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The contribution of oral infectious diseases in lacunar stroke based on meta-analysis and Mendelian randomization study

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To investigate the causal relationship between oral infectious diseases and lacunar stroke (LS) and to identify the role of interleukin-1α (IL-1α), interleukin-6 (IL-6), tumor necrosis factor (TNF), and C-reaction protein (CRP) as potential mediators. The meta-analysis incorporating cross-sectional studies was carried out. Additionally, two-sample Mendelian randomization (MR) analysis was performed to explore the associations between genetically predicted oral infectious diseases (including dental caries, periodontitis, and pulp and periapical diseases) and lacunar stroke, utilizing summary-level data from genome-wide association studies (GWAS). This was followed by a mediation analysis to explore the role of IL-1α, IL-6, TNF, and CRP. Meta-analysis suggested that individuals with periodontitis have a 5.16 times higher risk of developing LS compared to those without periodontitis (95%CI 3.68–7.24). Genetically predicted pulp and periapical diseases (Odds ratios [OR]: 1.20, 95% CI 1.03–1.41) and periodontitis (OR: 1.24, 95%CI 1.03–1.49) showed a moderate association with LS. However, the mediation analysis yielded negative results. The evidence derived from both the MR study and the meta-analysis suggested a potential association between periodontitis and LS. These findings indicated that periodontitis might play a role in the development of LS. However, given the limitations inherent in our research, further studies are necessary to validate these conclusions.

Keywords Oral infectious diseases, Periodontitis, Lacunar stroke, Mendelian randomization analysis, Meta-analysis

Abbreviations

MR	Mendelian randomization
GWAS	Genome-wide association studies
OR	Odds ratios
PRISMA	Preferred reporting items for systematic review and meta-analysis
IV	Instrumental variable
SNPs	Single nucleotide polymorphisms
AHRQ	The agency for healthcare research and quality
MD	Mean difference
GLIDE	Gene-lifestyle interaction in the dental endpoints
ISGC	International stroke genetics
SCALLOP	Systematic and combinatorial analysis of proteins in Olink
IEU	Integrative epidemiology unit
IVW-RE	Random effects inverse variance weighting
IVW-FE	Fixed effects inverse variance weighting
WM	Weighted median
ML	Maximum likelihood

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FDR	False discovery rate
MR-presso	MR pleiotropy residual sum and outlier
PD	Probing depth
CAL	Clinical attachment loss
PISA	Periodontal inflammatory surface area
FMBS	Full-mouth bleeding score
LPS	Lipopolysaccharide

Nowadays, the prevalence of aging and neurodegenerative diseases is on the rise. The 2015 World Population Ageing Report forecasted that the global population aged 60 years and older is expected to more than double over the next 35 years, reaching nearly 2.1 billion individuals[1]. This projection underscored the increasing prevalence of population ageing. Stroke, the second most common cause of death globally, occurs in approximately three-quarters of people aged ≥ 65 years. As a result of population ageing, the risk of stroke is expected to rise substantially within 20 years, especially in developing countries[2,3]. Lacunar stroke (LS), classified as a subtype of cerebral small vessel disease (CSVD), represents approximately 25% of all ischemic strokes[2,4]. LS is linked to cognitive decline and aging, and it exists in the early stage of them[5,6]. Therefore, the prevention of LS is crucial. Although the etiology of LS has not yet been adequately identified, inflammation and endothelial dysfunction are acknowledged as potential contributing factors[7].

Dental caries, pulp and periapical diseases, and periodontitis are significant contributors to oral inflammation. These conditions generate inflammatory mediators that may enter the systemic circulation, potentially affecting distant organs[8–11]. Several studies supported the effect of periodontitis, as well as pulp and periapical diseases, on the dysfunction of endothelial cells[12–15]. Therefore, oral infectious diseases can be considered a source of systemic inflammation and endothelial vascular stress. However, the association between oral infectious diseases and LS remains ambiguous. If the interaction between oral infectious diseases and LS is substantiated and reliable, then the proactive management of oral infectious diseases may enhance cerebrovascular health and facilitate the early detection of cerebrovascular disease[16].

Clinical studies have explored the relationship between periodontitis and LS[16,17]. The extraction of all available trial data can provide a more accurate evaluation of the relationship between oral infectious diseases and LS. Therefore, this study conducted a meta-analysis of the evidence on the association between oral infectious diseases and LS following the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines[18].

However, clinical studies are susceptible to various biases, confounding factors, and reverse causation, which can result in misleading associations. Despite meticulous research design and statistical adjustments, erroneous causal inferences may still occur[19]. Consequently, we used the summary statistics of a genome-wide association study (GWAS) and conducted a two-sample Mendelian randomization (MR) analysis to explore the association between oral infectious diseases and LS. MR analysis is a form of instrumental variable (IV) analysis that facilitates causal inference regarding the effects of exposure on outcomes derived from observational data[20]. The allocation of genotypes follows Mendelian's second law. MR analysis exploits genetic variants as IV to detect causal relationship by single nucleotide polymorphisms (SNPs). Therefore, it is not affected by epidemiological research bias[21].

Several studies have indicated a possible association between the levels of interleukin-1α (IL-1α), interleukin-6 (IL-6), tumor necrosis factor (TNF), and C-reactive protein (CRP) and the prognosis of patients with CSVD[22–24]. The meta-analysis conducted by Wiseman et al. revealed that the levels of IL-1α, TNF-α, and CRP in patients with LS were significantly elevated compared to those in individuals without LS[25]. These findings suggested that IL-1α, IL-6, TNF, and CRP may be implicated in the onset and progression of LS. The underlying mechanism may involve the ability of these inflammatory factors to facilitate the migration of leukocytes to the brain, resulting in microvascular occlusion and a gradual decline in cerebral blood flow. Additionally, these cytokines may influence vascular stiffness. So we speculated that IL-1α, IL-6, TNF, and CRP are potential mediators between oral infectious diseases and LS. Consequently, we performed a mediation analysis utilizing the MR study to investigate the potential mediating role of these cytokines in the association between oral infectious diseases and LS.

