1.14)	5.74 (1.14)	5.74 (1.13)
Missing, % (n)	4.48 (3 228)	4.58 (2 508)	4.59 (3 496)	4.70 (2 383)
Blood glucose, *mmol/L*	5.12 (1.20)	5.12 (1.17)	5.10 (1.17)	5.09 (1.13)
Missing, % (n)	12.51 (9 021)	12.75 (6 990)	12.74 (9 707)	13.12 (6 649)

GRS indicates genetic risk score; SD, standard deviation; TDI, Townsend deprivation index; BMI, body mass index; and SBP, systolic blood pressure.

Table S10: Statistical test of the proportional hazard assumption for one-sample Mendelian randomization (MR) Cox regression models.

	UK Biobank [†]		HUNT2 [‡]	
Sleep trait	**Correlation coefficient [*]**	**P**	**Correlation coefficient [*]**	**P**
Insomnia symptoms	-0.0047	0.676	0.0166	0.269
24-hour sleep duration (h)	0.0067	0.558	0.0165	0.260
Short sleep (≤6 h vs. 7-8 h)	-0.0070	0.560	0.0048	0.788
Long sleep (≥9 h vs. 7-8 h)	-0.0081	0.539	-0.0162	0.293
Chronotype (morning preference)	0.0033	0.772	-	-

[†] Using unweighted genetic risk score for each sleep trait in the MR Cox regression model, with adjustment for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
[‡] Using weighted genetic risk score for each sleep trait in the MR Cox regression model, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.
***** Values represent the Pearson's correlation coefficient between the first scaled Schoenfeld residual in the MR Cox regression and the rank-normalized natural logarithm of follow-up time.

Table S11: Statistical test of the proportional hazard assumption for 2x2 factorial Mendelian randomization (MR) Cox regression models.

	UK Biobank [†]		HUNT2 [‡]	
Sleep trait combination	**Correlation coefficient [*]**	**P**	**Correlation coefficient [*]**	**P**
Insomnia symptoms – Short sleep	-0.0084	0.479	0.0259	0.153
Insomnia symptoms – Long sleep	-0.0144	0.280	-0.0016	0.917
Insomnia symptoms – Chronotype [#]	0.0029	0.795	-	-
Short sleep – Chronotype [#]	-0.0061	0.610	-	-
Long sleep – Chronotype [#]	0.0051	0.701	-	-

[†] Using unweighted genetic risk score for the sleep traits in the factorial MR Cox regression model, with adjustment for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
[‡] Using weighted genetic risk score for each sleep trait in the factorial MR Cox regression model, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.
***** Values represent the Pearson's correlation coefficient between the first scaled Schoenfeld residual in the factorial MR Cox regression and the rank-normalized natural logarithm of follow-up time.
[#] Chronotype genetic risk score calculated using alleles for morning preference.

Table S12: One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with sleep traits in HUNT2 using weighted and unweighted genetic risk scores for sleep traits.

	Weighted genetic risk score		Unweighted genetic risk score	
Sleep trait	**N (incident cases)**	**Hazard ratio (95% CI)***	**N (incident cases)**	**Hazard ratio (95% CI)***
Insomnia symptoms #	44 728 (4 488)	1.23 (1.00, 1.55)	44 728 (4 488)	1.24 (1.00, 1.59)
24-hour sleep duration (h)	44 728 (4 488)	0.76 (0.31, 1.79)	44 728 (4 488)	0.72 (0.30, 1.64)
Short sleep # (≤6 h vs. 7-8 h)	33 243 (3 058)	0.87 (0.15, 3.24)	33 243 (3 058)	0.82 (0.06, 6.53)
Long sleep # (≥9 h vs. 7-8 h)	41 945 (4 209)	0.53 (0.01, 8.28)	41 945 (4 209)	0.85 (0.29, 1.83)

CI indicates confidence interval.
* Adjusted for age, gender, 20 genetic principal components, and genotyping chip.
# Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait.

Table S13: Associations between genetic risk scores and potential confounders in UK Biobank.

