



# Association between telomere length and risk of ischemic stroke: a systematic review and meta-analysis

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## ABSTRACT

**Background:** Telomere shortening reflects biological aging and cumulative cellular stress and has been proposed as a marker of ischemic stroke susceptibility.

**Objective:** This systematic review and meta-analysis evaluated the association between leukocyte telomere length and ischemic stroke risk.

**Methods:** PubMed, Scopus, CINAHL, OpenGrey, and ClinicalTrials.gov were searched for studies published from January 2003 to December 2025. Eligible studies included cohort, case-control, and Mendelian randomization studies assessing leukocyte or genetically predicted telomere length in relation to ischemic stroke. Two reviewers independently screened studies, extracted data, and assessed quality using the Newcastle-Ottawa Scale and Q-Genie tool. Odds ratios were pooled using a random-effects model.

**Results:** Seven studies were included: one cohort study, one nested case-control study, and five Mendelian randomization studies. Telomere length was not significantly associated with ischemic stroke risk overall (OR = 1.00, 95% CI 0.92-1.07;  $p = 0.90$ ;  $I^2 = 20.8\%$ ). Subgroup analyses by ischemic stroke subtype also showed no significant associations for cardioembolic stroke, large-artery atherosclerosis, or small-vessel occlusion. Fixed-effect sensitivity analysis yielded consistent findings.

**Conclusions:** Current evidence does not provide strong support for an independent or causal association between leukocyte telomere length and ischemic stroke risk. However, confidence is limited by the small number of studies, heterogeneous study designs, and variation in telomere measurement methods. Further studies using standardized methods and diverse populations are warranted.

**Registration:** PROSPERO CRD420251230165.

**Keywords:** Telomere length; ischemic stroke; Mendelian randomization; meta-analysis; systematic review

## 1. Introduction

Stroke continues to impose a substantial global health burden and remains one of the most important causes of death and persistent disability. The World Stroke Organization estimates that approximately 12 million new cases and 7 million deaths occur each year.<sup>1</sup> Ischemic stroke accounts for the majority of events, representing about 65% of all strokes.<sup>1</sup> Although established risk factors such as smoking, hypertension, diabetes mellitus, and older age are central to risk assessment, they do not completely explain why some individuals are more susceptible than others.<sup>2</sup> This gap has encouraged interest in biomarkers that may better reflect underlying biological processes.

Telomeres are repetitive DNA sequences (TTAGGG) that cap chromosome ends and help preserve genomic integrity by limiting degradation and chromosomal fusion.<sup>3</sup> Because telomeres shorten with repeated cell division, telomere length is widely viewed as an indicator of biological aging.<sup>4</sup> In addition to genetic influences, telomere length is shaped by environmental and lifestyle exposures, including oxidative stress and chronic inflammation.<sup>5</sup> Telomere shortening contributes to vascular aging, endothelial dysfunction, and atherosclerosis, all of which are relevant to the pathogenesis of ischemic stroke.<sup>6</sup> For this reason, leukocyte telomere length has attracted attention as a potential biomarker linking biological aging with cerebrovascular disease.

A systematic review of observational studies has suggested that shorter-than-expected telomeres may be associated with an increased risk of ischemic stroke, as well as other chronic disorders such as diabetes, hypertension, and malignancy.<sup>7</sup> However, the published findings remain inconsistent. Some investigations have reported that the shorter the telomeres, the higher the risk of stroke.<sup>8,9</sup> Others have found no clear association or have reported conflicting results across stroke subtypes.<sup>10,11</sup> Such variation may stem from differences in population characteristics, methods for telomere measurement, and analytical strategies. Conventional observational designs are

also vulnerable to residual confounding because telomere length is influenced by many factors that are themselves related to stroke risk. In addition, when telomere length is measured after stroke onset, reverse causation becomes a concern.

Mendelian randomization (MR) offers a complementary strategy for addressing some of these limitations by using genetic variants associated with telomere length as instrumental variables.<sup>12</sup> Because such variants are allocated at conception, MR analyses are generally less prone to confounding and reverse causation, thereby providing a stronger framework for causal inference.<sup>13</sup> Even so, MR evidence on telomere length and ischemic stroke remains limited and has not been comprehensively synthesized alongside observational studies. Therefore, this study systematically reviewed and meta-analyzed the association between leukocyte telomere length and the risk of ischemic stroke. We also explored findings across ischemic stroke subtypes and considered evidence from both observational studies and Mendelian randomization analyses to provide a more complete appraisal of the question.

## 2. Materials and Methods

### 2.1 Search strategy and data sources

The protocol was prospectively registered in PROSPERO (registration number: CRD420251230165). We searched PubMed, CINAHL, Scopus, OpenGrey, and Clinical Trials.gov for relevant studies published from January 2003 through December 2025. Search terms captured both ischemic stroke and telomere biology and were adapted to the indexing system of each database. The search strategy was developed iteratively and tailored to each database to maximize sensitivity while maintaining specificity. Controlled vocabulary, such as MeSH when available, was combined with free-text keywords, and no geographic restrictions were applied. Full database-specific search strategies, including Boolean operators, keywords, and indexing terms, are provided in Supplementary Material 1. The review was conducted in accordance with the PRISMA 2 020

guidelines. A completed PRISMA checklist is provided in Supplementary Material 3.

## 2.2 Eligibility criteria

Observational studies (cohort or case-control) and Mendelian randomization investigations that evaluated the association between leukocyte telomere length and the risk of ischemic stroke were included. Eligible studies were required to: (1) use a cohort, case-control, or Mendelian randomization design; (2) include participants without a prior history of stroke at baseline, where applicable; (3) assess leukocyte telomere length or genetically predicted telomere length; (4) report ischemic stroke outcomes, either overall or by subtype; and (5) provide unadjusted or adjusted effect estimates with 95% confidence intervals, or sufficient data for effect size derivation. We excluded non-original reports, studies without accessible full text, reports based on overlapping datasets, and studies that combined ischemic stroke with broader cardiovascular outcomes without providing stroke-specific results.

## 2.3 Study selection

After removing duplicates in EndNote (Version 20), records were screened in two sequential stages: first by title and abstract, and then through full-text review. Two reviewers independently evaluated eligibility, and disagreements were solved through discussion until consensus was reached. The study selection pathway is summarized in the PRISMA flow diagram.

