Sleep Duration May Not Have Any Effect on The Risk of Stroke: Insights from Mendelian Randomization and Prospective Cohort Studies

Type

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Keywords

sleep duration, Mendelian Randomization, Stroke, Ischemic stroke

Abstract

Introduction
Due to contentious associations between sleep and stroke risk we performed a meta-analysis of cohort studies and utilized Mendelian randomization (MR).

Material and methods
For the meta-analysis we pooled prospective studies and then reviewed the largest genome-wide association studies regarding self-reported or accelerometer-derived sleep duration with stroke [ischemic (IS), cardioembolic (CES), large artery (LAS), small vessel (SVS)]. Inverse variance weighted method (IVW), weighted median (WM)-based method, MR-Egger and MR-Pleiotropy RESidual Sum and Outlier (PRESSO) were performed. To determine the impact of single nucleotide polymorphisms (SNPs) leave-one-out method was applied.

Results
Pooled prospective studies demonstrated shorter (<7h) [n=25 studies, I2 = 71.4, p <0.001; risk ratio (RR): 1.18, 95%CI: 1.08-1.30, p <0.001] and longer (>8h) [n=16 studies, I2 = 53.6, p <0.001; RR: 1.38, 95%CI: 1.24-1.53, p <0.001] sleep increased stroke risk (compared with 7-8h), but were subject to high levels of heterogeneity. In MR, self-reported sleep duration had no significant effect on IS (IVW: beta = -0.031, p = 0.747), CES (IVW: beta = -0.039, p = 0.849), LAS (IVW: beta = -0.246, p = 0.328) and SVS (IVW: beta = -0.102, p = 0.667) risk. This was also observed for short and long accelerometer-derived sleep (all p >0.126). Estimated associations had no significant heterogeneity and MR-PRESSO revealed no outliers. There was low likelihood of pleiotropy (all estimations p >0.539) and associations were not driven by single SNPs.

Conclusions
Meta-analysis revealed shorter and longer sleep increased total stroke risk, but with high heterogeneity. MR analysis showed no causal associations between sleep duration and stroke risk.

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Running title: *Sleep duration and the risk of stroke*

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INTRODUCTION:

Stroke is a major cardiovascular (CV) event that can lead to brain tissue damage and functional disability, as well as death (1). Due to these detrimental effects of stroke, evaluating stroke risk factors can be of high importance to prevent this potentially fatal CV disease (CVD) or determine its outcomes.

Sleep duration is recognised as a major factor for a healthier lifestyle and improved cardiac function (2). In this context, sleeping patterns (i.e. short or long duration) have been closely linked to morbidity and mortality (3, 4), as well as with chronic cardiometabolic disease, including obesity, hypertension, diabetes, respiratory diseases, coronary heart disease and reduced renal function (CHD) (2, 5-9). There are several parameters that can influence sleep duration, such as cultural, social, psychological, behavioral, pathophysiological and environmental factors (10, 11).

Considering the potential harmful effects of unfavourable sleep duration on public health, it is clinically important to establish whether causality exists between sleep duration and CVD. Recent studies showed that sleep duration may be a risk factor for CVD (12). Both short (<7 h) and long (>8 h) sleep duration have been related to a greater risk of myocardial infarction (MI) (12-14). However, the National Health and Nutrition Examination Survey (NHANES), the Nurses' Health Study (NHS) and the Monitoring Trends and Determinants on Cardiovascular Disease (MONICA) Augsburg cohort study reported inconsistent findings regarding the association between sleep duration and CHD risk (5, 15, 16). A prospective study of 461,347 UK Biobank (UKB) individuals found that short and long sleep duration had a 20 and 34% higher risk of incident MI compared with sleeping 6 - 9 h, respectively, independent of individuals' genetic predisposition to CHD (17).

With regards to stroke, epidemiological studies showed a significant association between long sleep duration and stroke risk (16, 18, 19). In contrast, no significant relationship between sleep duration and stroke risk was observed in another prospective cohort study of 2,282 males (20). Previous systematic reviews evaluated the links between sleep duration, CV outcomes and all-cause death (11, 12), reporting that both short and long duration of sleep were associated with a greater risk of total mortality and/or CVD morbidity, including CHD and stroke. However, the high heterogeneity between the studies was a limitation, making it difficult to draw safe conclusions or support causal inference.

Given the controversial evidence on the potential role of sleep duration on CVD outcomes and its importance for public health, there is an unmet need to elucidate this link in terms of developing effective approaches (including drug therapy) for stroke prevention and management. Epidemiological

studies provide only simple estimates of the associations between sleep duration and disease risk, and they are prone to bias. Therefore, Mendelian Randomization (MR) studies can evaluate these relationships with the use of a large-scale genetic data [involving single nucleoid polymorphisms (SNPs) as proxies for lifetime exposure] to provide a more reliable indication of a causal role of sleep duration on stroke risk (21).

In the present study, a comprehensive systematic review and meta-analysis was conducted to examine the associations between sleep duration and the risk of total stroke based on data from prospective cohort studies. Second, a MR analysis was performed to assess the associations between sleep duration (<7 vs >8 h) and different types of stroke, surpassing the limitations of the epidemiological studies (i.e. residual bias, confounding factors and reverse causation) (22).

METHODS

[A] Cohort studies

Literature search and study selection

The meta-analysis was designed, conducted and reported, according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (23) (the MOOSE checklist is provided in **Supplementary Table 1**). The primary exposure of interest was sleep duration and stroke was the primary outcome. Prospective cohort studies published up to 31 October 2019 (without language restriction) were searched using the PubMed, Embase, and Scopus databases; the query search syntax is shown in **Supplementary Table 2**. When required, further searches were performed regarding the reference list of eligible articles, previous reviews or meta-analyses, as well as email correspondence with authors for additional data.

Study Selection

Predefined inclusion criteria (see below) were used to determine prospective cohort studies regarding the association between sleep duration and stroke risk. Duplicates were removed and then titles and abstracts were screened by two reviewers (MM and NS). To avoid bias, both reviewers were blinded to the names, qualifications or the affiliations of the study authors. The overall agreement between both reviewers was excellent (*Kappa* index: 0.91; p <0.001) and a meeting was held to resolve any disagreements between reviewers prior to articles being retrieved (a flow chart outlining this process is available in **Figure 1**).

We included studies if the following criteria were met: (1) the topic of interest was sleep duration; (2) the studies were population-based cohort studies which reported stroke risk data; (3) relative risk (RR), hazard ratio (HR) or odds ratio (OR) estimates with 95% confidence interval (CI) adjusted for multivariable factors were available or could be calculated.

We excluded studies if the following criteria were met: (1) narrative reviews, reviews, comments, opinion papers, methodological reports, editorials, letters, or any other publications lacking primary data and/or specific descriptions of the methods which were used; (2) not population-based cohort studies; (3) RR, HR or OR estimates with 95%CI were either not available or could not be calculated.

Data extraction and management

The full text of studies meeting the inclusion criteria were retrieved and eligibility was determined via screening by two reviewers (MM and NS). An assessment of study quality was determined using the Newcastle-Ottawa Scale (NOS) (**Supplementary Table 3**) (24). Furthermore, sources of funding pertaining to each eligible study was investigated. Following an assessment of methodological quality, both reviewers (MM and NS) extracted data using a purpose-designed data extraction form. Using this data the most important results from each study were summarized by each reviewer independently. Both summaries were then compared, and a third reviewer (MB) to then consulted to resolve any differences. Any necessary additional calculations regarding the study data, were performed by the first reviewer (MM) and verified by the second reviewer (NS). Specific information which was extracted from each eligible study included: author, year and references, study name, proportion male, mean age, follow-up time (Years), assessment of sleep duration, main confounders (**Table 1**).

Data synthesis and statistical analyses

For those studies which reported results from a variety of multivariable-adjusted models, only the model with the most confounding factors was incorporated into the meta-analysis. A random-effect model was employed to generate pooled RRs, 95%CI and p values for heterogeneity. RRs comparing the highest and lowest categories were combined across studies to determine summary associations. The extent of heterogeneity across studies was examined using the I^2 test (25-27); an I^2 >50% with a two-sided p <0.05 indicated significant heterogeneity (25-27).