In summary, a meta-analysis and MR study were conducted to reveal the relationship between oral infectious diseases and LS. This was achieved by synthesizing data from clinical observational studies and employing genetic methodologies, thereby strengthening the evidence for this previously under-explored association from dual perspectives.

Materials and methods

Meta-analysis

The analysis plan, crafted by all authors, was registered with PROSPERO (CRD42024563379). In accordance with the PRISMA guidelines[26], our review was conducted following the PICO framework: adults (P-patients); dental caries, pulp and periapical diseases, periodontitis (I-intervention); patients without oral infectious diseases (C-comparison); lacunar stroke (O-result).

Search strategy

We conducted a comprehensive search of PubMed, Cochrane Library, EMBASE, and Web of Science up to August 2024, carried out by two authors. Furthermore, we screened the reference lists of relevant papers to identify potentially eligible research articles. In addition, gray literature was sourced from Google Scholar (https://schlar.google.com). Unpublished trials were retrieved from the trial registry (EU Clinical Trials Registry: https://www.clinicaltrialsregister.eu/). The main search terms utilized include "Periodontitis", "Periodontal disease",

"Pulp and Periapical Disease", "Dental Pulp Diseases", "Dental Caries", "Tooth Decay", "Stroke, Lacunar", and "Lacunar Stroke", using both medical subject headings and entry terms. The comprehensive overview of the search strategy was shown in Supplementary Table S1.

Eligibility criteria

1. Cross-sectional, cohort, or case-control design;
2. All included patients were adults;
3. Patients with periodontitis and LS have a clear diagnosis;
4. Data on at least one periodontal clinical indicator was provided, including probing depth (PD), clinical attachment loss (CAL), periodontal inflammatory surface area (PISA), and full-mouth bleeding score (FMBS).

Also, other types of stroke or neurological disorders were ruled out.

Study selection and data extraction

Two authors (Q.Z. and S.L.) conducted an independent literature search and selected relevant studies based on their titles and abstracts. Subsequently, these authors performed a cross-check of the initially included literature, and the final selection of articles was made through a comprehensive review of the full texts. Any disagreements were resolved by the third author (T.X.). Following the determination of the final literature, the two authors (Q.Z. and S.L.) utilized a prepared data extraction checklist to gather pertinent details and results from the research.

The quality analysis of cross-sectional studies was based on evaluation criteria recommended by the Agency for Healthcare Research and Quality (AHRQ)[27]. This evaluation comprised 11 items, a score of 1 was assigned if the answer was yes while a score of 0 was assigned if the answer was no or unclear, resulting in a total possible score of 11. Literature with scores ranging from 4 to 7 was classified as medium-quality, whereas literature with scores ranging from 8 to 11 was classified as high-quality.

Statistical analysis

The meta-analysis was carried out by Review Manager 5.3. Odds ratio (OR) with a 95% confidence interval (CI) was adopted to assess dichotomous outcomes, and overall mean difference (MD) was applied to assess continuous data.

Heterogeneity was analyzed with the I^2 index and the Cochrane Q statistic. If there was no significant heterogeneity ($P > 0.10$, $I^2 < 50\%$), a fixed-effects model was applied for meta-analysis. Otherwise, a random effects model was chosen. Publication bias observed through funnel plot.

Mendelian randomization analysis

Study overview

GWAS summary statistics were used to perform a two-sample MR analysis to examine the causal effect of oral infectious diseases, including dental caries, pulp and periapical diseases, and periodontitis, on LS. Mediation analysis was performed using a two-step MR analysis to explore whether cytokines mediate the causal pathway from oral infectious diseases to LS (Fig. 1). In addition, there are three fundamental assumptions that MR must follow: (i) the IVs ought to be closely linked to exposures ($P < 5 \times 10^{-8}$); (ii) the IVs are independent of potential confounding cytokines; (iii) the IVs affect outcomes only via exposures, not via confounders[28] (Fig. 1). The MR analysis was conducted in accordance with the guidelines established by the Strengthening the Reporting of Observational Studies in Epidemiology[29].

Data sources

Oral infectious diseases The genetic variations associated with periodontitis were identified through GWAS data analysis conducted by the Gene-Lifestyle Interaction in Dental Endpoints (GLIDE) Consortium[30]. In this study, to ensure the homogeneity of the cohorts' ancestry, we excluded data related to the Hispanic Community Health Study/Study of Latinos, resulting in a final sample of 12,289 cases and 22,326 controls from Europe.

Data regarding dental caries were also acquired from the GLIDE Consortium. This dataset encompassed decayed, missing, and filled tooth surfaces. The information was derived from clinical dental records and synthesized from a comprehensive study involving 26,792 volunteers across nine queues[30].

Summary statistics for pulp and periapical diseases were obtained from the FinnGen Biobank. The dataset included 271,312 European, comprising 12,078 cases and 259,234 controls. The identification code for this dataset is "finn-b-K11_PULP_PERIAPICAL" (https://www.finngen.fi/en, version R9).

Lacunar stroke Summary statistics for LS were derived from a study that encompassed 7,338 cases identified by MRI alongside 254,798 controls. The available GWAS statistics were obtained from acute stroke admissions and outpatient cases within the International Stroke Genetics Consortium (ISGC)[31].

Cytokines GWAS data for IL-1α, IL-6, and TNF were sourced from Zhao et al.[32]. The authors utilized data from the Systematic and Combinatorial Analysis of Proteins in Olink (SCALLOP) consortium. This study involved 14,824 participants and examined 91 plasma proteins quantified using the Olink Target platform. We obtained genetic data for CRP from the Integrative Epidemiology Unit (IEU), which encompassed a sample size of 57,531 European.