## 2.4 Data extraction

Two reviewers extracted data independently using a prespecified collection form. Information recorded from each study included design characteristics, participant and population details, methods used to measure telomere length, stroke outcomes, reported effect estimates, and covariates included in adjusted analyses. When several estimates were available, we selected the most fully adjusted result. Any discrepancies during extraction were resolved by consensus.

## 2.5 Quality assessment

Methodological quality was appraised independently by two reviewers. Observational

studies were assessed with the Newcastle-Ottawa Scale (NOS), whereas Mendelian randomization studies were evaluated with the Q-Genie tool.<sup>14,15</sup> Differences in assessment were resolved through discussion. We explored possible publication bias by visually examining funnel plots.

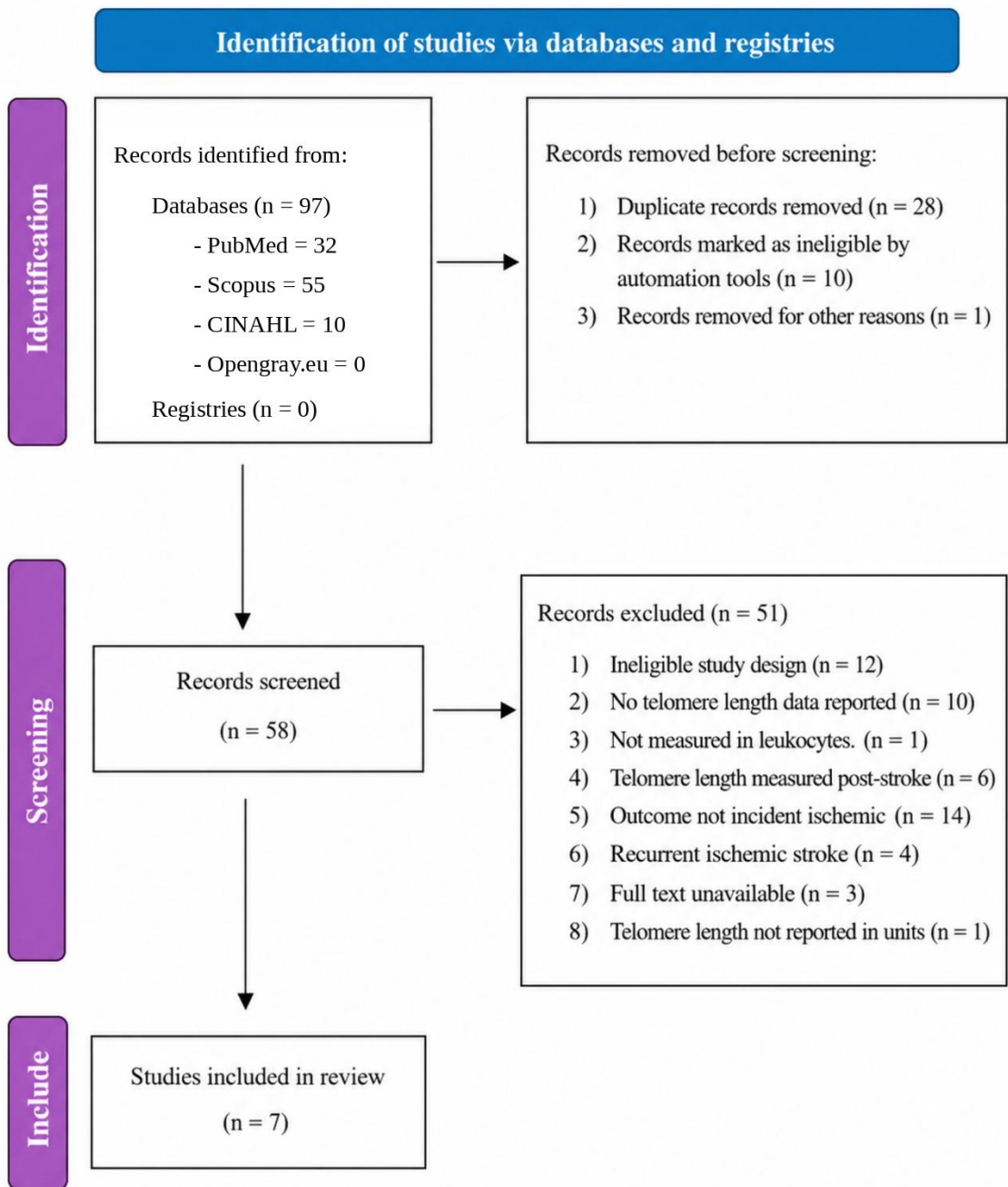
## 2.6 Statistical analysis

Meta-analysis was conducted using MetaAnalysisOnline/NetMetaEasy, an online web-based program for meta-analysis.<sup>16</sup> Summary effect estimates were analyzed with a random-effects model using the DerSimonian-Laird approach. Odds ratios were chosen as the common effect measure, and relative risks and hazard ratios were treated as approximately equivalent because ischemic stroke was a relatively infrequent outcome in the source populations. Statistical heterogeneity was quantified with the  $I^2$  statistic. Prespecified subgroup analyses were performed according to study design and ischemic stroke subtype when data allowed. Statistical significance was defined as a two-sided  $p$ -value  $< 0.05$ . A fixed-effect sensitivity analysis was additionally performed using Review Manager (RevMan), version 5.4.1.

# 3. Results

## 3.1 Study selection and characteristics

The database search identified 97 records. Fifty-eight full-text reports were assessed for eligibility; 51 were excluded for the following reasons: ineligible study design ( $n = 12$ ), absence of telomere length data ( $n = 10$ ), telomere length not measured in leukocytes ( $n = 1$ ), measurement after stroke onset ( $n = 6$ ), outcomes not specific to ischemic stroke ( $n = 14$ ), focus on recurrent stroke ( $n = 4$ ), unavailable full text ( $n = 3$ ), and missing telomere length units ( $n = 1$ ). Seven studies met the inclusion criteria and were included in the final analysis (Figure 1). Detailed database-specific search outputs are provided in Supplementary Material 1.



**Fig. 1.** PRISMA flow diagram for the systematic review of the association between telomere length and risk of ischemic stroke.