Publication bias

To determine potential publication bias Begg's funnel plot asymmetry was investigated by visual inspection and Begg's rank correlation and Egger's weighted regression tests were also used (28). To adjust for the effects of publication bias the Duval and Tweedie trim method was utilized (28). Comprehensive Meta-Analysis (CMA) V3 software (Biostat, NJ) was used to conduct the meta-analysis (29).

[B] Mendelian Randomization

Study design

For our MR study a two-sample MR study design was used and the largest genome wide association studies (GWAS) on sleep duration (objectively and subjectively measured) and interested outcomes were used to obtain summary statistics. We then applied methods to estimate the unbiased effect of sleep traits on the risk of different types of stroke [i.e. ischemic (IS), cardioembolic (CES), large artery (LAS), small vessel (SVS)].

Genetic instruments for sleep duration

Procedures pertaining to genotyping, quality control, and imputation which have been used in the UKB have been previously described (30). From the largest GWAS, 78 SNPs were identified to be associated with sleep duration (self-reported) among individuals of European ancestry (n = 446,118) (**Supplementary Table 4**) (31). We hypothesized that the link between sleep duration and stroke risk might be non-linear; therefore, we also used data pertaining to 27 SNPs associated with short sleep (<7 h; n = 106,192 cases/305,742 controls) and 8 SNPs related to long sleep (>8 h; n = 34,184 cases/305,742 controls) in the two-sample MR analysis (31). We applied on the GWAS (n = 85,205 participants), performed in the UKB for genetic variants known to be robustly associated with accelerometer-driven sleep trait data (nocturnal sleep duration), which were compared with causal estimates obtained by the genetic variants associated with self-reported sleep duration (**Supplementary Table 4**). Data on this procedure has been previously published (32). We identified proxy SNPs with a minimum linkage disequilibrium (LD) r2 = 0.8 if a SNP was unavailable for the outcome GWAS summary statistics. We restricted our genetic instrument to independent SNPs not in linkage disequilibrium (p = 0.0001) to minimize bias in effect estimates induced by correlation between SNPs. Herein we will refer to a set of SNPs which act as a proxy for sleep duration as "genetic instruments."

Association of genetic instruments with outcome

We utilized the largest available extensively genotyped dataset, i.e. the METASTROKE, a collaboration of the International Stroke Genetics Consortium to determine genetic associations with different ischemic stroke types (33). This dataset included GWAS data on 34,217 IS cases and 404,630 controls of European ancestry from across 15 studies [subtyped into SVS (n = 5,386), LAS (n = 4,373) and CES (n = 7,193)] (33). The majority of the IS cases involved brain imaging confirmation. Approximately 50% of the cases had IS subtype information (2,365 CES, 2,167 LAS and 1,894 SVS cases) based on the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification (34). Additional phenotype descriptions and details of individual studies included in the dataset are available elsewhere (33).

MR analysis

The effect of 5 instruments were combined using the inverse variance weighted (IVW) method as implemented using the TwoSampleMR package within the R environment [version 3.4.2 R Core Team (2017)]. Heterogeneity was assessed by the Q value for IVW. The potential effects of pleiotropic variants were addressed on the final effect estimate by performing a sensitivity analysis, including weighted median (WM) and MR-egger tests (35). To identify instruments which might drive the MR results sensitivity analysis was conducted using the leave-one-out method. The WM estimate provides correct estimates if SNPs accounting for ≥50% of the weight are valid instruments. To weight the variants inverse variance was used and to estimate Cis bootstrapping was applied (35). MR-egger analysis can define estimates even under the assumption that all SNPs are invalid instruments if the assumption of instrument strength independent of direct effect (InSIDE) is satisfied (35). That said, the InSIDE assumption is not easy to verify. The p value of the intercept term from the MR-Egger analysis was used to assess the average directional pleiotropy across genetic variants (35). Causal estimates in MR-Egger are less precise than those obtained by using IVW MR (36). Indeed, due to its lower statistical power the analysis using MR-Egger has a lower false-positive but a higher false-negative rate than IVW (37).

The Q' heterogeneity statistic (38) and the MR pleiotropy residual sum and outlier (MR-PRESSO) test (38) were both used to assess heterogeneity between individual genetic variant estimates. The Q' statistic uses modified 2nd order weights that are derived from a Taylor series expansion and considers the uncertainty in both numerator and denominator of the instrumental variable ratio (38). The MR-PRESSO framework detects and removes effect estimates which are outliers by regressing the variant-outcome associations on variant-exposure associations. Then a global heterogeneity test was implemented which compares the observed distance between residual sums of squares of all variants to the regression line with the distance expected under the null hypothesis of no pleiotropy (39). In

addition to this, we also applied an MR-Robust Adjusted Profile Score (RAPS) to correct for pleiotropy using robust adjusted profile scores. RAPS can also provide an unbiased causal estimate in the presence of weak instruments. We considered all results which were causal estimates that agreed in direction and magnitude across MR methods, as well as passing nominal significance in the IVW MR analysis and which did not, after applying heterogeneity tests, show evidence of bias from horizontal pleiotropy. To assess the instrumental variable analysis "exclusion-restriction" assumption, we used Ensembl release (http://useast.ensembl.org/index.html), which contains a base of SNP phenotypes.

Ethics

The present meta-analysis and MR analysis used published or publicly available summary data without involvement of participants or original data collection. Ethical approval for each of the studies included in these analyses can be found in the original publications (including informed consent from each participant). The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki**.**

RESULTS:

Meta-analysis and systematic review

Of 44 eligible full articles, 12 articles with 594,632 participants met the inclusion criteria (**Figure 1**). The included studies were published between 1997 (16) and 2016 (40), from different countries, including the United States of America (3 studies) (16, 19, 41), UK (1 study) (42), Japan (2 studies) (20, 43), Germany (2 studies) (44, 45), Australia (1 study) (46), China (2 studies) (40, 47) and Sweden (1 study) (48). A total of 3 studies presented sex-specific results, one study involved only men (20) and 2 studies only women (19, 47). The age of the participants ranged from 44 (20) to 63 years (46) and the follow up duration from 2 (41) to 14 years (20, 44). Basic characteristics of the 12 prospective cohort studies are shown in **Table 1**.

Sleep duration and risk of stroke

Compared with participants sleeping 7 - 8 h per night (reference group), individuals with <7 h sleep had a significantly higher risk of stroke (RR: 1.18, 95%CI: 1.08 - 1.30, p <0.001, n = 25 studies, **Figure 2**), but with a high level of heterogeneity (I^2 = 71.4, p <0.001). In gender analysis, stroke risk was insignificantly increased by 18% in men (RR: 1.18, 95%CI: 0.88 - 1.58, p = 0.263, n = 4 studies, I^2 = 43.5, p = 0.150) and by 20% in women (RR: 1.20, 95%CI: 0.98 - 1.46, p = 0.064, n = 7 studies, I^2 = 79.3, p <0.001).

We then pooled the risk of stroke for those with ≤5 h of sleep who had a significantly greater stroke risk compared with the reference group (RR: 1.34, 95%CI: 1.17 - 1.53, p <0.001, n = 13 studies, **Figure 3**) but again with a high level of heterogeneity (I^2 = 58.4, p <0.001). No association was observed for participants sleeping 6 h per night (compared with the reference group) (RR: 1.07, 95%CI: 0.98 - 1.18, p = 0.110, n = 12 studies, I^2 = 52.6, p = 0.016, **Figure 4**). Longer sleep duration (i.e. >8 h) was related to an increased stroke risk compared with 7 - 8 h sleep (RR: 1.38, 95%CI: 1.24 - 1.53, p <0.001, n = 16 studies, I^2 = 53.6, p <0.001, **Figure 5**). In gender analysis, the risk was 22% in men (RR: 1.22, 95%CI: 1.00 - 1.49, p = 0.042, n = 3 studies, I^2 = 0.0, p = 0.398) and 24% in women (RR: 1.24, 95%CI: 0.91 - 1.67, p = 0.159, n = 5 studies, I^2 = 81.6, p <0.001).