Instrument	Confounder	Coefficient (Beta)	SE	N	P	
Insomnia uwGRS	Marital status	0.057	0.051	230 590	0.270	
	Alcohol intake	-0.083	0.023	230 590	3.34e-04	*
	Smoking status	0.286	0.033	230 590	<2e-16	*
	BMI	0.053	0.005	230 590	<2e-16	*
	Physical activity	-0.014	0.029	230 590	0.625	
	TDI	0.030	0.008	230 590	1.46e-04	*
	Education	-0.215	0.023	230 590	<2e-16	*
	Shift work	0.061	0.102	230 590	0.547	
	Employment status	0.146	0.046	230 590	0.002	
	SBP	7.99e-04	0.001	230 590	0.512	
	Fasting time	-0.002	0.009	230 590	0.786	
	Serum cholesterol	-0.059	0.019	230 590	0.002	
	Blood glucose	0.020	0.018	230 590	0.280	
	Depression	0.632	0.071	230 590	<2e-16	*
	Anxiety	0.279	0.092	230 590	0.002	
	Sleep medication	0.413	0.231	230 590	0.074	
	Chronic illness	0.683	0.049	230 590	<2e-16	*
Sleep duration uwGRS	Marital status	-0.009	0.027	230 590	0.742	
	Alcohol intake	0.001	0.012	230 590	0.910	
	Smoking status	-0.014	0.017	230 590	0.410	
	BMI	-0.016	0.003	230 590	1.62e-09	*
	Physical activity	-0.009	0.016	230 590	0.553	
	TDI	-0.004	0.004	230 590	0.307	
	Education	0.048	0.012	230 590	8.95e-05	*
	Shift work	0.004	0.054	230 590	0.939	
	Employment status	-0.064	0.024	230 590	0.008	
	SBP	0.001	0.001	230 590	0.030	
	Fasting time	2.84e-04	0.005	230 590	0.953	
	Serum cholesterol	-0.002	0.010	230 590	0.860	
	Blood glucose	0.002	0.010	230 590	0.842	
	Depression	0.064	0.038	230 590	0.089	
	Anxiety	0.030	0.049	230 590	0.535	
	Sleep medication	-0.182	0.122	230 590	0.137	
	Chronic illness	-0.065	0.026	230 590	0.013	
Short sleep uwGRS	Marital status	0.005	0.016	213 418	0.768	
	Alcohol intake	-0.028	0.007	213 418	1.31e-04	*
	Smoking status	0.016	0.011	213 418	0.136	
	BMI	0.006	0.002	213 418	8.84e-05	*
	Physical activity	-0.006	0.009	213 418	0.534	
	TDI	0.002	0.002	213 418	0.494	
	Education	-0.060	0.007	213 418	1.38e-15	*
	Shift work	-0.001	0.032	213 418	0.977	
	Employment status	0.004	0.015	213 418	0.760	
	SBP	4.93e-04	3.90e-04	213 418	0.206	
	Fasting time	0.001	0.003	213 418	0.732	
	Serum cholesterol	-0.007	0.006	213 418	0.267	
	Blood glucose	-0.006	0.006	213 418	0.352	
	Depression	0.042	0.023	213 418	0.068	
	Anxiety	0.002	0.029	213 418	0.837	
	Sleep medication	0.072	0.076	213 418	0.345	
	Chronic illness	0.040	0.016	213 418	0.011	
Long sleep uwGRS	Marital status	-0.020	0.008	177 512	0.014	
	Alcohol intake	0.004	0.004	177 512	0.288	
	Smoking status	0.002	0.005	177 512	0.660	
	BMI	-0.007	7.87e-04	177 512	<2e-16	*
	Physical activity	-0.019	0.005	177 512	4.58e-05	*
	TDI	4.06e-04	0.001	177 512	0.748	
	Education	-0.005	0.004	177 512	0.153	
	Shift work	-0.009	0.017	177 512	0.591	
	Employment status	-0.004	0.007	177 512	0.558	
	SBP	2.27e-04	1.92e-04	177 512	0.236	
	Fasting time	0.002	0.001	177 512	0.141	
	Serum cholesterol	0.008	0.003	177 512	0.012	
	Blood glucose	3.81e-04	0.003	177 512	0.898	
	Depression	-0.013	0.011	177 512	0.238	
	Anxiety	0.024	0.015	177 512	0.099	
	Sleep medication	0.030	0.040	177 512	0.453	
	Chronic illness	-9.32e-05	0.008	177 512	0.991	
Chronotype (morning preference) uwGRS	Marital status	0.129	0.058	230 590	0.028	
	Alcohol intake	-0.055	0.026	230 590	0.038	
	Smoking status	-0.047	0.037	230 590	0.209	
	BMI	0.015	0.006	230 590	0.006	
	Physical activity	0.202	0.033	230 590	1.53e-09	*
	TDI	-0.051	0.009	230 590	1.18e-08	*
	Education	-0.070	0.026	230 590	0.008	
	Shift work	-0.104	0.115	230 590	0.368	
	Employment status	-0.027	0.052	230 590	0.601	
	SBP	-0.001	0.001	230 590	0.331	