Of these, two were observational studies conducted in the United States: one prospective cohort (n = 2,518) by Mwasongwe et al.<sup>17</sup> and one nested case-control study (1,008 participants; 504 pairs) by Schürks et al.<sup>18</sup> Both included populations with a mean age above 50 years and compared shorter versus longer telomere length, reporting hazard ratios or odds ratios. Findings from observational studies were inconsistent. In the cohort study, longer telomere length was associated with a lower risk of ischemic stroke (adjusted HR 0.69, 95% CI 0.48-0.99);<sup>17</sup> however, the association was attenuated and no longer significant after multivariable adjustment. Similarly, the nested case-control study found no significant association between relative telomere length and incident ischemic stroke (adjusted OR 0.82, 95% CI 0.52-1.32), with no clear dose-response relationship.<sup>18</sup>

Five studies applied MR approaches, including four two-sample designs and one one-sample design. These studies were conducted in populations of European or Asian ancestry and utilized genome-wide association study (GWAS) summary statistics or hospital-based datasets. Across the included MR studies, genetically predicted telomere length was not significantly associated with ischemic stroke. Two-sample MR analyses using large GWAS datasets, including Cao et al.<sup>19</sup> and Imahori et al.<sup>20</sup> reported no significant associations with overall ischemic stroke or its subtypes. Similarly, Dang et al.<sup>21</sup> which focused on lacunar stroke, reported a non-significant

association using inverse-variance weighted analysis (adjusted OR 0.90, 95% CI 0.67-1.22). A one-sample MR study by Cao et al.<sup>22</sup> based on a hospital-based case-control dataset, also reported no significant association between telomere length and ischemic stroke (adjusted OR 1.31, 95% CI 0.98-1.76). In addition, Maimaiti et al.,<sup>23</sup> which incorporated both observational and MR analyses with DNA methylation-based measures, reported no causal effect of telomere length on ischemic stroke.

All included studies evaluated overall ischemic stroke, with additional subtype analyses reported in some studies: cardioembolic stroke (n = 2), large-artery atherosclerosis (n = 2), and small-vessel occlusion (n = 3) (Table 1). Definitions of shorter telomere length varied across observational studies, including absolute thresholds (<6.33 kb) and relative telomere length measures (e.g., RTL < 0.540). For quality assessment, both observational studies had high methodological quality. However, MR studies had several methodological limitations, including incomplete reporting of Hardy-Weinberg equilibrium testing, limited description of sampling procedures, and insufficient reporting of sample size or power calculations in some studies (Table 2). Overall, evidence from both observational and genetic approaches did not support a robust association between telomere length and the risk of ischemic stroke.

**Table 1.** Characteristics of the included studies.

| No. | Author (Year)          | Setting                | Study design/Population        | Telomere Length Assessment Method       | Stroke type     | MR analysis technique | Genetic instrument | No. SNV | Exposure data source                                  | Outcome data source                                                                  | Effect estimate                                   |
|-----|------------------------|------------------------|--------------------------------|-----------------------------------------|-----------------|-----------------------|--------------------|---------|-------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------|
| 1   | Schürks et al (2013)   | USA (female)           | Nested case-control; 504 pairs | qPCR RTL (T/S ratio from leukocyte DNA) | Ischemic stroke | N/A                   | N/A                | N/A     | Peripheral blood leukocyte DNA (Nurses' Health Study) | Incident ischemic stroke (case-control)                                              | OR 0.82 (95% CI 0.52-1.32)                        |
| 2   | Mwasongwe et al (2017) | USA (African American) | Prospective cohort (n=2,518)   | Southern blot (LTL from leukocyte DNA)  | Ischemic stroke | N/A                   | N/A                | N/A     | Peripheral blood leukocytes (Jackson Heart Study)     | Incident ischemic stroke within Jackson Heart Study; follow-up approximately 9 years | HR 0.69 (95% CI 0.48-0.99), age- and sex-adjusted |

Table 1. (Cont).

| No. | Author (Year)         | Setting             | Study design/Population                                                              | Telomere Length Assessment Method                                                                                                       | Stroke type                                                                     | MR analysis technique                                                                   | Genetic instrument                                                                                                            | No. SNV | Exposure data source                                                                                     | Outcome data source                                                                                     | Effect estimate                                          |
|-----|-----------------------|---------------------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|---------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|----------------------------------------------------------|
| 3   | Cao et al (2019)      | Europe              | Two-sample Mendelian randomization study (GWAS-based; European ancestry)             | Microarray (GWAS); telomere length by qPCR                                                                                              | Ischemic stroke; large-artery stroke; cardioembolic stroke; small-vessel stroke | Two-sample MR (WM, PWM, IVW, PIVW, RIVW, MR-Egger)                                      | GWAS SNPs for TL, including TERC, TERT, NAF1, OBF1, ZNF208, RTEL1, and ACYP2                                                  | 7       | GWAS meta-analysis for telomere length (n approximately 37,684)                                          | MEGASTROKE Consortium (40,585 cases / 406,111 controls)                                                 | OR approximately 1.00; non-significant across MR methods |
| 4   | Cao et al (2020)      | China (Han Chinese) | One-sample MR study; case-control dataset (431 ischemic stroke cases / 304 controls) | qPCR (LTR)                                                                                                                              | Ischemic stroke                                                                 | One-sample MR (linear: IVW, maximum likelihood, mode-based; non-linear: semiparametric) | GWAS SNPs: ZNF676 (rs412658) and ACYP2 (rs11125529)                                                                           | 2       | Jidong Oil-field Hospital and Beijing Tiantan Hospital; selected from NHGRI-EBI GWAS Catalog and Ensembl | Hospital-based case-control cohort (431 cases / 304 controls)                                           | OR 1.31 (95% CI 0.98-1.76), non-significant              |
| 5   | Dang et al (2023)     | Europe              | Two-sample MR study (GWAS-based; European ancestry; n approximately 254,459)         | qPCR (LTR)                                                                                                                              | Lacunar stroke (small-vessel stroke subtype)                                    | Two-sample MR (IVW, MR-Egger, WM, simple mode, weighted mode, MR-PRESSO)                | GWAS SNPs including PARP1, SENP7, MOB1B, TERT, CARMIL1, POT1, STN1/OBF1, ATM, TERF2, RFWD3, MPHOSPH6, ZNF208, and RTEL1/STMN3 | 13      | GWAS LTL (n=78,592)                                                                                      | Cerebrovascular Disease Knowledge Portal / GWAS lacunar stroke dataset (6,030 cases / 248,929 controls) | OR 0.90 (95% CI 0.67-1.22), non-significant (IVW)        |
| 6   | Maimaiti et al (2024) | China               | Observational cohort + MR study                                                      | Genetically predicted telomere length (GWAS-derived; MR analysis); DNA methylation measured using Illumina HumanMethylation450 BeadChip | Ischemic stroke, including LAS, CES, and SVS subtypes                           | Two-sample MR                                                                           | GWAS-derived SNPs (not specified)                                                                                             | 133     | Whole blood DNA methylation (Illumina 450K array; GEO dataset)                                           | Multi-ancestry GWAS meta-analysis of ischemic stroke (67,162 cases / 454,450 controls)                  | No causal effect of telomere length on ischemic stroke   |
| 7   | Imahori et al (2025)  | Europe (Sweden)     | Two-sample MR (GWAS-based)                                                           | Genetically predicted telomere length (GWAS-derived)                                                                                    | Ischemic stroke                                                                 | IVW, weighted median, MR-Egger, MR-PRESSO                                               | SNPs associated with aging biomarkers (GWAS-derived)                                                                          | 138     | Public GWAS summary statistics (European ancestry)                                                       | MEGASTROKE Consortium (approximately 40,585 cases / 406,111 controls)                                   | No causal effect of telomere length on ischemic stroke   |

