SYSTEMATIC REVIEWS

DNA Methylation Analysis in Predicting Postoperative Delirium in Head Surgery Patients: A Systematic Review

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ABSTRACT

Objectives: To assess whether DNA methylation (DNAm) analysis can serve as a predictive biomarker for postoperative delirium (POD) in patients undergoing head surgery, and to synthesize current evidence linking perioperative epigenetic alterations with POD incidence.

Study design: Systematic review of prospective observational studies conducted in accordance with PRISMA guidelines. Eligible studies included cohort designs evaluating DNAm profiles in relation to POD. Risk of bias was assessed using the ROBINS-E tool. Due to methodological heterogeneity and limited sample size, meta-analysis was not performed.

Data sources: A comprehensive search of PubMed, Google Scholar, and ScienceDirect was conducted for studies published between January 2020 and December 2025. Reference lists of included articles were also screened to identify additional relevant studies.

Data synthesis: Four prospective cohort studies met the inclusion criteria. Across studies, POD was associated with differential DNAm in immune- and inflammation-related pathways, including cytokine signaling and immune system regulation. Epigenetic alterations were identified in both central nervous system tissue and peripheral samples. Dysregulation of glial cell–related pathways and surgery-induced methylation changes in proinflammatory genes were consistently reported. Although no individual CpG site achieved genome-wide significance, pathway-level analyses and composite methylation indices demonstrated moderate discriminatory potential for POD.

Conclusions: DNAm alterations are associated with POD following head surgery, particularly through immune and neuroinflammatory mechanisms. Current evidence remains exploratory and limited by small sample sizes and heterogeneity. Larger, well-designed longitudinal studies are required to validate the clinical utility of epigenetic biomarkers for POD prediction.

Registration: This review was not prospectively registered.

Keywords: biomarker; DNA methylation; epigenetics; head surgery; postoperative delirium

INTRODUCTION

Postoperative delirium (POD) has been associated with increased morbidity, higher mortality, prolonged hospitalization, and increased healthcare costs.[1] Patients undergoing head surgery are particularly vulnerable. A meta-analysis reviewing the incidence after intracranial surgery reported a rate of 19%, with a significant range of 12% to 26% depending on clinical characteristics and assessment methods used. Furthermore, in specific cohorts such as glioblastoma patients, the incidence of POD reached 7% and was associated with longer length of stay and decreased survival.[2]

The pathogenesis of POD is believed to be multifactorial, involving a complex interaction between individual genetic susceptibility, pre-existing neurodegenerative pathology (such as Aβ deposits and hyperphosphorylation of Tau protein), and inflammatory stressors triggered by surgical trauma.[3] In the context of pathophysiology, DNAm has emerged as an important epigenetic mechanism that may bridge the gap between stress and neurodegenerative processes. environmental factors (surgical trauma) and gene expression responsible for neuroinflammation and cognitive function.[4] DNAm is a chemical modification of cytosine that typically occurs at CpG sites, which functions to regulate gene transcription without altering the underlying DNA sequence.[5] Changes in DNAm patterns can be triggered by perioperative factors, including anesthetic agents and the postoperative inflammatory response, which can have long-term effects on cognitive function.[3]

Recent genomic studies support the epigenetic hypothesis, showing that surgical trauma can significantly alter DNAm profiles, particularly in pro-inflammatory genes such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6).[2] Genome-wide studies have shown promising DNAm signals associated with immune and inflammatory responses in POD patients, explicitly supporting a role for epigenetics in the molecular mechanisms of delirium pathogenesis.[4]

Given that most findings regarding DNAm and POD come from small-scale observational studies and often use heterogeneous surgical cohorts, there is an urgent need for structured data synthesis.[1] Therefore, this systematic review aims to assess the role of DNAm analysis as a predictor of POD incidence in head surgery patients.

METHODS

Article review process

The article review was conducted systematically in accordance with the PRISMA guidelines. This process involved several stages: (1) defining inclusion and exclusion criteria; (2) performing a comprehensive literature search; (3) screening and selecting relevant studies; (4) evaluating the quality of included studies; (5) extracting data; and (6) conducting qualitative analysis.

Search strategy

Relevant studies were retrieved from several databases, including PubMed, Google Scholar, and ScienceDirect. The search utilized Boolean operators with combined keywords such as (“DNAm” OR “epigenetic modification”) AND (“postoperative delirium”) AND (“neurosurgery” OR “brain surgery”).