All data utilized in this study were publicly accessible, as approved by the relevant ethical and institutional review boards. The exposure and outcome data were sourced from different databases, leading to a reduced

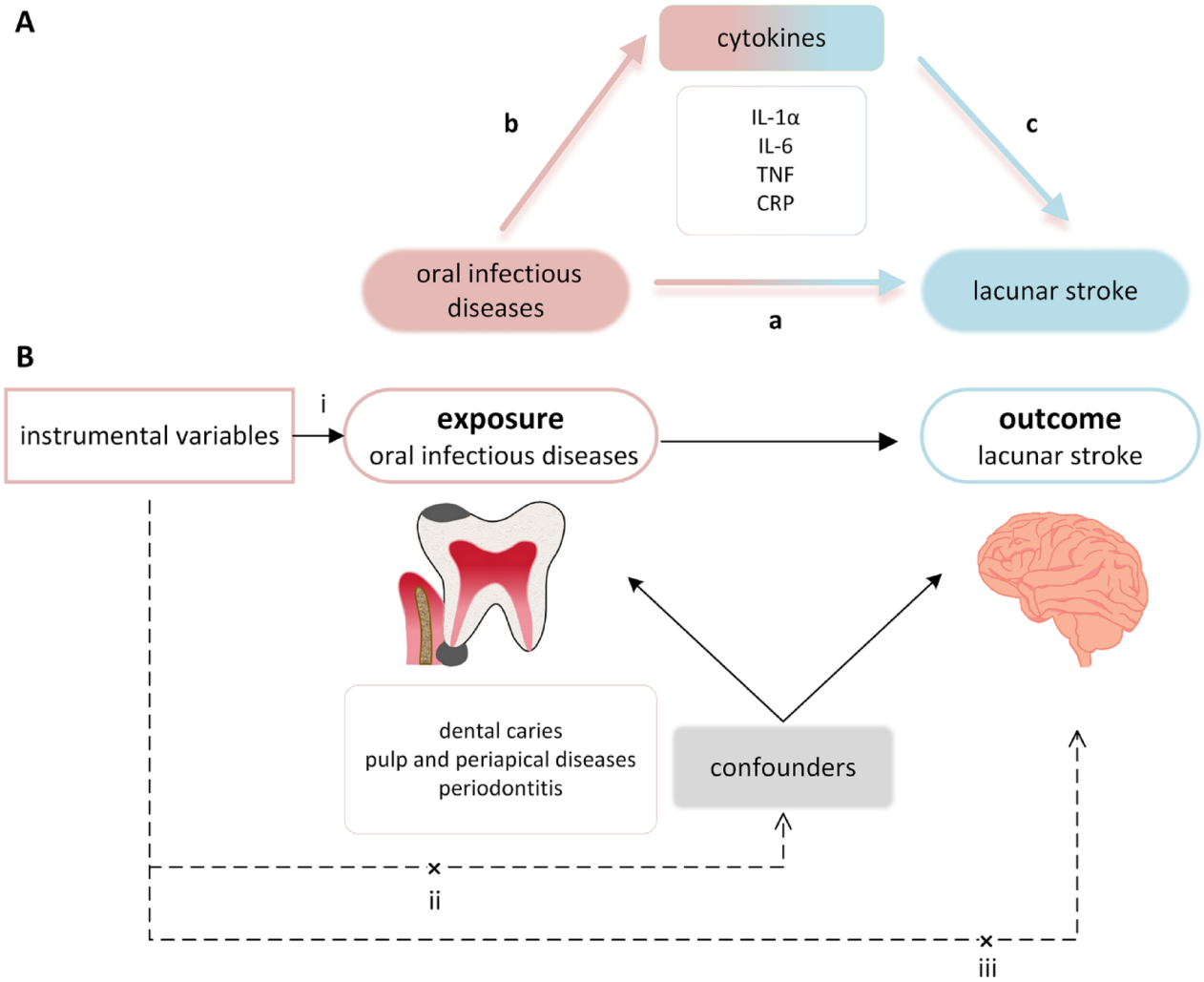

Fig. 1. Overview of procedures employed to investigate the causal association between oral infectious diseases and lacunar stroke by Mendelian randomization analysis. Hypothetical model diagram exploring the association between oral infectious diseases and LS (**A**). The total effect of oral infectious diseases on LS (a). Two-step MR study of the mediating role of four common cytokines (b, c). Three fundamental assumptions of Mendelian randomization (**B**). i, the instrumental variables are closely linked to exposures; ii, the instrumental variables are independent of potential confounders; iii, the instrumental variables affect outcomes only via exposures.

overlap within the sample. To ensure genetic homogeneity, the majority of the ethnic groups involved in all cases were of European descent. The detailed information of GWAS data was presented in Table 1.

Instrumental variable selection

The SNPs utilized as IVs in MR study must satisfy the three conditions outlined in Fig. 1. Firstly, SNPs representing exposure must meet genome-wide significance. However, there was a limited number of SNPs that exhibited a strong association with oral infectious diseases ($P < 5e\text{-}8$). Consequently, a threshold of $P < 5e\text{-}6$ was established as the criterion for the selection of SNPs associated with oral infectious diseases. Linkage disequilibrium was excluded to ensure the independence of SNPs. This was accomplished by pruning SNPs within a 10,000 kb window using a threshold of $r^2 < 0.001$. Subsequently, SNPs associated with confounding factors related to oral infections (such as hypertension), and those associated with LS (such as diabetes), were systematically queried and eliminated using the PhenoScanner website (https://www.phenoscanner.medschl.cam.ac.uk/). In this study, the SNP rs2976950 was excluded from consideration.