Compared with the reference group, >9 h of sleep also significantly increased stroke risk (RR: 1.35, 95%CI: 1.22 - 1.50, p <0.001, n = 13 studies, I^2 = 41.5, p = 0.043), whereas 10 h of sleep did not affect the risk (RR: 1.40, 95%CI: 0.95 - 2.07, p = 0.082, n = 3 studies, I^2 = 75.7, p = 0.016). It should be noted that in all the above comparisons, there was a high level of heterogeneity between the studies.

Sensitivity analysis

In the leave-one-out sensitivity analyses, the pooled effect estimates remained similar for the effect of both shorter and longer (in comparison with the reference group) sleep duration (RR: 1.18, 95%CI: 1.08 - 1.30, and 1.38, 95%CI: 1.24 - 1.53, respectively). This confirms that the significant difference between the studied groups is the overall effect of all included studies.

Publication bias

Egger's linear regression also supported the absence of any publication bias (intercept = 2.33, 95%CI = 1.77, -5.22, p = 0.450). Furthermore, Begg's rank correlation test (Kendall's Tau with continuity correction = 0.412, z = 0.832, p = 0.452) was not indicative for publication bias.

Mendelian Randomization

The list of all instruments associations for sleep duration (subjectively and objectively assessed) is shown in **Supplementary Table 4**. The results, expressed as *beta*-coefficient for sleep duration per one standard deviation (SD) increase in outcomes (i.e. stroke risk) are presented in **Tables 2-5**. Self-reported sleep duration had no significant effect on the risk of IS (IVW: beta = -0.031, p = 0.747), CES (IVW: beta = -0.039, p = 0.849), LAS (IVW: beta = -0.246, p = 0.328) and SVS (IVW: beta = -0.102, p = 0.667) (**Table 2**). The same pattern was observed for the accelerometer recorded sleep duration (for IS IVW: beta = 0.033,

p = 0.739; for CES IVW: beta = -0.072, p = 0.713; for LAS IVW: beta = 0.109, p = 0.664 and for SVS IVW: beta = 0.367, p = 0.149) (**Table 3**).

Short sleep duration had also no significant impact on the risk of different strokes (for IS IVW: beta = 0.343, p = 0.335, for CES IVW: beta = -0.384, p = 0.614, for LAS IVW: beta = 1.321, p = 0.156 and for SVS IVW: beta = -0.009, p = 0.990) (**Table 4**). Similar results were found for long sleep duration (for IS IVW: beta = -0.219, p = 0.805, for CES IVW: beta = 1.895, p = 0.279, for LAS IVW: beta = 2.126, p = 0.389 and for SVS IVW: beta = 1.369, p = 0.508) (**Table 5**).

None of the IWV estimates showed any heterogeneity (**Tables 2-5**). The MR-PRESSO analysis also did not show any possibility of outlier for all the estimates. Furthermore, the pleiotropy test, with very negligible intercept and insignificant p value, also indicated low chance of the pleiotropy for all estimations (all p >0.539, **Tables 2-5**). The results of the MR-RAPS were identical with the IVW estimates in almost all cases, highlighting again a low likelihood of pleiotropy. The results of the leave-one-out method demonstrated that the observed associations were not driven by single SNPs.

DISCUSSION:

In the present study, we performed a comprehensive systematic review and meta-analysis of the available prospective cohort studies, as well as conducted a MR analysis to evaluate the potential effects of sleep duration on the risk of stroke in a causal model. By pooling prospective studies, we showed that shorter and longer sleep duration might be considered as a significant risk factor for stroke. However, these results were subjected to a high level of heterogeneity, thus minimizing their validity, and highlighting the need for further, more reliable, statistical analyses. In this context, the MR analysis showed no association between sleep duration and the risk of different types of stroke, with low levels of heterogeneity and pleiotropy.

Sleep duration is generally not regarded as a traditional risk factor for CVD, but previous studies reported a negative association between short and long sleep duration with CVD morbidity (48) and CVD risk factors (49). Indeed, sleep duration and circadian rhythm disorders, as well as insomnia have been related to stroke (49,50) with approximately half of stroke survivors potentially having insomnia (50).

Growing evidence suggests that short and long sleep duration may be linked to adverse health outcomes, including total mortality (3, 4), CVD events (4, 15, 18, 51), diabetes (52) and hypertension (7, 8). The exact underlying mechanisms of a potential association between sleep duration and CVD have not been established yet. In this context, short sleep duration may increase leptin and ghrelin levels (53), leading to increased appetite and caloric intake and reduced energy expenditure, both of which

may contribute to the development of obesity (54). Increased cortisol secretion and changes in growth hormone metabolism have also been reported in relation to short sleep duration (55), as well as low-grade inflammation, which can predispose to CVD (55,56). Furthermore, epidemiological studies have shown that short sleep duration may be related to higher levels of hemoglobin A1C (56), total cholesterol, triglycerides and blood pressure (57), which are all CVD risk factors. Sleep disorders have also been suggested to affect the outcomes of CVD events (58)

Depressive symptoms, low socio-economic status, unemployment and low level of physical activity have been linked to long sleep duration and may confound the relationship of sleep duration with morbidity and mortality (59, 60). In this context, the observed harmful effects of longer sleep were confounded by depression or socioeconomic status (61).

One previous meta-analysis (n = 74 studies; 3,340,684 participants) reported no significant differences in stroke events in relation to self-reported sleep duration shorter or longer than 7 h, whereas a moderate increase in stroke mortality was observed in those sleeping ≥7 h (2). A study in 2,282 males healthy aged 35 - 54 years found no significant association between any sleep duration and stroke risk during 14 years of follow-up, after adjustment for potential confounders, including traditional CVD risk factors and working status (20). In contrast, another systemic review and meta-analysis of 15 prospective studies (n = 474,684 male and female individuals; follow-up 6.9 - 25 years) reported that both short and long sleep duration were significantly related to a greater risk of stroke (12).

Ikehara *et al.* performed a study which included 98,643 individuals (41,489 men and 57,145 women) aged 40 to 79 years and found that long sleep duration (≥10 h) was associated with a 1.5 to 2-fold increased mortality from total and ischemic stroke compared with 7 h of sleep (18). In a 10-year follow up of the NHANES cohort (n = 7844; aged ≥32 years), long (>8 h) sleep duration was related to a higher risk of stroke compared with the reference group (6 to 8 h of sleep) (16). In another meta-analysis of 16 prospective studies, the lowest stroke risk was observed in those sleeping 7 h per night; of note, in cases of >7 h sleep, total stroke risk was increased by 13% for every 1 h increase in sleep duration (61). Furthermore, the Women's Health Initiative Observational Study reported that long sleep duration was significantly associated with ischemic stroke among postmenopausal women (19). In motor-impaired, right hemisphere stroke patients, sleep latencies were longer and sleep efficiency was worse in comparison to age-and sex-matched controls (62). Furthermore, in a Taiwan administrative data study which included 21,438 participants with insomnia and 64,314 age and sex-matched without insomnia, those with insomnia had a 54% increased risk of stroke (63).

The present findings should be extrapolated with caution since our analysis has some limitations. In this context, we were unable to thoroughly evaluate individual-level confounding factors. Although we used different methods in our MR analysis, there might be still a chance of horizontal pleiotropy. Furthermore, a high level of heterogeneity was observed in the present meta-analysis. Despite this, the present analysis has strengths, including the fact that the MR analysis reduced the risk of potential reverse causation and genotypes were assumed to be randomly distributed with respect to confounders.

In conclusion, pooled data from prospective cohort studies showed that sleep duration may increase the risk of stroke, but the high level of heterogeneity observed between studies significantly minimizes the validity of these findings. Furthermore, the MR analysis reported no associations between sleep duration and the risk of different ischemic stroke types, with a low level of heterogeneity and pleiotropy.