Eligibility criteria

Studies were considered eligible if they met the following criteria: (1) published in English; (2) released between 2020 and 2025; and (3) available as open-access articles. Exclusion criteria included: (1) lack of relevance to the research objective; (2) absence of full-text availability; (3) publication in the form of conference proceedings, and (4) any review article. The selection process was guided by predefined PICO criteria (Table 1).

Risk of bias assessment

To evaluate methodological quality, the risk of bias was assessed using the ROBINS-E tool, specifically for non-randomized and cohort studies.

Data collection

The final phase involved systematic data extraction. Information collected included author and publication year, sample size, and participant characteristics, methods of DNA analysis, along with the biological samples used, and outcome measurements across study groups. Data were manually extracted, organized into tables, and subsequently analyzed using qualitative synthesis.

Table 1. PICO criteria

Population	Neurosurgery Patient
Intervention/ Exposure	DNA methylation (DNAm)
Comparison	Neurosurgery patient without POD
Outcome	POD

RESULTS

Study selection

A total of 69 records were initially retrieved from databases, including PubMed, Google Scholar, and ScienceDirect. Following the preliminary screening, 25 articles underwent further assessment, of which 4 studies met the inclusion criteria and were included in the final analysis (Figure 1).

Risk of bias in studies

The methodological quality of each included study was evaluated using the ROBINS-E tool, which is designed for non-randomized and cohort designs. Overall, the included studies demonstrated a low to moderate risk of bias (Figure 2).

Participants

Across the included studies, patient baseline characteristics were generally comparable. Each study involved a moderate sample size, with a substantial proportion of patients experiencing POD. The study populations consisted predominantly of young to middle-aged adults, with a slight predominance of female patients. Mean body mass index values indicated that most patients were overweight to obese. Operative profiles were similar across studies, with consistently prolonged anaesthesia and procedure durations, reflecting the complexity of the surgical interventions. Intraoperative blood loss was moderate and comparable between cohorts. Additionally, a considerable proportion of patients had a history of alcohol consumption and tobacco use, suggesting the presence of lifestyle-related risk factors that may influence perioperative outcomes (Table 2).

Methylation DNA analysis method

All included studies assessed DNAm using Illumina array–based platforms, predominantly the Infinium Methylation EPIC BeadChip, enabling epigenome-wide analysis of CpG sites. DNA was extracted from various samples, but dominantly from peripheral blood samples (Table 3).

Alterations in immune and inflammatory pathways associated with POD

Across all included studies, POD was consistently associated with epigenetic alterations involving immune and inflammatory pathways. DNAm analyses revealed differential methylation patterns in genes and pathways related to immune system regulation, cytokine signaling, and inflammatory responses. These findings were observed in both central (brain tissue) and peripheral samples (blood, saliva, buccal cells), indicating that POD involves a systemic immune-inflammatory response rather than a purely localized neurological phenomenon.[6,7,8,9]

Glial cell dysregulation and neuroinflammatory signatures

Two studies demonstrated that POD is characterized by marked changes in glial cell–related pathways. In brain tissue, epigenetic and transcriptomic analyses identified enhanced microglial activation, strongly implicating neuroinflammation in POD pathophysiology. Astrocytes also exhibited functional alterations, particularly in synapse-related processes and cellular migration. Enrichment analyses further highlighted pathways related to glial cell differentiation, supporting the role of glial dysregulation as a central mechanism underlying POD.[6,7]

Surgery and anesthesia-related epigenetic changes

Two studies showed that surgical intervention and anesthesia induced significant DNAm changes, particularly in proinflammatory cytokine genes. Alterations in methylation levels of TNF, IL1B, and IL6 were more pronounced in patients who developed POD compared with those who did not. These findings suggest that perioperative stressors may trigger maladaptive epigenetic responses in susceptible individuals, contributing to the development of POD.[8,9]

Identification of potential epigenetic biomarkers

Several studies explored the potential clinical utility of epigenetic markers for POD detection. Specific CpG sites and composite indices based on postoperative DNAm patterns demonstrated moderate to good discriminatory performance in identifying POD.[9] Although some genome-wide analyses did not yield CpG sites reaching strict significance thresholds, pathway-level enrichment consistently pointed toward biologically relevant immune and inflammatory processes, supporting the translational potential of epigenetic biomarkers.[7]