Then to avoid the effects of weak instrumental bias, the strength of the IVs was assessed using F-statistics. SNPs with F-statistics greater than 10 were required[33,34]. Proxy SNPs were employed in instances where the exposed SNP was not obtained from the result. Finally, the exposure and outcome data were harmonized to coordinate the direction of their influence, and the palindromic sequences were excluded[35].

Trait		Consortium	Years	Ethnicity	Sample size
Oral infectious diseases	Periodontitis	GLIDE	2019	European	34,615
	Dental caries	GLIDE	2019	European	26,792
	Diseases of the pulp and periapical tissues	FinnGen	NA	European	271,312
LS		ISGC etc.	2021	European	62,136
Cytokines	IL-1α	SCALLOP	2023	European	14,824
	IL-6	SCALLOP	2023	European	14,824
	TNF	SCALLOP	2023	European	14,824
	CRP	IEU	2022	European	575,531

Table 1. Details of genome-wide association study included in this analysis.

Mendelian randomization study

To quantify the impact of oral infectious diseases on LS, a two-sample MR was employed. The MR analysis and sensitivity analysis were conducted utilizing R software (version 4.3.0, https://www.r-project.org/) along with the packages "Two Sample MR", "tidyr", and "MRPRESSO"[35]. The effect estimates of four distinct methods were compared: random effects inverse variance weighting (IVW-RE), fixed effects inverse variance weighting (IVW-FE), weighted median (WM), and maximum likelihood (ML). As the most widely used and effective method in MR analysis, IVW-FE was employed as the primary analytical approach[36]. Statistical significance was determined using a p-value adjusted for the false discovery rate (FDR) method ($P<0.05$).

Sensitivity analysis

The sensitivity analysis was adopted to confirm the robustness of the findings. Given the diverse origins of SNPs from various GWAS datasets, conducting a heterogeneity test was essential to evaluate the heterogeneity in outcomes. The Cochran Q statistic was applied to quantify the results[37–39]. Subsequently, a horizontal pleiotropy test was conducted by identifying outlier SNPs with both the MR-Egger intercept and the MR pleiotropy residual sum and outlier (MR-presso) method[40]. Lastly, "leave-one-out" analysis was undertaken to find out if an SNP is a strong driver of the outcome.

Mediation analysis

We further used a two-step MR to investigate whether the cytokines (IL-1α, IL-6, TNF, and CRP) mediate the relationship between oral infectious diseases on LS. The overall effect of oral infectious diseases on LS was decomposed into direct and indirect effects.

Results

Meta-analysis

Study selection

Following the selection of studies, only those that reported on the relationship between periodontitis and LS were summarized and analyzed. No studies addressing the association between dental caries and LS were identified, neither pulp and periapical diseases and LS. The search yielded 38 studies (Fig. 2). Following the removal of duplicate entries, a total of 22 studies were evaluated based on their titles and abstracts. Of these, 6 studies fulfilled the predetermined inclusion criteria. Upon further examination, five articles were ultimately included in the analysis, as one article was excluded due to the absence of a definitive diagnosis of LS.

A descriptive summary of the five studies was presented in Table 2. These studies were cross-sectional in design, with periodontitis considered an exposure factor and LS considered the outcome. The relationship between periodontitis and LS was analyzed by comparing the incidence of periodontitis and the clinical indicators of periodontitis in patients with and without LS. All five studies[8,41–44] were conducted in Japan and the Republic of Chile, published between 2013 and 2022. Sample sizes ranged from 110 to 370. Three studies[8,42,43] have reported the number of individuals affected by periodontitis. Additionally, four studies[8,41–43] have reported PD levels, four studies[8,41–43] have provided CAL values, two studies[8,43] have reported PISA, and three studies[8,42,43] have published FMBS. The studies assessed using the AHRQ checklist received a score of 7 points, suggesting that the quality of the literature included is moderate (Table 3).

Periodontitis may serve as a potential risk factor for lacunar stroke

Three articles[8,42,43] indicated that the prevalence of periodontitis was higher in the LS group compared to the non-LS group, suggesting that periodontitis may be a potential risk factor for LS. In the absence of significant heterogeneity ($I^2=0\%$, $P=0.97$), a fixed-effects model was applied. The combined OR was 5.16, with a 95% CI (3.68, 7.24) and $P<0.001$ (Fig. 3). However, the data presented in the three articles originated from the same research team, it is essential to be cautious when drawing conclusions. No publication bias was observed on the funnel plot (Supplementary Fig. S1).

The periodontitis surface area and the total mouth bleeding score in individuals with lacunar stroke were significantly higher than in those without lacunar stroke

The meta-analysis revealed a significant increase in PD[8,41–43], CAL[8,41–43], PISA[8,43], and FMBS[8,42,43] in the group with LS compared to the group without LS (PD: MD = 0.98, 95% CI [0.57, 1.38], $P<0.001$; CAL: MD = 1.34, 95%