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Table 1. Characteristics of the Prospective Cohort Studies included in the meta-analysis.

Author, year and References	Country, region/cohort	Men (%)	Mean Age	follow-up time (Years)	Assessment of sleep duration	Main confounders
Chen, 2008 (1)	Prospective cohort study, USA	0	unclear	7.5	Interview questioning	Age, race, education, family income, employment status, depression, smoking, exercise, use of hormone therapy, prior cardiovascular disease, diabetes mellitus, hypertension, high cholesterol level requiring pills and body mass index.
Hamazaki, 2011 (2)	Prospective cohort study, Japan	100	44	14	Self-administered questionnaire	Age, type of job, working hours, mental workload, body mass index, mean blood pressure, HbA1c, total cholesterol, current smoking habit, drinking habit, leisure-time physical activity, medication for hypertension, diabetes, hypercholesterolemia
Helbig, 2015 (3)	Prospective cohort study, Germany	50	48	14	Interview questioning	Age, survey, education , physical activity, alcohol consumption, current smoking activity, body mass index, hypertension, diabetes and dyslipidemia
Leng, 2015 (4)	Prospective cohort study, UK	46	62	9.5	Questionnaire	Age, sex, social class, education, marital status, smoking, alcohol intake, hypnotic drug use, family history of stroke, body mass index, physical activity, depression, systolic blood pressure, diastolic blood pressure, preexisting diabetes, myocardial infarction, cholesterol level and hypertension drug use.

Magee, 2011 (5)	Prospective cohort study, Australia	47	63	unclear	Self-reported questionnaire	Age, sex, country of birth, marital status, education, employment status, remoteness, body mass index, physical activity, smoking, alcohol and screen time.
Qureshi, 1997 (6)	Prospective cohort study, USA	36	unclear	10	Participants were interviewed	Age, sex, race, education, cigarette smoking, systolic blood pressure, serum cholesterol level, diabetes and body mass index.
Ruiter Petrov, (7)2014	Prospective cohort study, USA	44	61	2	Participants were questioned on sleep	Demographics, stroke risk factors, psychological symptoms, health behaviors and diet quality.
Song, 2016 (8)	Prospective cohort study, China	79	51	7.9	Interviews, death certificates, discharge summaries and medical records	Age, sex, marital status, income, education level, smoking status, physical activity, family history of stroke, body mass index, blood pressure, blood glucose, total cholesterol, lipid-lowering drug use, hypoglycemic drug use, history of myocardial infarction, snoring status, C-reactive protein and atrial fibrillation.
Tu, 2012 (9)	Prospective cohort study, China	0	60	4	Participants were questioned on sleep	Age, education level, occupational status, night-shift work, annual income, menopausal status, marital status, number of live births, physical activity, passive smoking tea consumption, energy intake, time spend watching TV and vitamin supplement use.
Von Ruesten, 2012 (10)	Prospective cohort study, Germany	39	49	8	Participants were interviewed about their sleep	Age, sex, sleeping disorders, alcohol intake, smoking status, walking cycling sports, employment status, education, body mass index, waist-to-hip ratio, hypertension, high blood lipid, caffeinated beverages,

						satisfaction with life, satisfaction with health and intake of antidepressants
Amagai, 2010 (11)	Prospective cohort study, Japan	39	55	10.7	Interview for sleep duration	Age, systolic blood pressure, total cholesterol, body mass index, smoking habits and alcohol drinking habits
Westerlund, 2013(12)	Prospective cohort study, Sweden	35	unclear	13	Questionnaire	Age, sex, education, employment status, smoking, alcohol, snoring, work schedule, depressive symptoms, self-rated health, physical activity, body mass index, diabetes, lipid disturbance and hypertension.

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Table 2. Results of the Mendelian Randomization analysis on the associations between self-reported sleep duration and different types of stroke.

Exposures		MR				Heterogeneity			Pleiotropy		
		Method	**beta**	**SE**	**p**	**Method**	**Q**	**p**	**Intercept**	**SE**	**p**
sleep duration (self-reported)	IS	**MR Egger**	-0.229	0.341	0.504	**MR Egger**	48.668	0.445	0.0034	0.0057	0.547
		WM	-0.019	0.154	0.901						
		IVW	-0.031	0.098	0.747	**IVW**	49.040	0.471			
		RAPS	0.004	0.103	0.967						
	CES	**MR Egger**	0.537	0.725	0.462	**MR Egger**	58.130	0.150	-0.010	0.012	0.409
		WM	0.163	0.287	0.569						
		IVW	-0.039	0.208	0.849	**IVW**	58.967	0.155			
		RAPS	0.055	0.216	0.796						
	LAS	**MR Egger**	-0.658	0.878	0.457	**MR Egger**	51.42584	0.341	0.0072	0.014	0.626
		WM	-0.131	0.382	0.731						
		IVW	-0.246	0.251	0.328	**IVW**	51.68298	0.369			
		RAPS	-0.249	0.259	0.336						
	SVS	**MR Egger**	-0.369	0.832	0.658	**MR Egger**	53.354	0.275	0.0046	0.013	0.738
		WM	-0.175	0.367	0.634						
		IVW	-0.102	0.238	0.667	**IVW**	53.479	0.306			
		RAPS	-0.159	0.258	0.536						

Weighted median: WM, Inverse variance weighted: IVW, SE: standard error, beta: beta-coefficients, RAPS: MR-Robust Adjusted Profile Score, MR: Mendelian randomization, IS: ischemic stroke, CES: cardioembolic stroke, LAS: large artery stroke, SVS: small vessel stroke

Table 3. Results of the Mendelian Randomization analysis on the associations between accelerometer derived sleep duration and different types of stroke.

Exposures		**MR**				**Heterogeneity**			**Pleiotropy**		
		Method	**beta**	**SE**	**p**	**Method**	**Q**	**p**	**Intercept**	**SE**	**p**
sleep duration (accelerometer derived**)**	IS	**MR Egger**	-0.043	0.261	0.874	**MR Egger**	3.344	0.764	0.0037	0.011	0.759
		WM	-0.006	0.132	0.963						
		IVW	0.033	0.102	0.739	**IVW**	3.446	0.840			
		RAPS	0.034	0.105	0.746						
	CES	**MR Egger**	0.233	0.523	0.671	**MR Egger**	2.190	0.901	-0.014	0.023	0.550
		WM	-0.039	0.256	0.878						
		IVW	-0.072	0.198	0.713	**IVW**	2.590	0.920			
		RAPS	-0.073	0.205	0.720						
	LAS	**MR Egger**	-0.351	0.683	0.625	**MR Egger**	6.404	0.379	0.0223	0.030	0.492
		WM	-0.132	0.336	0.693						
		IVW	0.109	0.253	0.664	**IVW**	6.975	0.431			
		RAPS	0.056	0.273	0.835						
	SVS	**MR Egger**	0.038	0.720	0.959	**MR Egger**	7.557	0.272	0.0157	0.031	0.639
		WM	0.252	0.320	0.430						
		IVW	0.367	0.254	0.149	**IVW**	7.863	0.344			
		RAPS	0.353	0.266	0.185						

Weighted median: WM, Inverse variance weighted: IVW, SE: standard error, beta: beta-coefficients, RAPS: MR-Robust Adjusted Profile Score, MR: Mendelian randomization, IS: ischemic stroke, CES: cardioembolic stroke, LAS: large artery stroke, SVS: small vessel stroke

Table 4. Results of the Mendelian Randomization analysis on the associations between short sleep duration and different types of stroke.