Abbreviations: CI, confidence interval; GWAS, genome-wide association study; HR, hazard ratio; IVW, inverse-variance weighted; LTL, leukocyte telomere length; MR, Mendelian randomization; MR-Egger, Mendelian randomization–Egger regression; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; N/A, not applicable; OR, odds ratio; qPCR, quantitative polymerase chain reaction; RTL, relative telomere length; SNP, single nucleotide polymorphism; SNV, single nucleotide variation; WM, weighted median. Stroke subtypes: LAS, large-artery atherosclerosis; CES, cardioembolic stroke; SVS, small-vessel stroke.

**Table 2.** Quality assessment results.**Newcastle-Ottawa Scale****Cohort study**

| Author, Year          | Representativeness of exposed cohort | Selection of non-exposed cohort | Ascertainment of exposure | Outcome not present at beginning | Comparability of cohorts | Assessment of outcome | Follow-up duration | Adequacy of follow-up | Total (9) |
|-----------------------|--------------------------------------|---------------------------------|---------------------------|----------------------------------|--------------------------|-----------------------|--------------------|-----------------------|-----------|
| Mwasongwe et al, 2017 | *                                    | *                               | *                         | *                                | **                       | *                     | *                  | *                     | 9         |

**Case-control study**

| Author, Year        | Adequacy of case definition | Representativeness of cases | Selection of controls | Definition of controls | Comparability of case-control | Ascertainment of exposure | Same ascertainment method | Non-response rate | Total (9) |
|---------------------|-----------------------------|-----------------------------|-----------------------|------------------------|-------------------------------|---------------------------|---------------------------|-------------------|-----------|
| Schürks et al, 2013 | *                           | *                           | *                     | *                      | **                            | *                         | *                         | *                 | 9         |

**Quality of genetic association studies (Q-Genie): Mendelian randomization studies**

| Author, Year         | Rationale (7) | Outcome selection/definition (7) | Comparison groups (7) | Technical exposure classification (7) | Non-technical exposure classification (7) | Other bias (7) | Sample size/power (7) | A priori analyses (7) | Statistical methods/confounding (7) | Testing assumptions/inferences (7) | Appropriateness of inferences (7) | Total (77) |
|----------------------|---------------|----------------------------------|-----------------------|---------------------------------------|-------------------------------------------|----------------|-----------------------|-----------------------|-------------------------------------|------------------------------------|-----------------------------------|------------|
| Cao et al, 2019      | 5             | 5                                | 5                     | 1                                     | 3                                         | 5              | 4                     | 5                     | 5                                   | 4                                  | 6                                 | 48         |
| Cao et al, 2020      | 5             | 3                                | 5                     | 5                                     | 1                                         | 7              | 1                     | 7                     | 3                                   | 7                                  | 6                                 | 50         |
| Dang et al, 2023     | 7             | 4                                | 5                     | 1                                     | 3                                         | 5              | 7                     | 5                     | 5                                   | 4                                  | 7                                 | 53         |
| Maimaiti et al, 2024 | 5             | 5                                | 5                     | 2                                     | 3                                         | 5              | 5                     | 5                     | 7                                   | 7                                  | 5                                 | 54         |
| Imahori et al, 2025  | 7             | 5                                | 5                     | 3                                     | 3                                         | 7              | 7                     | 5                     | 7                                   | 4                                  | 6                                 | 59         |

Scores  $\leq 35$  indicate poor quality,  $>35$  to  $\leq 45$  indicate moderate quality, and  $>45$  indicate good quality.

### 3.2 Overall meta-analysis of telomere length and risk of ischemic stroke

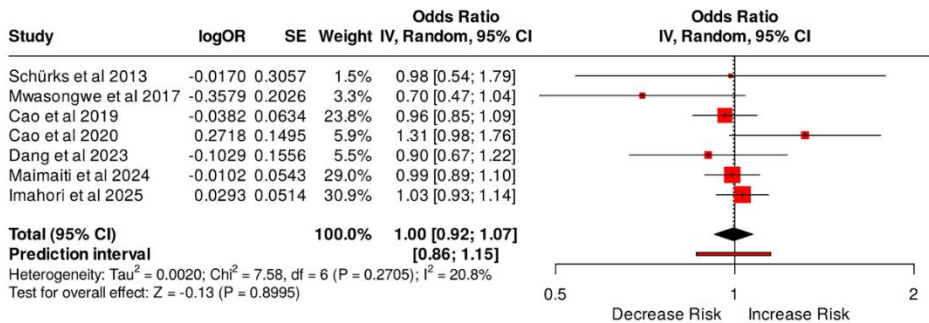
Pooled analysis showed no evidence of an association between telomere length and ischemic stroke risk (OR 1.00, 95% CI 0.92–1.07;  $p = 0.90$ ;  $n = 7$ ). This estimate indicates a null effect, with telomere length neither increasing nor decreasing stroke risk. Between-study heterogeneity was low ( $I^2 = 20.8\%$ ,  $p = 0.27$ ), suggesting good consistency across studies. The overall forest plot is presented in Figure 2, whereas subgroup forest plots stratified by stroke subtype and study design are provided in Supplementary Material 2.