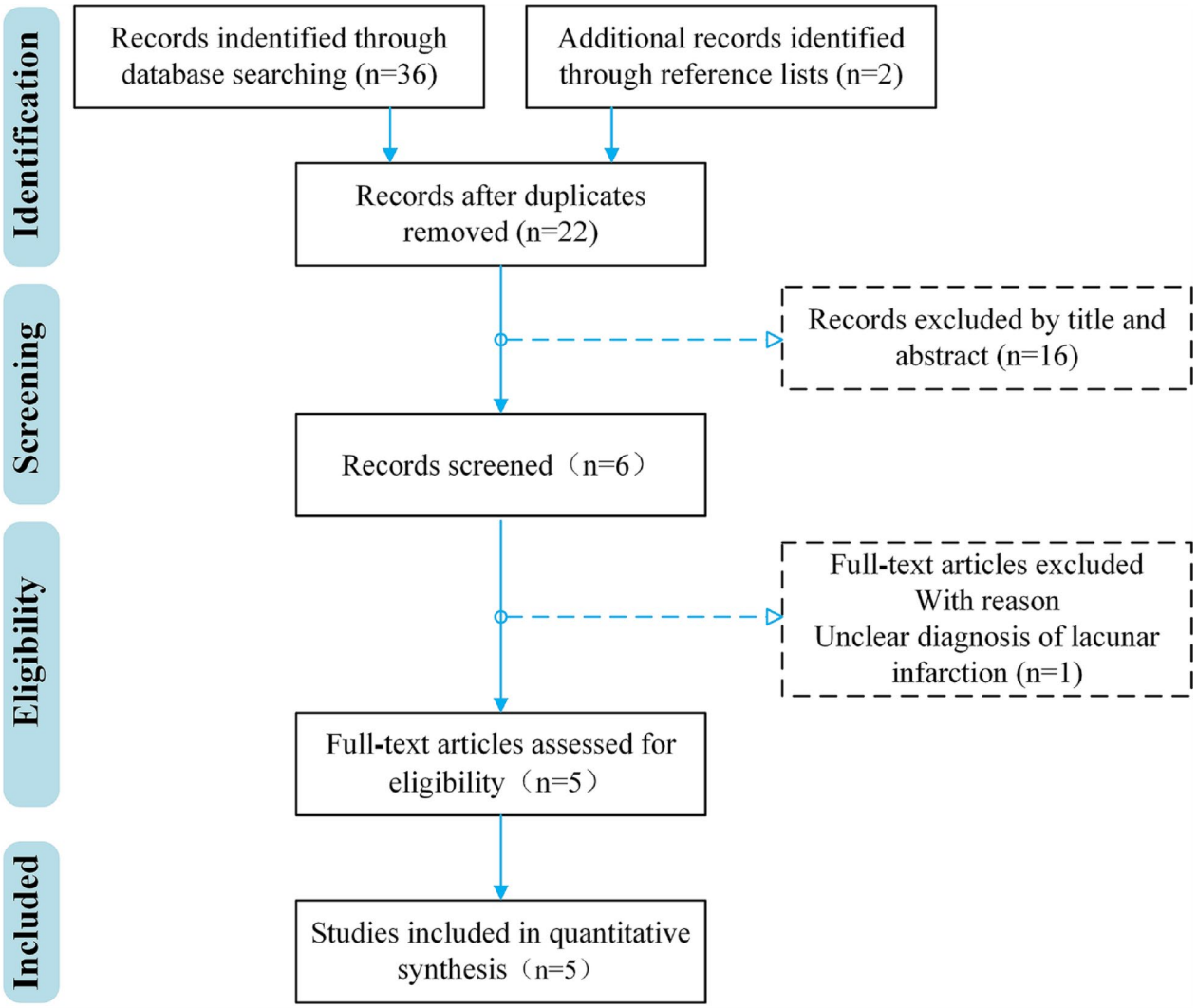

Fig. 2. Flowchart of article retrieval process.

References	Country	Type of study	Sample size	Male/Female	Mean age ± SD	LS diagnostic criteria	Clinical periodontal indices
Taguchi et al.[41]	Japan	cross-sectional study	110	52/58	57.8 ± 11.5	T2-images showed hyperintense lesions with corresponding hypointense centers and a hyperintense rim on FLAIR, < 20 mm in diameter, inconsistent with clinical findings.	(1)
Leira et al.[42]	Republic of Chile	cross-sectional study	122	86/36	68	The patient presented with a typical lacunar syndrome, a neurological deficit lasting over 24 h, no signs of cortical dysfunction, and a computed tomography /magnetic resonance imaging (CT/MRI) showing either normal results or a deep focal infarction ≤ 15 mm in the correct location.	(1) (2) (3)
Leira et al.[8]	Republic of Chile	cross-sectional study	277	190/87	66.4 ± 9.9/65.4 ± 9.9	The patient exhibited one of the typical clinical lacunar syndromes, characterised by a neurological deficit persisting for more than 24 h, absence of cerebral cortical dysfunction, and either normal CT/MRI findings or evidence of a deep focal infarction in a suitable location, measuring ≤ 15 mm in diameter.	(1) (2) (3) (4)
Leira et al.[43]	Republic of Chile	cross-sectional study	240	168/72	66.4 ± 9.9/64.9 ± 10.0	The patient exhibited a lacunar syndrome lasting > 24 h, without cortical dysfunction, and CT/MRI revealed a normal brain or a deep focal infarction ≤15 mm in the correct area.	(1) (2) (3) (4)
Ito et al.[44]	Japan	cross-sectional study	370	179/191	61	At least one infarction lesion, 320 mm in diameter, was observed as a high-intensity area on MRI T2-weighted images and as a low-intensity area on MRI T1-weighted and FLAIR images.	(2)

Table 2. Characteristics of included researches. Clinical periodontal indices: (1): probing depth; (2): clinical attachment loss; (3): periodontal inflammatory surface area; (4): full-mouth bleeding score.

References	Items											Total score	Quality
	1	2	3	4	5	6	7	8	9	10	11		
Taguchi et al.[41]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	7	Moderate
Leira et al.[42]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	7	Moderate
Leira et al.[8]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	7	Moderate
Leira et al.[43]	Yes	Yes	Yes	Yes	No	Yes	Yes	Unclear	No	Yes	No	7	Moderate
Ito et al.[44]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	7	Moderate

Table 3. Agency for healthcare research and quality (AHRQ) checklist (cross-sectional) for studies. Yes: a score of 1; NO or Unclear: a score of 0; overall risk of bias: low (score > 8), moderate (score 4–7), or high (score ≤ 3). Items scored: (1) Define the source of information (survey, record review); (2) List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications; (3) Indicate time period used for identifying patients; (4) Indicate whether or not subjects were consecutive if not population-based; (5) Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants; (6) Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements); (7) Explain any patient exclusions from analysis; (8) Describe how confounding was assessed and/or controlled; (9) If applicable, explain how missing data were handled in the analysis; (10) Summarize patient response rates and completeness of data collection; (11) Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained.