Exposures		MR				Heterogeneity			Pleiotropy		
		Method	**beta**	**SE**	**p**	**Method**	**Q**	**p**	**Intercept**	**SE**	**p**
Short sleep duration	IS	**MR Egger**	1.123	1.70	0.515	**MR Egger**	15.057	0.820	-0.0053	0.0114	0.643
		WM	0.261	0.461	0.571						
		IVW	0.343	0.356	0.335	**IVW**	15.278	0.850			
		RAPS	0.323	0.373	0.386						
	CES	**MR Egger**	2.089	3.732	0.581	**MR Egger**	26.571	0.185	-0.0169	0.0250	0.505
		WM	-1.590	0.986	0.106						
		IVW	-0.384	0.763	0.614	**IVW**	27.152	0.205			
		RAPS	-0.755	0.764	0.322						
	LAS	**MR Egger**	2.098	4.559	0.650	**MR Egger**	24.291	0.2791	-0.0053	0.0307	0.863
		WM	1.583	1.191	0.183						
		IVW	1.321	0.932	0.156	**IVW**	24.327	0.3303			
		RAPS	1.437	0.935	0.124						
	SVS	**MR Egger**	-0.047	3.901	0.990	**MR Egger**	21.220	0.445	0.00026	0.0263	0.992
		WM	-0.004	1.098	0.996						
		IVW	-0.009	0.822	0.990	**IVW**	21.220	0.507			
		RAPS	-0.136	0.865	0.875						

Weighted median: WM, Inverse variance weighted: IVW, SE: standard error, beta: beta-coefficients, RAPS: MR-Robust Adjusted Profile Score, MR: Mendelian randomization, IS: ischemic stroke, CES: cardioembolic stroke, LAS: large artery stroke, SVS: small vessel stroke

Table 5. Results the Mendelian Randomization analysis on the associations between long sleep duration and different types of stroke.

Exposures		MR				Heterogeneity			Pleiotropy		
		Method	**beta**	**SE**	**p**	**Method**	**Q**	**p**	**Intercept**	**SE**	**p**
Long sleep duration	IS	**MR Egger**	1.836	2.531	0.495	**MR Egger**	2.311	0.888	-0.012	0.0139	0.418
		WM	-0.132	1.096	0.903						
		IVW	-0.219	0.891	0.805	**IVW**	3.064	0.878			
		RAPS	-0.222	0.929	0.810						
	CES	**MR Egger**	-3.836	5.247	0.492	**MR Egger**	4.742	0.577	0.0328	0.0283	0.290
		WM	2.074	2.229	0.352						
		IVW	1.895	1.751	0.279	**IVW**	6.085	0.529			
		RAPS	2.396	1.845	0.194						
	LAS	**MR Egger**	16.35	6.517	0.045	**MR Egger**	3.113	0.794	-0.0823	0.0355	0.059
		WM	3.095	2.898	0.285						
		IVW	2.126	2.472	0.389	**IVW**	8.521	0.288			
		RAPS	3.084	2.702	0.253						
	SVS	**MR Egger**	5.993	5.924	0.350	**MR Egger**	3.877	0.693	-0.027	0.0325	0.436
		WM	1.313	2.640	0.619						
		IVW	1.369	2.072	0.508	**IVW**	4.571	0.712			
		RAPS	1.394	2.178	0.522						

Weighted median: WM, Inverse variance weighted: IVW, SE: standard error, beta: beta-coefficients, RAPS: MR-Robust Adjusted Profile Score, MR: Mendelian randomization, IS: ischemic stroke, CES: cardioembolic stroke, LAS: large artery stroke, SVS: small vessel stroke

Supplementary Table 1. MOOSE Reporting Checklist for Authors, Editors, and Reviewers of Meta-analyses of Observational Studies

Reporting of background should include:	**Page number:**
Problem definition	5
Hypothesis statement	5
Description of study outcome(s)	5
Type of exposure or intervention used	5
Type of study designs used	5
Study population	5
Reporting of search strategy should include:	**Page number:**
Qualifications of searchers (eg, librarians and investigators)	1
Search strategy, including time period included in the synthesis and keywords Effort to include all available studies, including contact with authors Databases and registries searched	Appendix 1 6-7 6-7
Search software used, name and version, including special features used (eg, explosion) Use of hand searching (eg, reference lists of obtained articles)	6-7 6-7
List of citations located and those excluded, including justification Method of addressing articles published in languages other than English Method of handling abstracts and unpublished studies	Figure 1 - 6-7
Description of any contact with authors	-
Reporting of methods should include:	**Page number:**

Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	6-7
Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	6-7
Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	6-7
Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	6-7
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	6-7
Assessment of heterogeneity	6-7
Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	6-7
Provision of appropriate tables and graphics	Tables and Figures
Reporting of results should include:	**Page number:**
Graphic summarizing individual study estimates and overall estimate	Figures
Table giving descriptive information for each study included	Table
Results of sensitivity testing (eg, subgroup analysis)	8-12
Indication of statistical uncertainty of findings	8-12
Reporting of discussion should include:	**Page number:**
Quantitative assessment of bias (eg, publication bias)	13-16
Justification for exclusion (eg, exclusion of non–English-language citations)	-
Assessment of quality of included studies	13-16
Reporting of conclusions should include:	**Page number:**
Consideration of alternative explanations for observed results	16-17
Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	16-17
Guidelines for future research	-
Disclosure of funding source	17

Supplementary Table 2. Full search terms and strategy for papers indexed in PUBMED.		
No	**Concept**	**Search terms**
1	**Stroke**	cerebrovascular[tiab] OR stroke[tiab] OR TIA[tiab] OR transient ischemic*[tiab] OR CVA[tiab] OR cerebral infarction[tiab] OR Cerebrovascular accident [Mesh:NoExp] OR stroke[Mesh:NoExp]
2	**Sleep duration**	sleep duration [tiab]
3	**Combination Exposure And Outcome**	#1 AND #2
4	**Limit**	Rats[Mesh:NoExp]) OR Mice[Mesh:NoExp]) OR rat[Title/Abstract]) OR rats[Title/Abstract]) OR mouse[Title/Abstract]) OR mice[Title/Abstract]) OR vivo[Title/Abstract]) OR vitro[Title/Abstract])
5	**Limit**	#3 NOT #4

NEWCASTLE – OTTAWA QUALITY ASSESSMENT SCALE - COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average *healthy adults* in the community★

b) somewhat representative of the average *healthy adults* in the community ★

c) selected group of users *e.g. nurses, volunteers, vegetarian*

d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort

a) drawn from the same community as the exposed cohort★

b) drawn from a different source

c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure

a) secure record (*e.g. 7 day food diary)* ★

b) structured interview/≥ 2 *dietary recalls/diet history/ food frequency questionnaire validated for dairy components* ★

c) written self-report (*e.g. <2 dietary recalls/non-validated food frequency questionnaire or not reported whether food frequency questionnaire was validated*)

d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes★

b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for *age, sex, smoking, total energy intake, and body mass index*★

b) study controls for any additional factor (*e.g. physical activity, alcohol intake, family history of diabetes, dietary factors*) ★

Outcome

1) Assessment of outcome

a) independent blind assessment (e.g. clinical diagnosis/complete medical information available)★
b) record linkage/*medical record or validated self-report* ★
c) non-validated self-report
d) no description
2) Was follow-up long enough for outcomes to occur
a) yes/ *follow up period for outcome of interest is 10 years or over* ★
b) no
3) Adequacy of follow-up of cohorts
a) complete follow-up - all subjects accounted for ★
b) subjects lost to follow-up unlikely to introduce bias - small number lost ≤*20%* follow-up, or description provided of those lost ★
c) follow-up rate <*80%* or no description of those lost
d) no statement

Supplemental Table 3. Quality assessment of selected cohorts studies.