Sensitivity analysis using a fixed-effect model showed similar results to the primary random-effects analysis. The pooled odds ratio from the fixed-effect model was 1.00 (95% CI 0.94–1.06;  $I^2 = 21\%$ ), which was comparable to the random-effects model (OR

1.00, 95% CI 0.92–1.07;  $I^2 = 20.8\%$ ) (Figure S3).

### 3.3 Subgroup analysis by ischemic stroke subtypes

Subgroup analyses by stroke subtype likewise showed no significant associations. For cardioembolic stroke, the pooled OR was 0.99 (95% CI 0.84–1.18;  $I^2 = 0\%$ ). For large-artery atherosclerosis, the pooled OR was 0.88 (95% CI 0.72–1.07;  $I^2 = 0\%$ ). For small-vessel occlusion, the pooled OR was 0.93 (95% CI 0.80–1.10;  $I^2 = 0\%$ ). There was no evidence of differences between subtypes ( $p$  for subgroup differences = 0.64). These findings suggested that telomere length was not associated with specific ischemic stroke mechanisms. Detailed subgroup analyses are presented in Figures S1 and S2 (Supplementary Material 2).

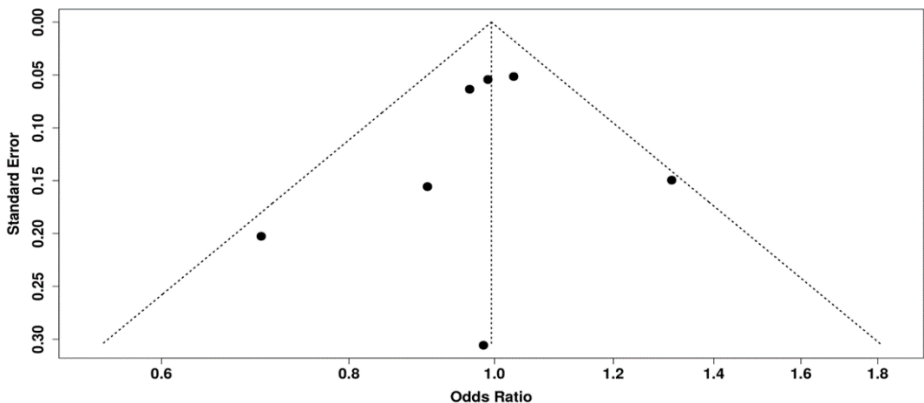


**Fig. 2.** Forest plot showing the pooled odds ratio and 95% confidence interval for the association between telomere length and the overall risk of ischemic stroke.

### 3.4 Assessment of publication bias

Publication bias was assessed using visual inspection of the funnel plot (Figure 3). Some asymmetry was observed, including a relative absence of studies in the lower right region and clustering of larger studies with

smaller standard errors. However, given the limited number of included studies, the reliability of funnel plot interpretation is constrained. These findings should therefore be interpreted with caution.



**Fig. 3.** Funnel plot for assessment of publication bias.

### 4. Discussion

This systematic review and meta-analysis, which incorporated seven studies spanning observational and Mendelian randomization designs and more than 1.2 million participants, found no evidence that leukocyte telomere length is associated with ischemic stroke risk. The null pattern was consistent across cardio-embolic stroke, large-artery atherosclerosis, and small-vessel occlusion. Collectively, these findings contradict a meaningful role for telomere length as a determinant of ischemic stroke.

Earlier observational literature has produced mixed results. For instance, a case-control study from Southern China reported that shorter telomeres were associated with atherothrombotic stroke but appeared inversely related to lacunar infarction, underscoring inconsistency across subtypes.<sup>8</sup> Likewise, a prior meta-analysis of observational studies suggested an overall association between shorter telomere length and stroke, but the signal was stronger in retrospective studies and varied among populations, raising concerns about bias and heterogeneity.<sup>9</sup> The

previous review included 11 prospective and retrospective observational studies and evaluated broader stroke outcomes, including ischemic and cardioembolic stroke, but did not incorporate MR analyses. In contrast, the present study focused specifically on ischemic stroke and included MR studies to further evaluate the potential causal relationship between telomere length and ischemic stroke risk. The lower heterogeneity observed in the current analysis may also reflect differences in study selection, outcome definition, and analytical approaches. By comparison, Mendelian randomization evidence offers little support for a causal effect of telomere length on stroke. A systematic review of MR studies did not identify a consistent causal relationship between genetically predicted telomere length and stroke outcomes.<sup>10</sup> Similarly, broader MR analyses focused on cardiovascular disease have generally reported null associations for stroke despite the use of large datasets and established genetic instruments.<sup>11</sup> One plausible explanation for the difference between observational and MR findings is methodological bias. Observational studies remain susceptible to residual confounding because age, metabolic health, lifestyle, and inflammatory burden all influence telomere length and are also strongly linked to stroke risk.<sup>4</sup> Reverse causation is another concern when telomere length is measured after the disease has occurred.<sup>24</sup> In contrast, MR uses germline variants fixed at conception, which reduces vulnerability to these sources of bias. When its core assumptions are met, including instrument relevance, independence from confounders, and absence of horizontal pleiotropy, MR provides a stronger basis for causal inference.<sup>12</sup> The consistent null MR findings therefore suggest that leukocyte telomere length is unlikely to be a direct causal factor in ischemic stroke.

From a biological standpoint, telomere length may be better interpreted as a general indicator of accumulated aging-related exposure than as an active driver of stroke. Telomere shortening has been associated with cellular senescence and systemic aging, but ischemic

stroke develops through complex vascular pathways involving atherosclerosis, endothelial dysfunction, and thrombogenesis.<sup>25</sup> These processes are shaped predominantly by established clinical risk factors such as hypertension, diabetes, dyslipidemia, and adverse lifestyle exposures. The absence of a clear signal in the present analysis, therefore, supports the view that telomere length behaves more like a non-specific marker of biological aging than a mechanistic contributor to stroke pathogenesis. This interpretation is also consistent with the overlapping determinants of telomere shortening and stroke risk. Smoking, physical inactivity, unhealthy diet, obesity, and psychosocial stress have all been linked to accelerated telomere attrition and are also recognized contributors to ischemic stroke.<sup>26-28</sup> Pharmacological interventions that may influence telomere biology have also been explored in cardiovascular research. Statins have been reported to modulate telomerase activity in some studies.<sup>29,30</sup> In addition, antioxidant-related compounds, including resveratrol and polyphenols, may improve oxidative stress and metabolic factors associated with vascular disease.<sup>31-33</sup> However, current evidence remains insufficient to determine whether these interventions directly affect telomere shortening or reduce ischemic stroke risk, and no clinical studies have specifically evaluated these outcomes in ischemic stroke. Telomere length may therefore capture the cumulative burden of these exposures rather than exerting its independent effect. The attenuation of associations in adjusted observational analyses, together with the absence of signal in MR studies, aligns with this explanation.