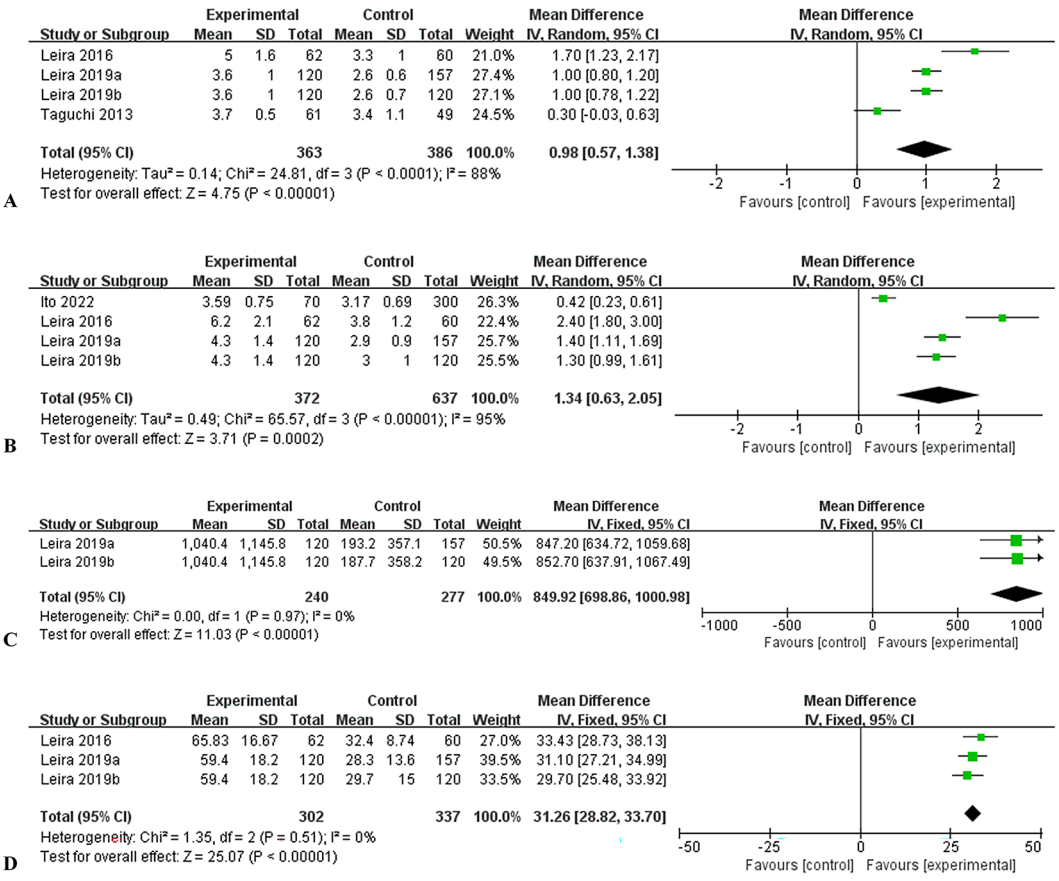

Fig. 3. The relationship between lacunar stroke and periodontitis.

CI [0.63, 2.05], $P < 0.001$; PISA: MD = 849.92, 95% CI [698.86, 1000.98], $P < 0.001$; FMBS: MD = 31.26, 95% CI [28.82, 33.70], $P < 0.001$). The meta-analysis results for PD and CAL demonstrated significant heterogeneity (PD: $I^2 = 88\%$, $P < 0.01$; CAL: $I^2 = 95\%$, $P < 0.01$), whereas the results for PISA and FMBS exhibited no heterogeneity (Fig. 4). No publication bias was observed on the funnel plot (Supplementary Fig. S1).

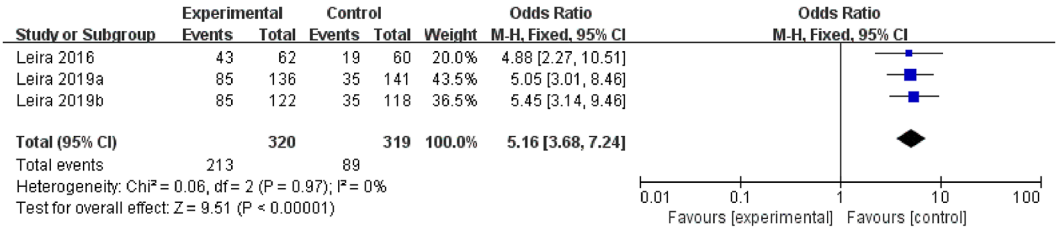

Fig. 4. The relationship between lacunar stroke and clinical periodontal indicators. Probing depth (**A**); clinical attachment loss (**B**); periodontal inflamed surface area (**C**); full-mouth bleeding on probing score (**D**).

Mendelian randomization analysis

Selection of instrumental variables

Ultimately, six SNPs associated with periodontitis, six associated with dental caries, and sixteen associated with pulp and periapical diseases were included in the analysis. All involved SNPs exhibited F-statistic values exceeding 20 (Supplementary Table S2).

Periodontitis and diseases of the pulp and periapical tissues may pose potential risk factors for lacunar stroke

Two-sample MR analysis showed a positive causal relationship between genetically predicted pulp and periapical diseases and LS (OR [95% CI] = 1.20[1.03,1.41], $P = 0.022$), adjusted $P = 0.033$ (Supplementary Table S3, Fig. 5). The sensitivity analysis did not reveal any horizontal pleiotropy (MR Egger: P-value = 0.35, MR-presso: Global test P-value = 0.48). No heterogeneity was observed according to Cochran's Q-test done by IVW and MR-Egger (IVW: Q P-value = 0.45, MR Egger: Q P-value = 0.45) (Supplementary Table S5).