Fasting time	-0.020	0.010	230 590	0.054	
Serum cholesterol	-0.050	0.022	230 590	0.022	
Blood glucose	-0.069	0.021	230 590	0.001	
Depression	-0.002	0.080	230 590	0.980	
Anxiety	0.071	0.104	230 590	0.496	
Sleep medication	-0.088	0.262	230 590	0.737	
Chronic illness	-0.208	0.056	230 590	1.87e-04	*

uwGRS indicates unweighted genetic risk score; SE, standard error; BMI, body mass index; TDI, Townsend deprivation index; SBP, systolic blood pressure.
Coefficients are in terms of an average-SNP increase in the allele score per unit/level increase in confounder.
* Associations surpassing multiple-testing corrected p-value threshold of 0.05/85 = 5.88e-04.

Table S14: Associations between genetic risk scores and potential confounders in HUNT2.

Instrument	Confounder	Coefficient (Beta)	SE	N	P	
Insomnia wGRS	Marital status	0.004	0.005	27 309	0.364	
	Alcohol intake	0.001	0.004	27 309	0.757	
	Smoking status	0.015	0.003	27 309	9.47e-06	*
	BMI	0.003	0.001	27 309	7.07e-05	*
	Physical activity	-0.007	0.003	27 309	0.029	
	Education	-0.001	0.004	27 309	0.770	
	Shift work	0.008	0.007	27 309	0.272	
	Employment status	0.002	0.007	27 309	0.824	
	SBP	-1.83e-04	1.61e-04	27 309	0.254	
	Fasting time	0.002	0.001	27 309	0.224	
	Serum cholesterol	-0.009	0.002	27 309	2.02e-04	*
	Blood glucose	-5.35e-04	0.002	27 309	0.805	
	HADS - Depression	-0.004	0.001	27 309	0.003	
	HADS - Anxiety	0.004	0.001	27 309	2.58e-04	*
	Sleep medication	0.034	0.013	27 309	0.007	
	Chronic illness	0.013	0.007	27 309	0.058	
Sleep duration wGRS	Marital status	-0.222	3.390	27 309	0.948	
	Alcohol intake	4.280	2.613	27 309	0.101	
	Smoking status	-3.574	2.399	27 309	0.136	
	BMI	-0.734	0.515	27 309	0.154	
	Physical activity	0.142	2.351	27 309	0.952	
	Education	-3.158	2.971	27 309	0.288	
	Shift work	-2.939	5.150	27 309	0.568	
	Employment status	2.412	4.930	27 309	0.625	
	SBP	-0.040	0.113	27 309	0.721	
	Fasting time	-0.830	1.023	27 309	0.417	
	Serum cholesterol	-1.245	1.765	27 309	0.481	
	Blood glucose	0.727	1.520	27 309	0.632	
	HADS - Depression	-0.330	0.838	27 309	0.694	
	HADS - Anxiety	0.077	0.740	27 309	0.917	
	Sleep medication	4.460	8.876	27 309	0.615	
	Chronic illness	-2.337	4.692	27 309	0.618	
Short sleep wGRS	Marital status	4.61e-04	0.001	21 020	0.723	
	Alcohol intake	-4.71e-04	0.001	21 020	0.638	
	Smoking status	0.001	9.15e-04	21 020	0.121	
	BMI	5.10e-04	2.01e-04	21 020	0.011	
	Physical activity	-0.001	8.91e-04	21 020	0.249	
	Education	-1.46e-04	0.001	21 020	0.898	
	Shift work	-0.001	0.002	21 020	0.456	
	Employment status	-0.003	0.002	21 020	0.115	
	SBP	2.11e-05	4.48e-05	21 020	0.638	
	Fasting time	4..73e-04	3.89e-04	21 020	0.224	
	Serum cholesterol	-0.002	6.85e-04	21 020	0.021	
	Blood glucose	1.20e-04	6.40e-04	21 020	0.851	
	HADS - Depression	-3.15e-04	3.28e-04	21 020	0.337	
	HADS - Anxiety	-1.63e-04	2.86e-04	21 020	0.568	
	Sleep medication	-7.08e-04	0.004	21 020	0.853	
	Chronic illness	8.66e-04	0.002	21 020	0.638	
Long sleep wGRS	Marital status	-6.48e-04	0.001	25 635	0.535	
	Alcohol intake	5.24e-04	8.00e-04	25 635	0.512	
	Smoking status	2.82e-04	7.36e-04	25 635	0.702	
	BMI	-3.87e-04	1.58e-04	25 635	0.014	
	Physical activity	-3.64e-04	7.21e-04	25 635	0.614	
	Education	0.002	9.08e-04	25 635	0.052	
	Shift work	7.98e-05	0.002	25 635	0.960	
	Employment status	-0.001	0.001	25 635	0.446	
	SBP	2.22e-05	3.45e-05	25 635	0.519	
	Fasting time	-1.98e-04	3.15e-04	25 635	0.530	
	Serum cholesterol	2.42e-04	5.40e-04	25 635	0.654	
	Blood glucose	-2.32e-04	4.63e-04	25 635	0.617	
	HADS - Depression	2.99e-04	2.59e-04	25 635	0.247	
	HADS - Anxiety	-1.40e-04	2.28e-04	25 635	0.540	
	Sleep medication	-0.003	0.003	25 635	0.231	
	Chronic illness	0.001	0.001	25 635	0.391	