This review has several strengths. It combines observational and genetic epidemiology, allowing the evidence on association and causality to be considered together. The low heterogeneity observed across analyses also supports the consistency of the pooled findings despite differences in design and population characteristics. In addition, analyses by stroke subtype offered further insight into whether any subtype-specific pattern might exist, and the uniformly null results across subtypes

reinforce the main conclusion. Several limitations should also be recognized. The number of eligible studies was small, reducing statistical power and limiting precision, particularly within subgroup analyses. Although comprehensive search strategies were applied, some relevant studies may have been missed. Publication bias also cannot be completely excluded. Because the number of included studies was relatively small, funnel plot interpretation was limited and may not reliably detect small-study effects. Although additional searches of gray literature and study registry sources were conducted, no eligible studies were identified from these sources, and some unpublished or negative studies may still not have been captured. Variation in study populations, subtype definitions, sample sizes, and telomere measurement methods may also have introduced heterogeneity. Measurement differences in telomere length method, including Southern blot versus qPCR and absolute length versus relative T/S ratio reporting, may have affected comparability across studies.<sup>34</sup> Similar methodological limitations related to inconsistent biomarker or outcome definitions have been recognized in studies evaluating complex clinical associations and pharmacogenomic outcomes.<sup>35,36</sup> In addition, studies differed in how telomere length was categorized and reported, including quartiles, relative measurements, and genetically predicted telomere length. Because of these methodological differences, together with the limited number of included studies and low observed heterogeneity ( $I^2 = 20\%$ ), subgroup analyses based on telomere length categories were considered methodologically limited and potentially underpowered. Moreover, observational studies and MR studies do not estimate the same construct: MR reflects lifelong genetically influenced differences in telomere length, whereas observational research usually measures telomere length at a single time point that can be modified by transient exposures or disease status. Although subgroup findings were consistent, this methodological contrast remains relevant when interpreting the pooled evidence. Generalizability may also be constrained because several included studies were conducted

in relatively homogeneous populations. Differences in ancestry and environmental exposures could influence both telomere dynamics and stroke susceptibility.<sup>37,38</sup> Finally, ischemic stroke is biologically complex, and telomere length alone is unlikely to reflect all relevant mechanisms. Other pathways, including epigenetic, proteomic, and inflammatory processes, may be more directly involved in disease development.

Clinically, the present findings do not support using telomere length as a stand-alone biomarker for ischemic stroke prediction. Risk stratification should continue to rely on established clinical and biochemical variables supported by stronger evidence. Based on the current literature, adding telomere length to existing prediction models is unlikely to provide meaningful incremental value. At the same time, the lack of association observed here does not lessen the importance of established prevention strategies. Smoking cessation, healthy dietary patterns, regular physical activity, weight management, and use of evidence-based pharmacologic therapy remain fundamental approaches to reducing stroke risk in high-risk populations.<sup>39</sup> Future studies should include more diverse populations to improve generalizability. Because stroke pathogenesis is multifactorial, integrative research that combines telomere biology with other molecular markers, such as epigenetic clocks, proteomic profiles, and metabolomic signatures, may be more informative.<sup>40-</sup>

<sup>42</sup> Greater use of standardized telomere measurement methods reported in absolute units may also improve cross-study comparability and interpretability. Further work is needed to determine whether only critically short telomeres, rather than modest variation across the normal range, have biological relevance for stroke risk. Telomere length may become important only once shortening reaches a threshold at which chromosomal stability is compromised.

## Acknowledgements

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## Conflicts of interest

The authors declare no conflict of interest.

## Ethical approval and informed consent

This study was reviewed and approved by the Burapha University Institutional Review Board (IRB No. IRB1-020/2569). Details of authors omitted for double-blind reviewing. The requirement for informed consent was waived because this study was based on previously published data and did not involve human participants or identifiable personal data.

## Data availability statement

The data underlying this study are derived from published studies and are available within the article and its supplementary materials. Additional data can be provided upon reasonable request.

## Supplementary materials

Supplementary Material 1: Detailed database-specific search strategies.

Supplementary Material 2: Additional forest plots, including subgroup analyses by ischemic stroke subtype and study design.

Supplementary Material 3: PRISMA 2020 checklist.

[Supplementary Material 4: Excluded full-text studies with reasons for exclusion.](https://li01.tci-thaijo.org/index.php/JBAP/article/view/271857/182247) (<https://li01.tci-thaijo.org/index.php/JBAP/article/view/271857/182247>)

Supplement 1-3 are included in this article while Supplement 4 is available in a separate file through the above link.

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# Supplementary Materials

## Association between Telomere Length and Risk of Ischemic Stroke: A Systematic Review and Meta-analysis

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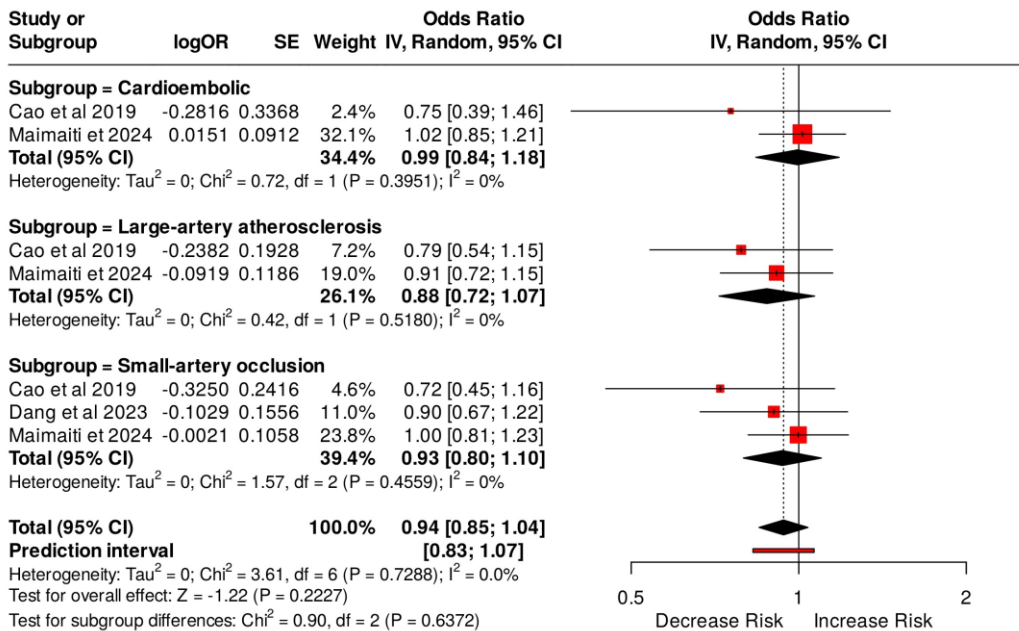
This supplementary file provides detailed search strategies, additional forest plots, and the PRISMA 2020 checklist supporting the manuscript. All supplementary materials are referenced in the main manuscript and are provided to enhance transparency and reproducibility.