Beyond that, the results suggested that periodontitis was a potential risk factor for LS (OR [95% CI] = 1.24 [1.03,1.49], $P = 0.021$), adjusted $P = 0.064$ (ML adjusted $P = 0.037$) (Supplementary Table S3, Fig. 5). No evidence of horizontal pleiotropy was detected through the implementation of sensitivity analysis (MR Egger: P-value = 0.48, MR-presso: Global test P-value = 0.34). No heterogeneity detected by Cochran's Q test (IVW: Q P-value = 0.32, MR Egger: Q P-value = 0.28) (Supplementary Table S5).

The scatter and funnel plots were showed in Supplementary Fig. S2 and S3. The leave-one-out analysis confirmed that no SNP dominated the overall effect (Supplementary Fig. S4). No significant causal association was found between dental caries and LS (Supplementary Table S3, Fig. 5).

No mediating role of IL-1α, IL-6, TNF, and CRP was observed in promoting lacunar stroke by periodontitis and diseases of the pulp and periapical tissues

The findings indicated a moderate level of causality between pulp and periapical diseases, periodontitis, and LS. Therefore, a two-step MR analysis was performed to investigate the potential bridging role of the cytokines in these relationships. Unexpectedly, IL-1α, IL-6, TNFα/β, and CRP did not demonstrate mediating effects (Supplementary Table S4). The results of the first MR step suggested that periodontitis led to an increase in IL-1α, but no association was found between IL-1α and LS in the second step (Fig. 6). In addition, no association between pulp and periapical diseases with these cytokines was found.

Discussion

In this research, we initially identified a correlation between oral infectious diseases and LS through a meta-analysis; however, the causal relationship between these two variables remained ambiguous. Consequently, we conducted a MR analysis to elucidate the potential influence of oral infectious diseases on the development of LS. Our integrated findings of MR study and meta-analysis provided evidence supporting a relationship between periodontitis and LS, generally consistent with previous observational studies. Additionally, the MR analysis demonstrated, preliminary evidence indicating a potential association between pulp and periapical diseases and the LS. This finding necessitates further validation through meticulous clinical investigations involving large sample sizes. Collectively, these complementary methodologies strengthen the corroboration that oral infectious diseases may influence LS from both epidemiological and genetic perspectives.

The mechanism connecting pulp and periapical diseases, periodontitis, and lacunar stroke remains unclear. However, a few studies have noted the association between oral infectious diseases and systemic inflammation, as well as their relationship with cardiovascular disease[45–47]. Endodontic infection has been shown to cause transient bacteremia, which results in the entry of bacteria into the bloodstream, thereby affecting the systemic inflammatory state. In addition to the direct entry of oral infectious disease pathogens into the circulatory system, bacterial virulence factors can also activate the immune system and exacerbate host inflammation. It has been reported that the lipopolysaccharide (LPS) of *Porphyromonas gingivalis* is associated with atherosclerosis and other cardiovascular diseases[16,48]. Moreover, LPS affects the influx and efflux of amyloid β protein (Aβ) across the blood-brain barrier, leading to the accumulation of Aβ, which is one of the characteristics of neurodegenerative diseases[49].The systemic inflammatory state induced by periodontitis could also be a contributing factor[9]. Furthermore, Dorn BR et al. found that *Porphyromonas endodontal* ATCC 35,406 can invade human coronary artery endothelial cells in vitro[50], which may trigger endothelial cell dysfunction and elevate the risk of LS. The oral-intestinal-brain axis also provides another possible way for oral infectious diseases to affect brain lesions[51].