wGRS indicates weighted genetic risk score; SE, standard error; BMI, body mass index; SBP, systolic blood pressure; HADS, Hospital Anxiety and Depression Scale.
Coefficients are in terms of an average-SNP increase in the allele score per unit/level increase in confounder.
* Associations surpassing multiple-testing corrected p-value threshold of 0.05/64 = 7.81e-04

Table S15: One-sample Mendelian randomization analysis for risk of incident acute myocardial infarction associated with sleep traits with and without adjustment for potential confounders in UK Biobank and HUNT2.

	UK Biobank				HUNT2			
	MR estimates [†]		MR estimates adjusted for potential confounders [*]		MR estimates [‡]		MR estimates adjusted for potential confounders [**]	
Sleep trait	**N (incident cases)**	**Hazard ratio (95% CI)**	**N (incident cases)**	**Hazard ratio (95% CI)**	**N (incident cases)**	**Hazard ratio (95% CI)**	**N (incident cases)**	**Hazard ratio (95% CI)**
Insomnia symptoms [#]	332 676 (7 813)	1.18 (1.07, 1.31)	265 998 (6 098)	1.04 (0.92, 1.17)	44 728 (4 488)	1.23 (1.00, 1.55)	37 860 (3 425)	1.13 (0.87, 1.47)
24-hour sleep duration (h)	332 676 (7 813)	0.97 (0.75, 1.29)	265 998 (6 098)	1.05 (0.76, 1.43)	44 728 (4 488)	0.76 (0.31, 1.79)	37 860 (3 425)	0.69 (0.29, 1.65)
Short sleep [#] (≤6 h vs. 7-8 h)	307 135 (7 028)	1.14 (0.97, 1.32)	246 136 (5 515)	1.11 (0.93, 1.32)	33 243 (3 058)	0.87 (0.15, 3.24)	28 776 (2 433)	0.87 (0.36, 2.07)
Long sleep [#] (≥9 h vs. 7-8 h)	253 811 (5 762)	0.83 (0.67, 0.99)	204 727 (4 526)	0.87 (0.70, 1.07)	41 945 (4 209)	0.53 (0.01, 8.28)	35 513 (3 209)	0.59 (0.20, 1.75)
Chronotype [#] (morning preference)	332 676 (7 813)	1.06 (0.99, 1.11)	265 998 (6 098)	1.06 (0.99, 1.13)	-	-	-	-

CI indicates confidence interval.