### Supplementary Material 1. Detailed search strategies

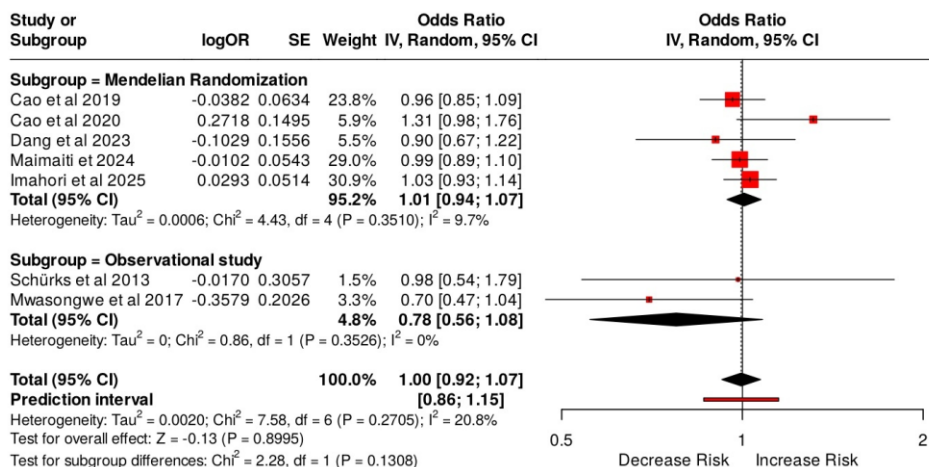
Search concept: “ischemic stroke” AND “telomere length”.

| Database | Date of search  | Records retrieved | Search strategy                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|----------|-----------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PubMed   | January 8, 2026 | 32                | (“Ischemic Stroke”[Mesh] OR “ischemic stroke” OR “cryptogenic strokes” OR “wake-up stroke” OR “Embolic Stroke”[Mesh] OR “embolic stroke” OR “cardioembolic stroke” OR “large artery atherosclerosis” OR “small vessel occlusion” OR “embolic strokes of undetermined source” OR “embolic stroke of undetermined source” OR “ESUS”) AND (“Telomere Shortening”[Mesh] OR (“Telomere”[Mesh] OR telomere*) AND (homeostasis OR length OR short OR shortening))) |
| Scopus   | January 8, 2026 | 55                | TITLE-ABS-KEY ((“ischemic stroke” OR “cryptogenic strokes” OR “wake-up stroke” OR “embolic stroke” OR “cardioembolic stroke” OR “large artery atherosclerosis” OR “small vessel occlusion” OR “embolic strokes of undetermined source” OR “embolic stroke of undetermined source” OR “ESUS”) AND (“Telomere Shortening” OR (telomere* AND (homeostasis OR length OR short OR shortening))))                                                                 |
| CINAHL   | January 8, 2026 | 10                | (“ischemic stroke” OR “cryptogenic strokes” OR “wake-up stroke” OR “embolic stroke” OR “cardioembolic stroke” OR “large artery atherosclerosis” OR “small vessel occlusion” OR “embolic strokes of undetermined source” OR “embolic stroke of undetermined source” OR “ESUS”) AND (“Telomere Shortening” OR (telomere* AND (homeostasis OR length OR short OR shortening)))                                                                                 |

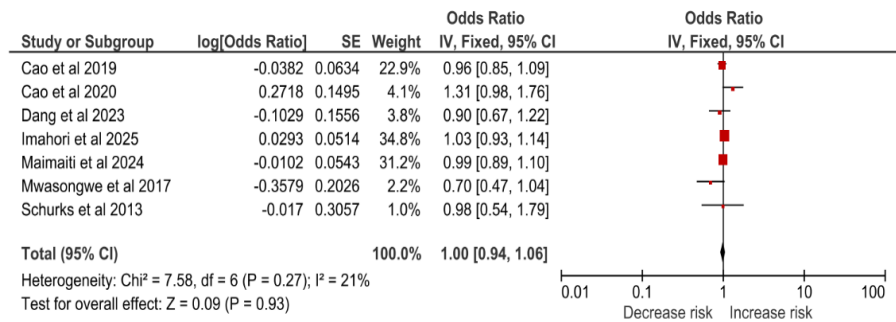
## Supplementary Material 2. Additional forest plots



**Figure S1.** Forest plot showing the pooled odds ratio and 95% confidence interval for the association between telomere length and ischemic stroke risk, stratified by stroke subtype, using a random-effects model.



**Figure S2.** Forest plot showing the pooled odds ratio and 95% confidence interval for the association between telomere length and ischemic stroke risk, stratified by study design (Mendelian randomization and observational studies), using a random-effects model.



**Figure S3.** Forest plot showing the pooled odds ratio and 95% confidence interval from the fixed-effect sensitivity analysis for the association between telomere length and ischemic stroke risk.