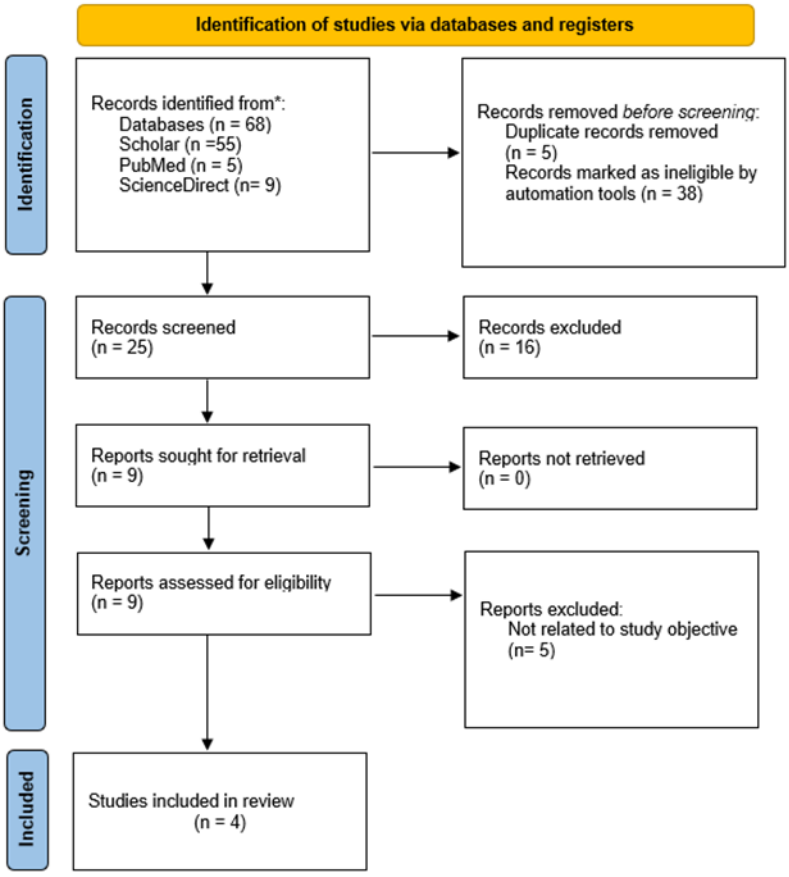

Figure 1. PRISMA flowchart

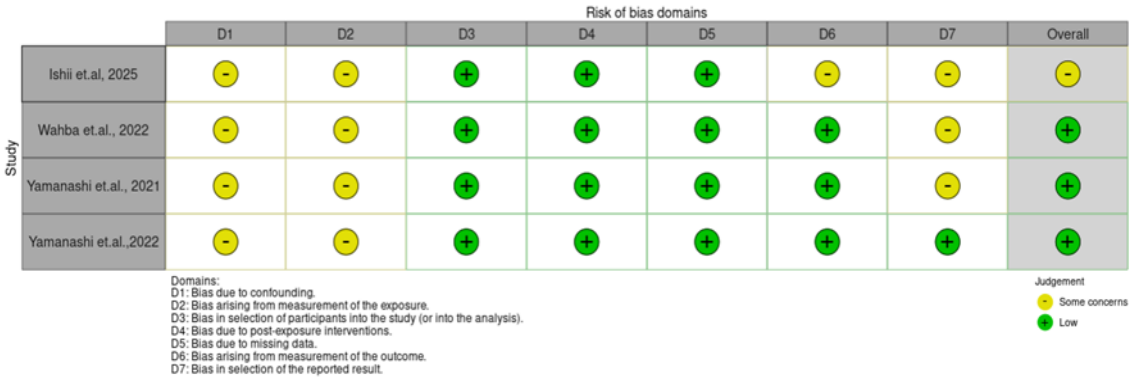

Figure 2. Risk of bias

Table 2. Characteristics of studied participants

	Ishii, et.al., 2025[6]	Wahba et.al, 2022[7]	Yamanashi et.al., 2022[8]	Yamanashi et.al., 2021[9]
Number of Patients (n)	18	37	39	37
POD patients (n)	9	10	10	10
Age (years) (mean ± SD)	36.4 ± 14.1	34.1 ± 16.3	33.4 ± 15.1	32.8 ± 15.3
Sex (female (n, %)	7 (38.9)	16 (44)	15 (38.5)	13 (0.4)
BMI (kg/m^2) (mean ± SD)	29.0± 8.5	N/A	29.8 ± 10.4	29.6 ± 10.4
Mean Anaesthesia time (min) (mean ± SD)	542.6 ± 64.6	531.4 ± 88.7	530.6 ± 91.0	535.9 ± 88.4
Mean Procedure time (min) (mean ± SD)	352.2 ± 76.8	N/A	348.1 ± 90.1	353.6 ± 88.0
Mean blood loss (mL) (mean ± SD)	182.5 ± 151.7	172.9 ± 142.7	165.0 ± 120.0	169.3 ± 120.8
History of alcohol (n, %)	6 (33.3)	9 (25)	12(30.8)	12 (32.4)
History of tobacco (n, %)	8 (44.4)	12 (33)	16 (41)	15 (40.5)

Table 3. Summary of studies

Author	Sample	Methylation method	Outcome	Study design
Ishii, et.al., 2025[6]	Brain tissue	Illumina Infinium Methylation EPIC BeadChip	• DNAm profiling demonstrated epigenetic alterations in genes associated with immune response and inflammatory pathways. • Single-nucleus RNA sequencing (snRNA-seq) revealed glial cell changes specific POD, with pronounced activation of microglia indicating heightened neuroinflammatory activity. • Astrocytes showed functional modifications related to synaptic processes and cellular migration. • Further downstream analyses suggested that glial cell profiles in POD share similarities with those observed in neurological conditions such as encephalitis and dementia.	Prospective observational cohort (post-test only)
Wahba et al., 2022[7]	Brain tissue, peripheral specimen (blood, buccal, saliva)	Illumina Infinium DNAm Array (EPIC/450K)	• The most significant CpG difference between control and POD groups was identified at site cg16526133, located near the *ADAMTS9* gene in brain tissue ($p = 8.66 \times 10^{-8}$). • However, no CpG sites reached genome-wide significance. • Enrichment analysis of the top 1,000 CpG sites ($p < 0.05$) across four tissue types revealed several notable biological pathways.	Prospective observational cohort (post-test only)