[†] Derived using unweighted genetic risk score for each sleep trait, with adjustment for age, gender, assessment center, 40 genetic principal components, and genotyping chip.

[‡] Derived using weighted genetic risk score for each sleep trait, with adjustment for age, gender, 20 genetic principal components, and genotyping chip.

[*] Additionally adjusted for alcohol intake frequency, smoking status, body mass index, physical activity, Townsend deprivation index, education, depression, and chronic illness.

[**] Additionally adjusted for smoking status, body mass index, serum cholesterol levels, and anxiety.

[#] Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait.

Table S16: Sensitivity analysis for risk of incident acute myocardial infarction associated with sleep traits in UK Biobank.

	UK Biobank [†]					
Sleep trait	**N (incident cases)**	**TSPS HR (95% CI) [§]**	**IVW HR (95% CI)**	**MR-Egger HR (95% CI)**	**Weighted Median HR (95% CI)**	**Weighted Mode-based HR (95% CI)**
Insomnia symptoms [#]	332 676 (7 813)	1.18 (1.07, 1.31)	1.09 (0.98, 1.20)	0.77 (0.62, 0.95)); Intercept 0.007 (0.003, 0.012)	1.04 (0.91, 1.18)	1.36 (0.84, 2.19)
24-hour sleep duration (h)	332 676 (7 813)	0.97 (0.75, 1.29)	0.94 (0.70, 1.27)	0.70 (0.25, 1.97); Intercept 0.005 (-0.012, 0.022)	0.77 (0.51, 1.16)	0.38 (0.14, 1.03)
Short sleep [#] (≤6 h vs. 7-8 h)	307 135 (7 028)	1.14 (0.97, 1.32)	1.13 (0.96, 1.33)	1.09 (0.61, 1.94); Intercept 0.001 (-0.019, 0.022)	1.24 (0.99, 1.55)	1.40 (0.88, 2.22)
Long sleep [#] (≥9 h vs. 7-8 h)	253 811 (5 762)	0.83 (0.67, 0.99)	0.83 (0.63, 1.08)	0.81 (0.40, 1.64); Intercept 0.002 (-0.048, 0.051)	0.79 (0.61, 1.02)	0.80 (0.59, 1.09)
Chronotype [#] (morning preference)	332 676 (7 813)	1.06 (0.99, 1.11)	1.06 (0.99, 1.11)	1.02 (0.88, 1.19); Intercept 0.001 (-0.003, 0.005)	1.01 (0.94, 1.09)	0.94 (0.76, 1.17)

TSPS indicates two-stage predictor substitution; IVW, inverse variance weighted; HR, hazard ratio; and CI, confidence interval.
For IVW, MR-Egger, weighted median and weighted mode-based estimates, the SNP-exposure and the SNP-outcome associations were obtained from the same participants.
[†] Adjusted for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
[§] Derived using unweighted genetic risk score for each sleep trait
[#] Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait

Table S17: Sensitivity analysis for risk of incident acute myocardial infarction associated with sleep traits in HUNT2.

	HUNT2 [†]					
Sleep trait	**N (incident cases)**	**TSPS HR (95% CI) [§]**	**IVW HR (95% CI)**	**MR-Egger HR (95% CI)**	**Weighted Median HR (95% CI)**	**Weighted Mode-based HR (95% CI)**
Insomnia symptoms [#]	44 728 (4 488)	1.23 (1.00, 1.55)	1.08 (0.99, 1.17)	1.16 (1.02, 1.32); Intercept -0.003 (-0.006, 0.001)	1.12 (0.99, 1.27)	1.15 (0.87, 1.51)
24-hour sleep duration (h)	44 728 (4 488)	0.76 (0.31, 1.79)	0.71 (0.48, 1.05)	0.92 (0.47, 1.82); Intercept -0.004 (-0.014, 0.005)	0.81 (0.45, 1.47)	0.79 (0.38, 1.65)
Short sleep [#] (≤6 h vs. 7-8 h)	33 243 (3 058)	0.87 (0.15, 3.24)	1.06 (0.88, 1.29)	1.07 (0.75, 1.52); Intercept -0.001 (-0.015, 0.014)	1.09 (0.80, 1.50)	1.07 (0.81, 1.42)
Long sleep [#] (≥9 h vs. 7-8 h)	41 945 (4 209)	0.53 (0.01, 8.28)	1.07 (0.61, 1.90)	0.95 (0.38, 2.39); Intercept 0.006 (-0.030, 0.042)	0.94 (0.50, 1.75)	1.02 (0.63, 1.65)