Studies	Selection				Comparability	Outcome			Total score
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	
Chen, 2008 (1)	C	A★	B★	A★	A★ B★	B★	B	B★	9
Hamazaki, 2011 (2)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Helbig, 2015 (3)	C	A★	B★	A★	A★ B★	B★	A★	B★	8

Leng, 2015 (4)	C	A★	B★	A★	A★ B★	B★	B	B★	9
Magee, 2011 (5)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Qureshi, 1997 (6)	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Ruiter Petrov, (7)2014	C	A★	B★	A★	A★ B★	B★	A★	B★	8
Song, 2016 (8)	B★	A★	C	A★	A★ B★	B★	A★	C	7
Tu, 2012 (9)	C	A★	B★	A★	A★ B★	B★	B	B★	9
Von Ruesten, 2012 (10)	B★	A★	C	A★	A★ B★	B★	A★	C	7
Amagai, 2010 (11)	C	A★	B★	A★	A★ B★	B★	B	B★	9
Westerlund, 2013(12)	C	A★	B★	A★	A★ B★	B★	A★	B★	8

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12. Westerlund A, Bellocco R, Sundström J, Adami H-O, Åkerstedt T, Lagerros YT. Sleep characteristics and cardiovascular events in a large Swedish cohort. European journal of epidemiology. 2013;28(6):463-73.

Supplementary Table 4. Summary results of the genetic loci of self-reported or derived by accelerometer sleep duration

SNP	GX	GX SE	EA	OA	EAF
Self-reported sleep duration					
rs915416	0.019259	0.002495	C	G	0.289947
rs269054	-0.01364	0.002293	T	A	0.577924
rs12567114	-0.01483	0.00254	G	A	0.724198
rs62120041	0.026111	0.004575	T	C	0.933902
rs374153	0.017612	0.003103	C	T	0.158085
rs75539574	-0.03625	0.004065	A	C	0.914208
rs7556815	-0.04072	0.00274	G	A	0.780856
rs12611523	0.012635	0.002276	A	G	0.545244
rs4538155	-0.01298	0.002374	C	T	0.352574
rs10173260	-0.01284	0.002313	T	C	0.393765
rs112230981	0.031528	0.005228	A	G	0.94984
rs17732997	0.012935	0.002288	C	G	0.569098
rs7644809	0.013062	0.002301	T	C	0.421606
rs13088093	-0.01627	0.002402	T	G	0.663683
rs2192528	0.013369	0.002269	A	G	0.480065
rs17427571	0.013826	0.002435	A	G	0.684313
rs35531607	-0.01284	0.002273	T	C	0.525917
rs13109404	0.031204	0.004408	T	G	0.928024
rs365663	0.014629	0.002279	A	G	0.545963
rs56372231	-0.01694	0.0024	C	T	0.665907
rs180769	0.012724	0.002294	T	C	0.424698
rs151014368	-0.01609	0.00282	G	A	0.793742
rs34556183	0.016923	0.002523	A	G	0.719606
rs80193650	-0.01684	0.003067	A	G	0.837534

rs9382445	0.014536	0.002334	T	C	0.62305
rs2231265	-0.01496	0.002699	A	G	0.227711
rs34731055	-0.01946	0.002948	C	T	0.81911
rs2079070	0.017548	0.002566	C	G	0.264613
rs7806045	0.014792	0.002626	T	C	0.754703
rs330088	-0.01447	0.002277	T	C	0.452988
rs10973207	-0.02043	0.003124	G	T	0.842323
rs1776776	0.019963	0.003411	T	C	0.873832
rs12246842	0.013395	0.002274	A	G	0.459815
rs10761674	0.012333	0.002266	C	T	0.477334
rs11190970	0.015379	0.002823	G	A	0.798661
rs7915425	0.019064	0.00299	T	C	0.174682
rs1517572	-0.01464	0.002295	A	C	0.419464
rs4592416	-0.01468	0.00227	A	G	0.535593
rs174560	-0.01358	0.002437	T	C	0.685785
rs12791153	-0.02355	0.004217	A	T	0.918911
rs1939455	0.020425	0.003561	G	T	0.879446
rs1263056	0.012799	0.002277	A	G	0.519099
rs34354917	0.013746	0.002501	C	A	0.710472
rs11614986	0.016379	0.002951	A	G	0.820952
rs6575005	0.015564	0.002642	T	C	0.757854
rs61985058	-0.01859	0.003229	C	T	0.856824
rs11621908	0.024095	0.004163	C	T	0.917141
rs8038326	0.01592	0.002541	A	G	0.72691
rs3095508	0.015352	0.002304	C	A	0.593529
rs11643715	-0.0139	0.002497	C	G	0.709058
rs9940646	0.016946	0.002291	C	G	0.577569
rs7503199	0.014745	0.002564	C	T	0.734267
rs1991556	0.016566	0.002724	G	A	0.773765

rs12607679	0.020139	0.002593	T	C	0.737717
rs10421649	-0.0133	0.002295	T	A	0.44303
rs2072727	0.013243	0.002285	T	C	0.43617
Accelerometer derived sleep duration					
rs2660302	0.041	0.006	A	T	0.811
rs113851554	0.11	0.011	G	T	0.943
rs62158170	0.054	0.006	G	A	0.217
rs17400325	0.066	0.012	T	C	0.958
rs72828540	0.041	0.005	T	C	0.752
rs9369062	0.033	0.005	C	A	0.292
rs2975734	0.027	0.005	C	G	0.561
rs13282541	0.032	0.005	C	T	0.739
rs2880370	0.028	0.005	A	T	0.67
rs800165	0.028	0.005	T	T	0.343
rs10138240	0.029	0.005	G	G	0.514
Short Sleep					
rs7524118	-0.00576	0.001054	T	C	0.291624
rs2186122	-0.00567	0.000972	A	T	0.438434
rs12567114	0.006325	0.001077	G	A	0.7246
rs2820313	-0.00601	0.00101	A	G	0.658888
rs1380703	-0.00676	0.001005	A	G	0.616469
rs2863957	0.01019	0.001161	C	A	0.781508
rs2014830	0.005786	0.00105	C	T	0.698128
rs17005118	-0.00648	0.001087	G	A	0.735064
rs13107325	-0.01327	0.001828	C	T	0.925472
rs12518468	-0.00589	0.001021	T	C	0.671544
rs3776864	0.005724	0.001019	A	C	0.66721
rs4585442	-0.00635	0.001036	A	G	0.688977
rs12661667	-0.00602	0.001087	C	T	0.736505

rs9367621	0.005445	0.00097	T	A	0.43104
rs9321171	0.005354	0.000966	C	T	0.540122
rs11763750	0.007212	0.001234	G	A	0.814346
rs1229762	-0.00724	0.001017	C	T	0.335499
rs60882754	0.011304	0.002001	A	T	0.938985
rs1607227	0.006369	0.001055	G	T	0.704938
rs7939345	0.006498	0.001182	T	G	0.207569
rs17388803	-0.00983	0.001587	A	C	0.894352
rs59779556	0.005491	0.000966	T	G	0.553827
rs205024	0.00551	0.000986	C	T	0.616724
rs12963463	0.007114	0.00106	C	T	0.299425
rs5757675	0.006455	0.001099	G	T	0.259528
Long Sleep					
rs10899255	-0.00562	0.001032	G	A	0.855558
rs149980149	-0.01192	0.002226	C	G	0.971827
rs5848	0.004465	0.00082	C	T	0.721799
rs62158160	-0.00501	0.00083	C	T	0.742431
rs72630583	-0.0047	0.000882	C	T	0.783789
rs73608603	-0.00598	0.001073	A	G	0.867766
rs75458655	-0.01673	0.002423	C	T	0.977027
rs8047395	0.003957	0.000728	G	A	0.50028

EA: effect allele; OA: other allele, EAF: effect allele frequency; GX: the per-allele effect on standard deviation units of the telomere length; GX SE: standard error of GX.