### Supplementary Material 3. PRISMA 2020 checklist

The checklist below indicates where each PRISMA 2020 reporting item is addressed in the manuscript.

| Section/topic | Item No. | PRISMA 2020 checklist item                                                                                                                                                                                                               | Reported location in manuscript                                     |
|---------------|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
| TITLE         | 1        | Identify the report as a systematic review.                                                                                                                                                                                              | Title page                                                          |
| ABSTRACT      | 2        | See the PRISMA 2020 for Abstracts checklist.                                                                                                                                                                                             | Abstract                                                            |
| INTRODUCTION  | 3        | Describe the rationale for the review in the context of existing knowledge.                                                                                                                                                              | Introduction, paragraphs 1-5                                        |
| INTRODUCTION  | 4        | Provide an explicit statement of the objective(s) or question(s) the review addresses.                                                                                                                                                   | Introduction, final paragraph                                       |
| METHODS       | 5        | Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.                                                                                                                              | Methods: Eligibility Criteria                                       |
| METHODS       | 6        | Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.                                | Methods: Search Strategy and Data Sources; Supplementary Material 1 |
| METHODS       | 7        | Present the full search strategies for all databases, registers and websites, including any filters and limits used.                                                                                                                     | Supplementary Material 1                                            |
| METHODS       | 8        | Specify the methods used to decide whether a study met the inclusion criteria, including how many reviewers screened each record and report, whether they worked independently, and any automation tools used.                           | Methods: Study Selection                                            |
| METHODS       | 9        | Specify the methods used to collect data from reports, including how many reviewers collected data, whether they worked independently, any processes for obtaining or confirming data from investigators, and any automation tools used. | Methods: Data Extraction                                            |
| METHODS       | 10a      | List and define all outcomes for which data were sought. Specify whether all results compatible with each outcome domain in each                                                                                                         | Methods: Eligibility Criteria; Data Extraction                      |

| Section/topic | Item No. | PRISMA 2020 checklist item                                                                                                                                                                                                                                  | Reported location in manuscript                             |
|---------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|
|               |          | study were sought and, if not, the methods used to decide which results to collect.                                                                                                                                                                         |                                                             |
| METHODS       | 10b      | List and define all other variables for which data were sought and describe any assumptions made about missing or unclear information.                                                                                                                      | Methods: Data Extraction                                    |
| METHODS       | 11       | Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and any automation tools used.                                | Methods: Quality Assessment                                 |
| METHODS       | 12       | Specify for each outcome the effect measure(s) used in the synthesis or presentation of results.                                                                                                                                                            | Methods: Statistical Analysis                               |
| METHODS       | 13a      | Describe the processes used to decide which studies were eligible for each synthesis.                                                                                                                                                                       | Methods: Eligibility Criteria; Statistical Analysis         |
| METHODS       | 13b      | Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics or data conversions.                                                                                                        | Methods: Statistical Analysis                               |
| METHODS       | 13c      | Describe any methods used to tabulate or visually display results of individual studies and syntheses.                                                                                                                                                      | Methods: Statistical Analysis; Results (Figures and Tables) |
| METHODS       | 13d      | Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. | Methods: Statistical Analysis                               |
| METHODS       | 13e      | Describe any methods used to explore possible causes of heterogeneity among study results.                                                                                                                                                                  | Methods: Statistical Analysis                               |
| METHODS       | 13f      | Describe any sensitivity analyses conducted to assess robustness of the synthesized results.                                                                                                                                                                | Methods: Statistical Analysis                               |
| METHODS       | 14       | Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).                                                                                                                                     | Methods: Quality Assessment                                 |
| METHODS       | 15       | Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.                                                                                                                                                       | Not assessed / not reported                                 |
| RESULTS       | 16a      | Describe the results of the search and selection process, from the number of records identified to the number of studies included, ideally using a flow diagram.                                                                                            | Results 3.1; Figure 1                                       |
| RESULTS       | 16b      | Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.                                                                                                                                 | Results 3.1 (reasons for exclusion summarised)              |
| RESULTS       | 17       | Cite each included study and present its characteristics.                                                                                                                                                                                                   | Results 3.1; Table 1                                        |
| RESULTS       | 18       | Present assessments of risk of bias for each included study.                                                                                                                                                                                                | Results 3.1; Table 2                                        |
| RESULTS       | 19       | For all outcomes, present, for each study: (a) summary statistics for each group, where appropriate; and (b) an effect estimate and its precision, ideally using structured tables or plots.                                                                | Results 3.1; Figure 2; Figures S1-S2; Table 1               |

| Section/topic     | Item No. | PRISMA 2020 checklist item                                                                                                                                                                                                                 | Reported location in manuscript                                                         |
|-------------------|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| RESULTS           | 20a      | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.                                                                                                                                     | Results 3.1-3.4                                                                         |
| RESULTS           | 20b      | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision and measures of statistical heterogeneity.                                                      | Results 3.2-3.3; Figure 2; Figures S1-S2                                                |
| RESULTS           | 20c      | Present results of all investigations of possible causes of heterogeneity among study results.                                                                                                                                             | Results 3.3 (subgroup analyses by stroke subtype and study design)                      |
| RESULTS           | 20d      | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.                                                                                                                                 | Sensitivity analyses not separately reported                                            |
| RESULTS           | 21       | Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.                                                                                                                    | Results 3.4; Figure 3                                                                   |
| RESULTS           | 22       | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.                                                                                                                                        | Not assessed / not reported                                                             |
| DISCUSSION        | 23a      | Provide a general interpretation of the results in the context of other evidence.                                                                                                                                                          | Discussion, paragraphs 1-4                                                              |
| DISCUSSION        | 23b      | Discuss any limitations of the evidence included in the review.                                                                                                                                                                            | Discussion, Limitations paragraphs                                                      |
| DISCUSSION        | 23c      | Discuss any limitations of the review processes used.                                                                                                                                                                                      | Discussion, Limitations paragraphs                                                      |
| DISCUSSION        | 23d      | Discuss implications of the results for practice, policy, and future research.                                                                                                                                                             | Discussion, Clinical implications and Future research paragraphs                        |
| OTHER INFORMATION | 24a      | Provide registration information for the review, including register name and registration number, or state that the review was not registered.                                                                                             | Methods: Search Strategy and Data Sources (PROSPERO registration number CRD42025123016) |
| OTHER INFORMATION | 24b      | Indicate where the review protocol can be accessed, or state that a protocol was not prepared.                                                                                                                                             | Protocol not reported                                                                   |
| OTHER INFORMATION | 24c      | Describe and explain any amendments to information provided at registration or in the protocol.                                                                                                                                            | No amendments reported                                                                  |
| OTHER INFORMATION | 25       | Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.                                                                                                              | Funding statement                                                                       |
| OTHER INFORMATION | 26       | Declare any competing interests of review authors.                                                                                                                                                                                         | Declaration of conflicting interest                                                     |
| OTHER INFORMATION | 27       | Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. | Data availability statement                                                             |

Abbreviation: PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.