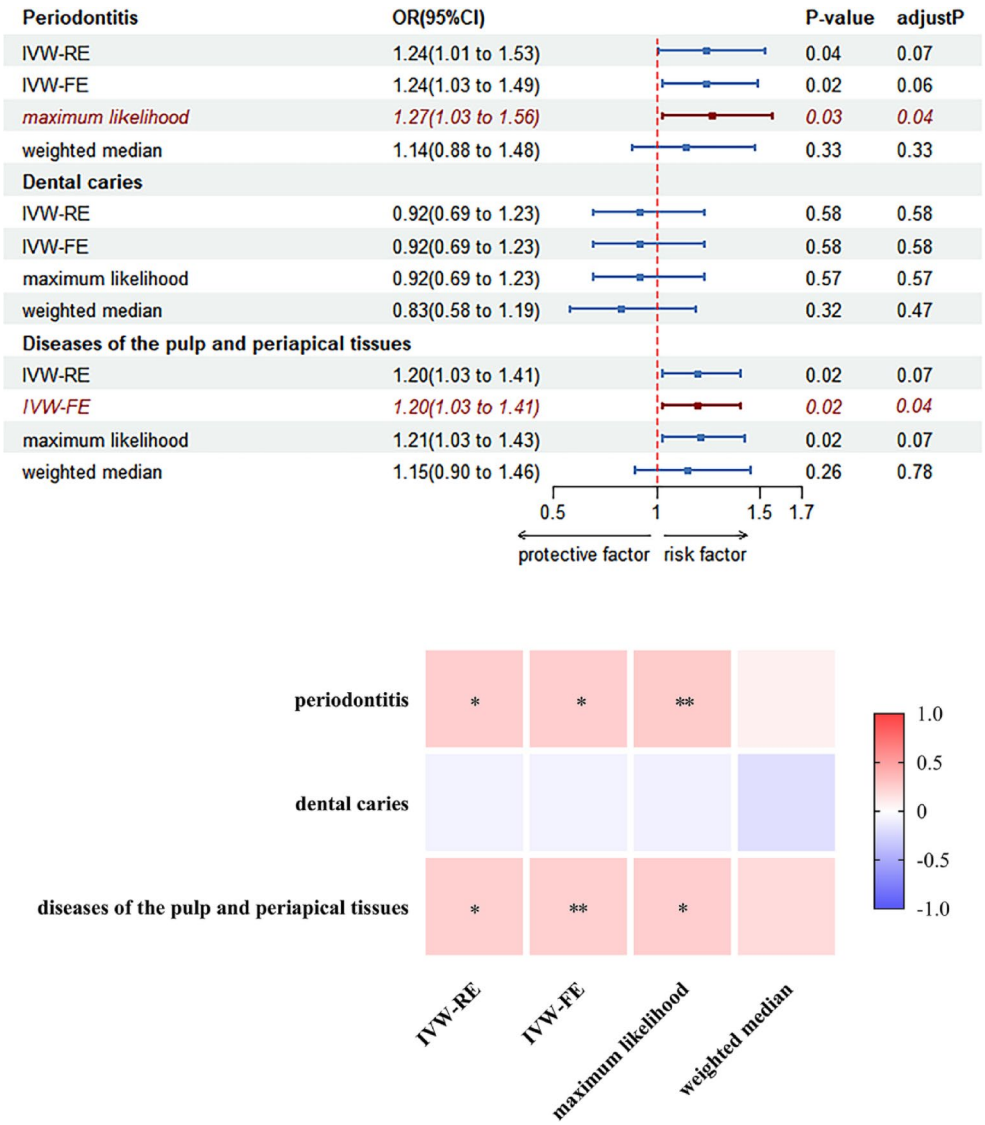

Fig. 5. The positive causal association between pulp and periapical diseases, periodontitis, and LS. Forest plot (**A**). Heatmap (**B**). OR: odds ratio; 95%CI: 95% confidence interval. Uncorrected p-value suggested possible association (*, $P < 0.05$), and FDR-corrected p-values suggested that the association were statistically significant (**, $P < 0.05$).

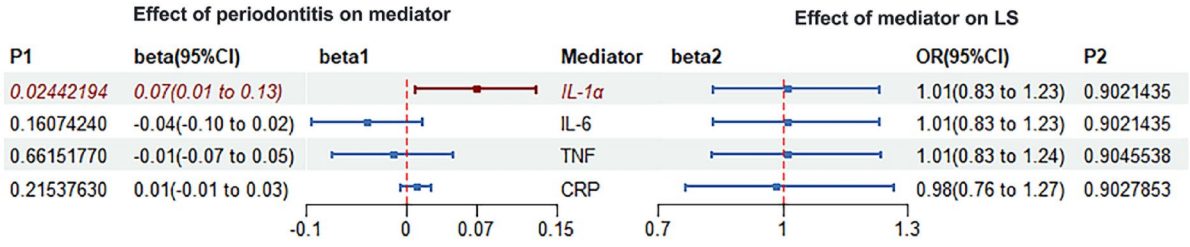

Fig. 6. Cytokines were not involved in the relationship between pulp and periapical diseases, as well as periodontitis and lacunar stroke. OR: odds ratio; 95%CI 95% confidence interval; LS: lacunar stroke.

Research conducted by Arboix et al. suggested the presence of a threshold for the incidence of LS among individuals aged between 55 and 85 years[52]. Additionally, a report by Ito et al. further suggested that 68 years of age serves as the threshold for a significantly elevated prevalence of LS[44]. The prevalence of periodontitis also increases with age and is characterized by a decrease in masticatory efficiency due to loosening and loss of teeth[53,54]. A previous study showed that diminished chewing motion may influence cerebral blood flow. Consequently, a reduction in blood flow to the cerebral cortex may diminish the capacity for cerebral vasodilation, leading to small arteriosclerosis, which is a contributing factor to lacunar infarction[55,56]. Therefore, this study hypothesized that with increasing age, the increased prevalence of periodontitis may be related to the higher incidence of LS.

Previous studies have suggested that IL-6 and CRP may serve as mediators in the relationship between oral infectious diseases and LS[57,43,58]. However, in our MR analysis, IL-1α, IL-6, TNF, and CRP did not serve as mediators. This underscored the necessity for additional experiments to validate our findings and to identify other factors or mediators in the relationship between oral infectious diseases and LS.

Our study has several limitations. In our MR analysis, only SNPs from European populations were included, necessitating caution when generalizing the conclusions to other racial groups. Additionally, the current meta-analysis revealed significant heterogeneity in some findings, attributable to the limited number of studies incorporated. It is noteworthy that the populations in the MR study and the meta-analysis differed, which may limit the universal applicability of our conclusions. Nevertheless, our research provided essential preliminary insights into this under-explored area by offering a comprehensive examination of genetic evidence through MR study and clinical evidence via meta-analysis.

Conclusion

The evidence derived from both the MR study and the meta-analysis suggested a potential association between periodontitis and LS. These findings indicated that periodontitis might play a role in the development of LS. However, given the limitations inherent in our research, further studies are necessary to validate these conclusions.

Data availability

Data supporting the results of this study are available from the corresponding author upon reasonable request.

Received: 29 October 2024; Accepted: 22 April 2025
Published online: 16 May 2025

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Author contributions

All authors contributed to the study's conception and design. Data collection, analysis, and writing-original draft were performed by Q.Z. Validation was done by T.X. Both S.L. and J.Z. collected part of the materials and helped with data curation. Supervision, and Visualization were performed by Y.Y. Methodology was suggested by A.J., D.Z. and Y.P. reviewed and edited the manuscript. All authors commented on previous versions of the manuscript and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China, grant number: 81970943.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
This study didn't involve the use of human or animal subjects. Therefore, an ethics statement was not provided. The study used publicly available GWAS data approved by relevant, ethical, and institutional review boards. All procedures were implemented in accordance with relevant laws and institutional guidelines.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-99742-x.

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