			• In brain tissue, pathways related to the positive regulation of glial cell differentiation were identified. • Blood samples indicated enrichment in immune-related pathways. • Meanwhile, saliva and buccal samples showed associations with circadian rhythm pathways, although these results were not statistically significant after FDR correction.	
Yamanashi et.al., 2022[8]	Peripheral blood	Illumina Infinium Methylation EPIC BeadChip	• Enrichment analysis identified significant associations with pathways related to immune response and inflammatory cytokine activity, including "cellular response to cytokine stimulus," "regulation of immune system processes," "regulation of cell activation," and "regulation of cytokine production." • After adjusting for potential confounding effects of surgery and anesthesia between POD and non-POD groups, significant pathways remained, particularly those involving immune response and T cell activation.	Prospective observational cohort (pre-post-test)

Yamanashi et.al., 2021[9]	Peripheral blood	Illumina Infinium Methylation EPIC BeadChip	• Several CpG sites within the TNF gene in preoperative blood samples demonstrated a negative correlation between DNAm and age in both POD and non-POD groups. • Postoperatively, this inverse relationship persisted only in patients with POD. • Neurosurgical procedures significantly influenced DNAm levels across multiple inflammatory genes, including 17 of 24 CpG sites in TNF, 8 of 14 in IL1B, and 4 of 14 in IL6. • The inflammatory methylation index (IMI), derived from post-surgical DNAm levels at five selected CpG sites, showed potential as a diagnostic marker for delirium, with moderate accuracy (AUC = 0.84).	Prospective observational cohort (pre-post-test)

DISCUSSION

POD remains a common and serious complication of neurosurgery, affecting roughly one in five patients after intracranial procedures.[10] Recent work has focused on blood and tissue biomarkers, including epigenetic signatures, to fill this gap.[8] The present review systematically evaluated whether DNAm changes can predict POD in head surgery patients. In doing so, we aimed to integrate emerging data on DNAm profiles in both brain and peripheral tissues, and to clarify how epigenetic dysregulation may underlie the neuroinflammatory processes known to trigger delirium.[11]