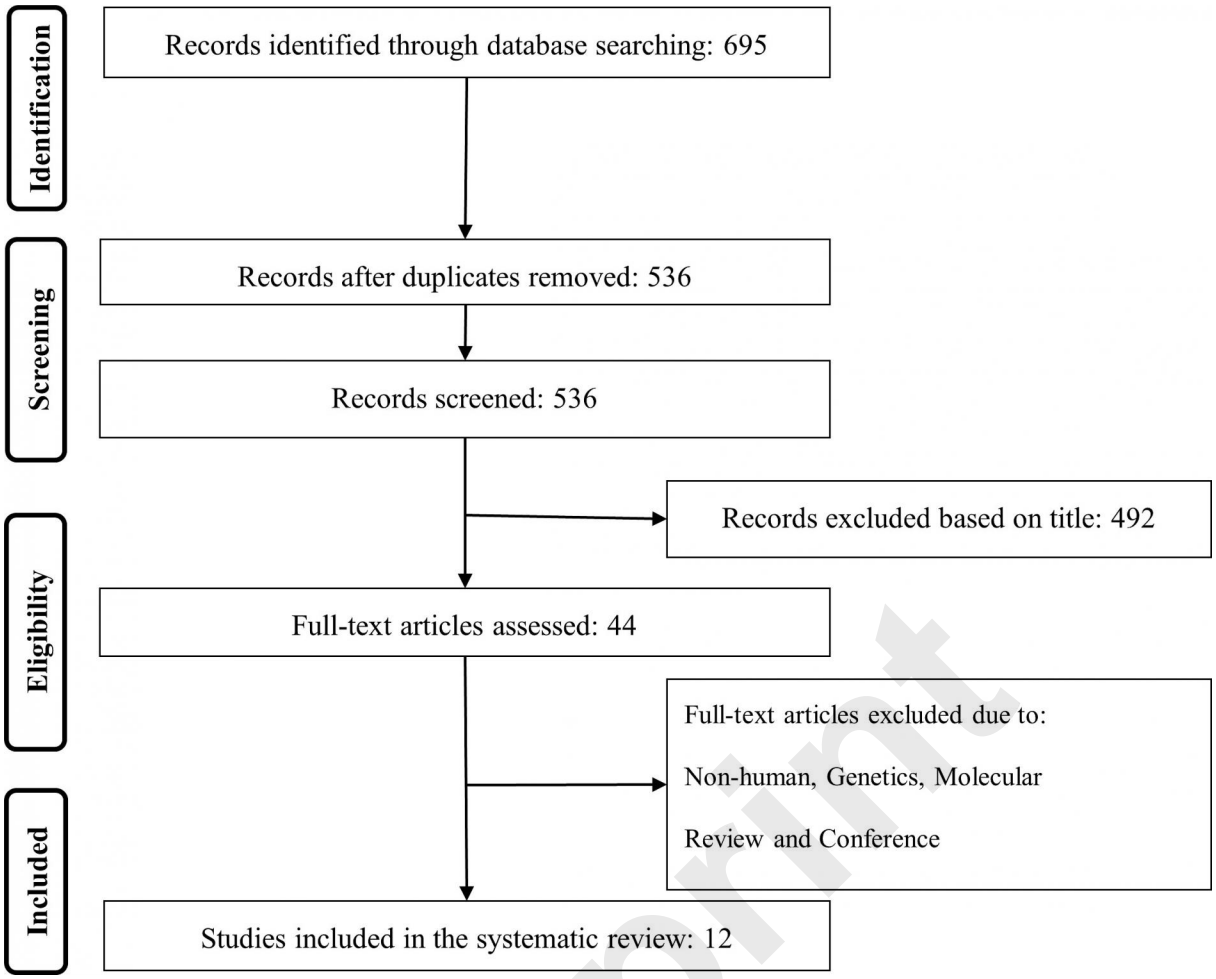

Figure 1. Flow chart of study selection.

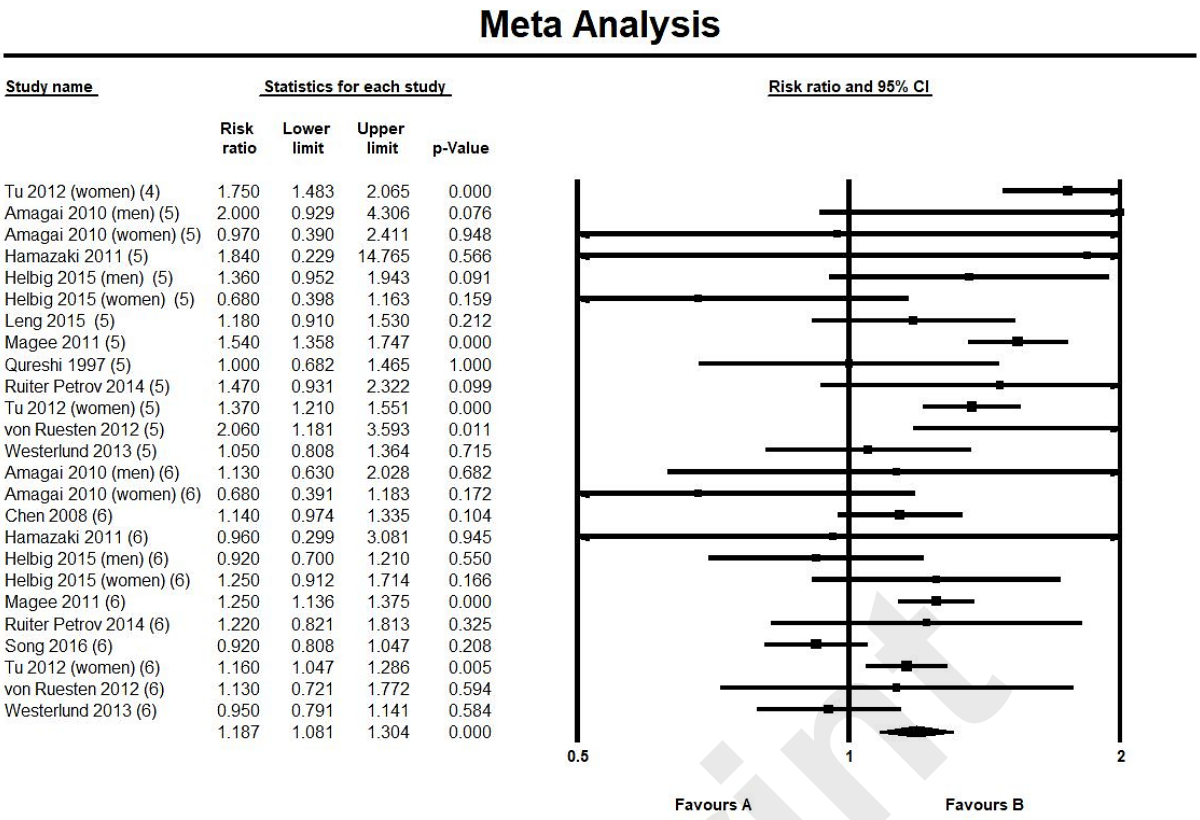

Figure 2: Forest plot of stroke risk associated with <7 hours sleep (Favours A) compared to 7-8 hours sleep (Favours B) from 25 studies. Results are expressed as risk ratios and 95% confidence intervals. Pooled analysis: p <0.001; heterogeneity test: I2 = 71.4, p <0.001. The area of each block is proportional to the weight attributed to each study.