TSPS indicates two-stage predictor substitution; IVW, inverse variance weighted; HR, hazard ratio; and CI, confidence interval.
For IVW, MR-Egger, weighted median and weighted mode-based estimates, the SNP-exposure and the SNP-outcome associations were obtained from the same participants.
[†] Adjusted for age, gender, 20 genetic principal components, and genotyping chip.
[§] Derived using weighted genetic risk score for each sleep trait.
[#] Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait.

Table S18: One-sample Mendelian randomization Cox regression analysis for risk of incident acute myocardial infarction associated with insomnia symptoms using instruments from Lane et al., 2019 [16] in UK Biobank and HUNT2.

	N (incident cases)	TSPS HR (95% CI) [§]	IVW HR (95% CI)	MR-Egger HR (95% CI)	Weighted Median HR (95% CI)	Weighted Mode-based HR (95% CI)
UK Biobank [†]	332 676 (7 813)	1.25 (1.09, 1.45)	1.12 (0.96, 1.31)	0.69 (0.50, 0.96); Intercept 0.013 (0.005, 0.022)	1.18 (0.96, 1.44)	1.15 (0.83, 1.60)
HUNT2 [‡]	44 728 (4 488)	1.39 (0.97, 2.41)	1.13 (0.98, 1.29)	0.97 (0.78, 1.19); Intercept 0.006 (0.001, 0.012)	1.11 (0.90, 1.37)	1.14 (0.92, 1.41)

TSPS indicates two-stage predictor substitution; IVW, inverse variance weighted; HR, hazard ratio; and CI, confidence interval.
Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait.
For IVW, MR-Egger, weighted median and weighted mode-based estimates, the SNP-exposure and the SNP-outcome associations were obtained from the same participants.
[§] Derived using unweighted genetic risk score for insomnia symptoms in UK Biobank and weighted genetic risk score for insomnia symptoms in HUNT2.
[†] Adjusted for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
[‡] Adjusted for age, gender, 20 genetic principal components, and genotyping chip.

Table S19: Sensitivity analysis for risk of incident acute myocardial infarction associated with insomnia symptoms and chronotype in UK Biobank using genetic variants genome-wide significant in 23andMe.

	UK Biobank †						
Sleep trait	**N (incident cases)**	**TSPS HR (95% CI) §**	**TSPS HR (95% CI) ***	**IVW HR (95% CI)**	**MR-Egger HR (95% CI)**	**Weighted Median HR (95% CI)**	**Weighted Mode-based HR (95% CI)**
Insomnia symptoms #	332 676 (7 813)	1.25 (1.08, 1.45)	1.33 (1.13, 1.56)	1.16 (0.99, 1.35)	0.78 (0.59, 1.02); Intercept 0.008 (0.003, 0.013)	1.10 (0.89, 1.35)	1.01 (0.61, 1.65)
Chronotype # (morning preference)	332 676 (7 813)	1.02 (0.94, 1.11)	0.99 (0.91, 1.09)	1.02 (0.92, 1.13)	1.14 (0.91, 1.43); Intercept -0.004 (-0.013, 0.004)	0.95 (0.84, 1.08)	0.92 (0.71, 1.18)

TSPS indicates two-stage predictor substitution; IVW, inverse variance weighted; HR, hazard ratio; and CI, confidence interval.
For IVW, MR-Egger, weighted median and weighted mode-based estimates, the SNP-exposure and the SNP-outcome associations were obtained from the same participants.
† Adjusted for age, gender, assessment center, 40 genetic principal components, and genotyping chip.
§ Derived using weighted genetic risk score for each sleep trait.
* Derived using unweighted genetic risk score for each sleep trait.
# Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait

Table S20: Sensitivity analysis for risk of incident acute myocardial infarction associated with insomnia symptoms in HUNT2 using genetic variants genome-wide significant in 23andMe.