The studies included in our review yielded consistent themes but few single-site biomarkers. In peripheral blood, major neurosurgery profoundly altered methylation at multiple CpGs in key cytokine genes (TNF, IL1B, IL6).[9] Similarly, pre-vs-post surgical DNAm changes in POD patients were enriched in immune response and cytokine signaling pathways.[8] More importantly, pathway analysis revealed that brain tissue CpGs were enriched in "positive regulation of glial cell differentiation", blood CpGs in immune-related pathways, and (to a lesser extent) saliva/buccal in circadian rhythm functions.[7] Although a study could not be fully accessed, their multi-omic analysis of resected brain tissue likewise highlighted POD-specific changes in glial cells, especially microglia, and strong neuroinflammatory gene signatures (paralleling encephalitis or dementia states).[6] Taken together, the main findings are that POD patients exhibit differential DNAm patterns in genes governing inflammation and brain immune function.[7,8,9]

These epigenetic findings resonate with the hypothesized neuroimmune etiology of delirium. Surgery and anesthesia trigger systemic inflammation, which can perturb the blood–brain barrier and activate brain immune cells (microglia, astrocytes).[10] DNAm provides a possible interface between these systemic insults and gene expression. In our review, changes in DNAm at inflammatory loci (TNF, IL1B, IL6) were prominent in POD cases, and methylation shifts also emerged in pathways for cytokine regulation and cell activation.[11-13] These epigenetic changes may reflect or even mediate a heightened immune response. Likewise, glial differentiation pathways were altered in POD brain tissue, consistent with single-nucleus transcriptomics, indicating over-activation of microglia and synaptic dysregulation in astrocytes during POD.[6,14] Notably, delirium-associated methylation differences have also been linked to cholinergic synapse pathways, fitting with clinical evidence that inflammation can disrupt acetylcholine-dependent attention networks. In summary, the reviewed data suggest that DNAm markers capture immune activation and neuroinflammation, reinforcing the concept that delirium is an epigenetically driven inflammatory brain syndrome.[11]

From a translational perspective, these results hint that methylation markers might eventually aid in POD risk stratification. For instance, the inflammatory methylation index (IMI) achieved moderate-to-high AUC for delirium detection.[9,14-16] In practice, a blood-based DNAm assay could supplement clinical scoring to identify high-risk patients preoperatively. The enrichment of immune and glial pathways also suggests targets for intervention. Specific cytokine-gene

methylation patterns confer vulnerability; one could monitor or modulate those axes (e.g., through anti-inflammatory prehabilitation).[17] Moreover, the peripheral vs central tissue findings raise the possibility of using accessible tissues (blood, buccal cells) as proxies for brain risk states. However, the predictive utility is still debatable. No single CpG has yet reached genome-wide significance across studies, so future biomarker panels will likely need to integrate multiple CpGs (and perhaps other data) for robust performance. Overall, these findings underscore an "epigenetic hypothesis" of delirium, where DNAm profiles may serve as both mechanistic indicators and clinical flags of POD risk.[11,13,18]

Nevertheless, the current evidence has some limitations. First, all studies had limited sample sizes, which may reduce statistical power, and indeed, no CpG met strict genome-wide significance in any study. Second, findings differed by tissue type. Brain tissue analysis (from epilepsy resections) may capture delirium-relevant changes, but this is feasible only in neurosurgical patients; blood, saliva, or buccal samples are easier to collect but may not fully reflect central neuroinflammation. Third, most designs were cross-sectional or immediate pre/post assessments, lacking long-term follow-up. Thus, it is unknown whether the observed DNAm differences are stable risk traits or acute responses that fade with recovery. Fourth, there is substantial inter-individual variability: DNAm patterns are strongly influenced by age, cell composition, genetics, and environment. Unless such factors are rigorously controlled or modeled, confounding could affect results. Finally, potential surgical or anesthetic confounders (residual effects of procedure type, anesthetic medications, or perioperative complications) may still bias DNAm measures.

This systematic review suggests that POD after head surgery is associated with DNAm changes in immune and inflammatory pathways. Although these epigenetic markers show promise for predicting POD, current evidence is limited by small, heterogeneous studies, and further validation is needed before clinical application.

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