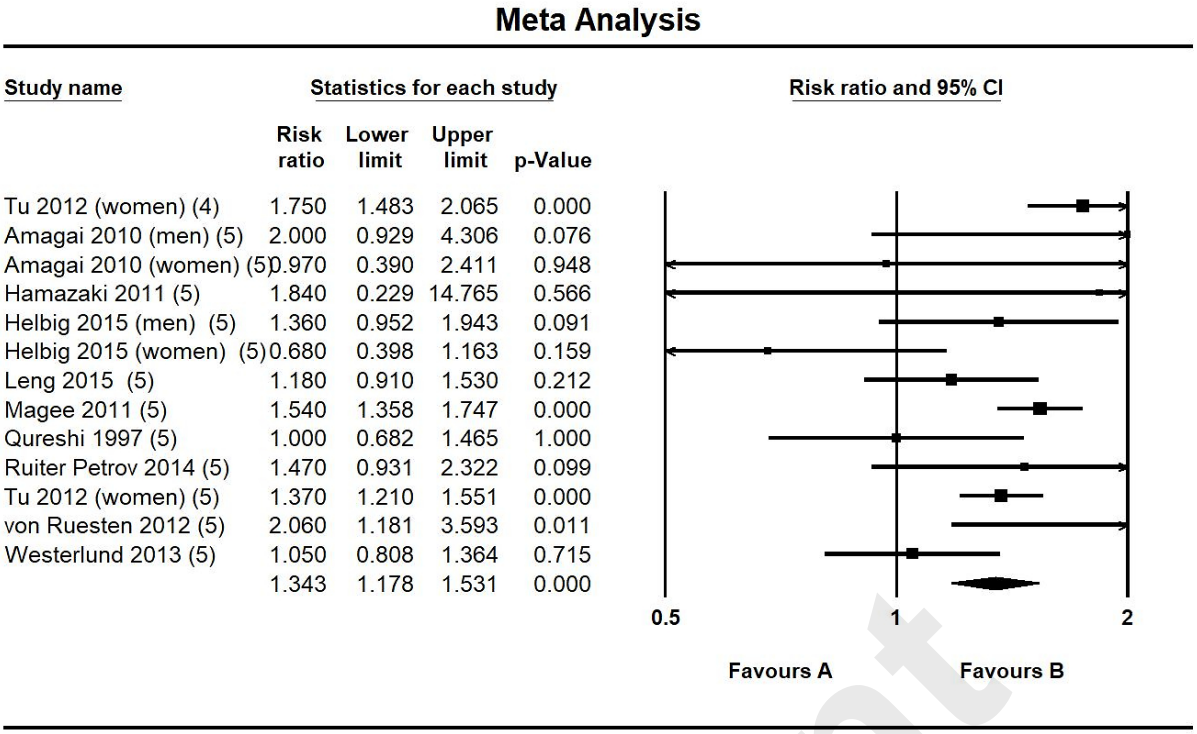

Figure 3: Forest plot of stroke risk associated with <5 hours sleep (Favours A) compared to 7-8 hours sleep (Favours B) from 13 studies. Results are expressed as risk ratios and 95% confidence intervals. Pooled analysis: p <0.001; heterogeneity test: I2 = 58.4, p <0.001. The area of each block is proportional to the weight attributed to each study.

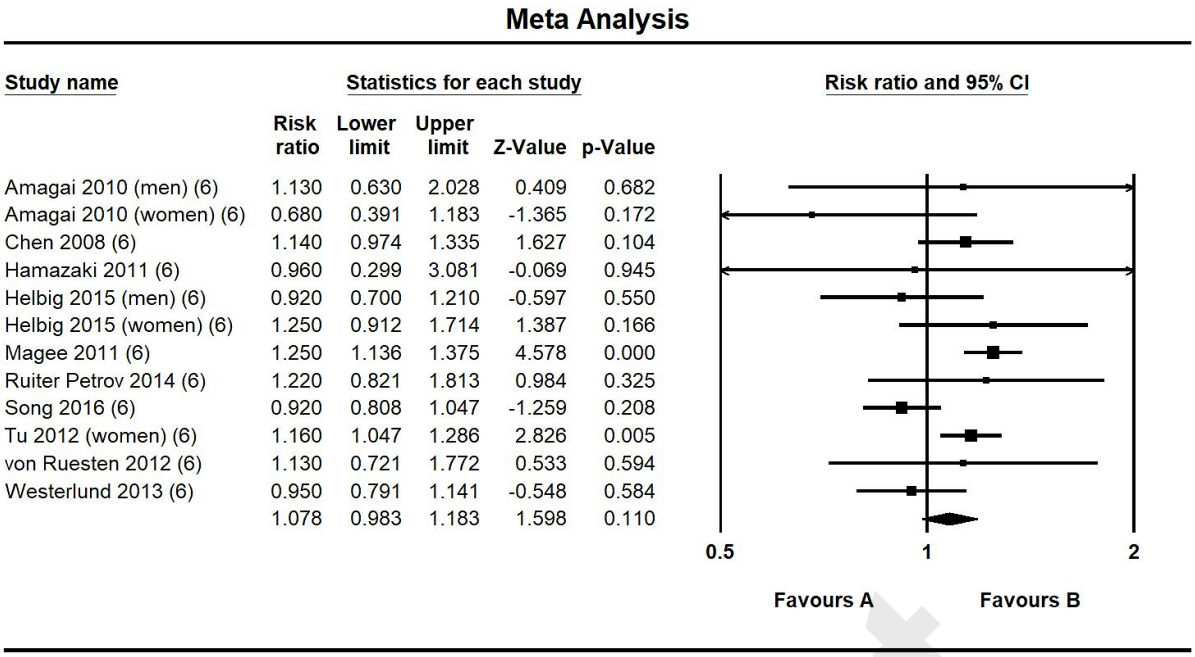

Figure 4: Forest plot of stroke risk associated with 6 hours sleep (Favours A) compared to 7-8 hours sleep (Favours B) from 12 studies. Results are expressed as risk ratios and 95% confidence intervals. Pooled analysis: p = 0.110; heterogeneity test: I2 = 52.6, p = 0.016. The area of each block is proportional to the weight attributed to each study.

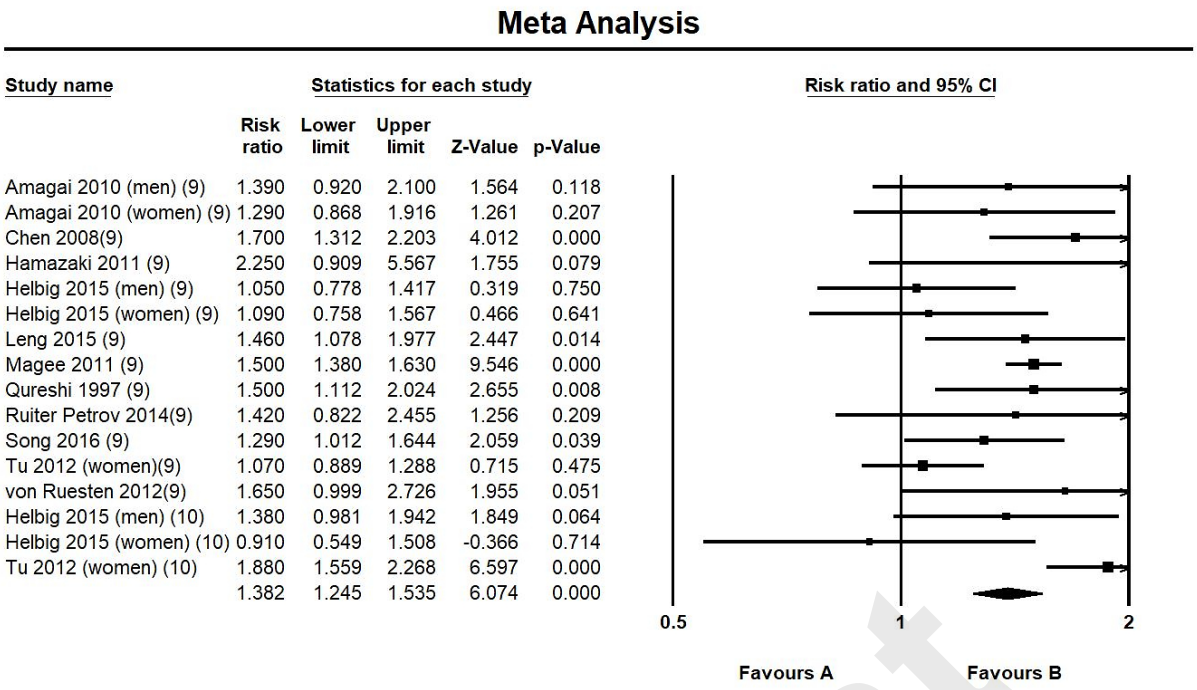

Figure 5: Forest plot of stroke risk associated with >8 hours sleep (Favours A) compared to 7-8 hours sleep (Favours B) from 16 studies. Results are expressed as risk ratios and 95% confidence intervals. Pooled analysis: p <0.001; heterogeneity test: I2 = 53.6, p <0.001. The area of each block is proportional to the weight attributed to each study.