	HUNT2 †						
Sleep trait	**N (incident cases)**	**TSPS HR (95% CI) §**	**TSPS HR (95% CI) ***	**IVW HR (95% CI)**	**MR-Egger HR (95% CI)**	**Weighted Median HR (95% CI)**	**Weighted Mode-based HR (95% CI)**
Insomnia symptoms #	44 728 (4 488)	1.15 (0.90, 1.46)	1.10 (0.85, 1.42)	1.11 (1.00, 1.25)	1.23 (1.03, 1.47); Intercept: -0.004 (-0.009, 0.001)	1.12 (0.95, 1.32)	1.18 (0.93, 1.51)

TSPS indicates two-stage predictor substitution; IVW, inverse variance weighted; HR, hazard ratio; and CI, confidence interval.
For IVW, MR-Egger, weighted median and weighted mode-based estimates, the SNP-exposure and the SNP-outcome associations were obtained from the same participants.
† Adjusted for age, gender, 20 genetic principal components, and genotyping chip.
§ Derived using weighted genetic risk score for insomnia symptoms.
* Derived using unweighted genetic risk score for insomnia symptoms.
# Hazard ratio (95% CI) scaled to per doubling in odds of the sleep trait.

Table S21: List of medications used to define the sleep medication covariate in UKBB.

Sleep medication	**Treatment/medication code** (UKBB field ID: 20003)
Oxazepam	1140863442
Meprobamate	1140863378
Medazepam	1140863372
Bromazepam	1140863318
Lorazepam	1140863302
Clobazam	1140863268
Chlormezanone	1140863262, 1140868274
Temazepam	1140863202
Nitrazepam	1140863182, 1140863104
Lormetazepam	1140863176
Diazepam	1140863152, 1141157496
Zopiclone	1140863144
Triclofos sodium	1140863140
Methyprylone	1140856040
Prazepam	1140855944
Triazolam	1140855914
Ketazolam	1140855860
Dichloralphenazone	1140855824
Clomethiazole	1140909798
Zaleplon	1141171404
Butobarbital	1141180444
Clonazepam	1140872150
Flurazepam	1140863110
Loprazolam	1140863120
Alprazolam	1140863308
Butobarbitone	1140882090

References

1. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381–95.

2. International Physical Activity Questionnaire (IPAQ): Guidelines for data processing analysis of the International Physical Activity Questionnaire (IPAQ) - Short and long forms. 2005. https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=540. Accessed 25 Oct 2021.

3. International Physical Activity Questionnaire (IPAQ): IPAQ scoring protocol. 2005. https://sites.google.com/site/theipaq/scoring-protocol. Accessed 25 Oct 2021.

4. Brumpton BM, Langhammer A, Ferreira MAR, Chen Y, Mai X-M. Physical activity and incident asthma in adults: the HUNT Study, Norway. BMJ Open. 2016;6:e013856.

5. Kurtze N, Rangul V, Hustvedt BE, Flanders WD. Reliability and validity of self-reported physical activity in the Nord-Trøndelag Health Study (HUNT 2). Eur J Epidemiol. 2007;22:379–87.

6. Townsend P. Poverty in the United Kingdom: A Survey of Household Resources and Standards of Living. London: Allen Lane and Penguin Books; 1979.

7. Holmen J, Midthjell K, Krüger Ø, Langhammer A, Holmen TL, Bratberg GH, et al. The Nord-Trøndelag Health Study 1995–97 (HUNT 2): objectives, contents, methods and participation. Norsk epidemiologi. 2003;13:19–32.

8. Daghlas I, Dashti HS, Lane J, Aragam KG, Rutter MK, Saxena R, et al. Sleep Duration and Myocardial Infarction. J Am Coll Cardiol. 2019;74:1304–14.

9. Elliott P, Peakman TC, UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International Journal of Epidemiology. 2008;37:234–44.

10. Skapinakis P. Hospital Anxiety and Depression Scale (HADS). In: Michalos AC, editor. Encyclopedia of Quality of Life and Well-Being Research. Dordrecht: Springer Netherlands; 2014. p. 2930–3.

11. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res. 2002;52:69–77.

12. Mykletun A, Stordal E, Dahl AA. Hospital Anxiety and Depression (HAD) scale: factor structure, item analyses and internal consistency in a large population. Br J Psychiatry. 2001;179:540–4.

13. Jansen PR, Watanabe K, Stringer S, Skene N, Bryois J, Hammerschlag AR, et al. Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways. Nat Genet. 2019;51:394–403.

14. Dashti HS, Jones SE, Wood AR, Lane JM, van Hees VT, Wang H, et al. Genome-wide association study identifies genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates. Nat Commun. 2019;10:1100.

15. Jones SE, Lane JM, Wood AR, van Hees VT, Tyrrell J, Beaumont RN, et al. Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms. Nat Commun. 2019;10:343.

16. Lane JM, Jones SE, Dashti HS, Wood AR, Aragam KG, van Hees VT, et al. Biological and clinical insights from genetics of insomnia symptoms. Nat Genet. 2019;51:387–93.

17. Ai S, Zhang J, Zhao G, Wang N, Li G, So H-C, et al. Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK Biobank. European Heart Journal. 2021;42:3349–57.

18. Larsson SC, Markus HS. Genetic Liability to Insomnia and Cardiovascular Disease Risk. Circulation. 2019;140:796–8.

19. Nikpay M, Goel A, Won H-H, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet. 2015;47:1121–30.

20. Liu X, Li C, Sun X, Yu Y, Si S, Hou L, et al. Genetically Predicted Insomnia in Relation to 14 Cardiovascular Conditions and 17 Cardiometabolic Risk Factors: A Mendelian Randomization Study. J Am Heart Assoc. 2021;10:e020187.

21. Yuan S, Mason AM, Burgess S, Larsson SC. Genetic liability to insomnia in relation to cardiovascular diseases: a Mendelian randomisation study. Eur J Epidemiol. 2021;36:393–400.

22. Yang Y, Fan J, Shi X, Wang Y, Yang C, Lian J, et al. Causal associations between sleep traits and four cardiac diseases: a Mendelian randomization study. ESC Heart Failure. 2022;9:3160–6.

Arora N, Brumpton BM, Åsvold BO, Loennechen JP, Malmo V, Bhatta L, Skarpsno ES, Richmond RC, Strand LB.

A Mendelian randomization study investigating the role of sleep traits and their joint effects on the incidence of atrial fibrillation

Manuscript

Paper III

Paper III

A Mendelian randomization study investigating the role of sleep traits and their joint effects on the incidence of atrial fibrillation

Nikhil Arora[1,2], Ben Michael Brumpton[1,3,4], Bjørn Olav Åsvold[1,3,5], Jan Pål Loennechen[6,7], Vegard Malmo[6,7], Laxmi Bhatta[1,8], Eivind Schjelderup Skarpsno[9,10], Rebecca Claire Richmond[2,11†] and Linn Beate Strand[9†]*

[1]K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway; [2]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom; [3]HUNT Research Centre, Department of Public Health and Nursing, Norwegian University of Science and Technology, Levanger, Norway; [4]Department of Medicine, St. Olavs Hospital, Trondheim, Norway; [5]Department of Endocrinology, Clinic of Medicine, St. Olavs Hospital, Trondheim, Norway; [6]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway; [7]Clinic of Cardiology, St. Olavs Hospital, Trondheim, Norway; [8]FIU-PH, Division of Mental Health Care, St. Olavs Hospital, Trondheim, Norway; [9]Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway; [10]Department of Neurology and Clinical Neurophysiology, St. Olavs Hospital, Trondheim, Norway; and [11]MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom.

* Corresponding author: e-mail: nikhil.arora@ntnu.no, or docnikhilarora@gmail.com

† These authors contributed equally to this work: Rebecca Claire Richmond, and Linn Beate Strand.

Appendices

Appendices

Genetic variants

The genetic information used for the main analysis in Papers II and III.

Paper 3 is awaiting publication therefore this information is not included in NTNU Open

ISBN 978-82-326-7488-6 (printed ver.)
ISBN 978-82-326-7487-9 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (online ver.)

NTNU

Norwegian University of
Science and Technology