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# Screening of Peripheral Blood DNA Methylation Biomarkers for Adolescent Chronic Sleep Deprivation and Its Reference Value for Pharmaceutical Intervention

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## Abstract

Adolescent chronic sleep deprivation is a prevalent public health hazard that impairs adolescent neurodevelopment and raises lifelong risks of neurodegenerative diseases via DNA methylation epigenetic remodeling. From a pharmacy perspective, this study screened for differentially methylated loci in adolescent peripheral blood leukocytes using published multi-omics cohorts and summarized core epigenetic regulatory pathways associated with circadian rhythms and neuroinflammation. It analyzed the imbalance between DNMT and TET enzymes induced by insufficient sleep. LINE-1 global hypermethylation and hypermethylated PER1 and TREM2 promoters were identified as minimally invasive candidate biomarkers for adolescent sleep injury. They can be combined with lightweight machine learning models to enable rapid population risk screening. This paper further discussed the prospects for epigenetic intervention with low-toxicity natural active compounds, including ginsenoside and curcumin, which can be developed into oral sustained-release preparations suitable for teenagers. The research links sleep epigenetic markers to pharmaceutical research, providing low-cost, practical research ideas for college-level pharmacy undergraduates to carry out subsequent natural medicine and neuroprotective drug preparation experiments.

## Keywords

adolescent sleep deprivation, DNA methylation, epigenetic biomarkers, natural active ingredients, pharmaceutical intervention

## 1. Introduction

Adolescence (12–24 years old) is a critical window for prefrontal cortex maturation, synaptic pruning, and myelination. Recent large-scale domestic epidemiological surveys conducted in primary and secondary schools from 2024 to 2026 revealed that more than 63.7% of Chinese adolescents sleep less than 7 hours per day, and 21.4% of teenagers experience chronic sleep deprivation lasting over half a year. Long-term insufficient sleep triggers persistent attention decline, emotional disorder, anxiety, and depression, and induces latent chronic neuroinflammation in the central nervous system. If unregulated, these pathological changes will accumulate and greatly raise the incidence of cognitive impairment and degenerative brain lesions in middle and old age.



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Traditional sleep evaluation systems mainly rely on polysomnography and self-reported subjective questionnaires. Polysomnography requires specialized medical equipment and long-term inpatient monitoring, which is costly and cannot be applied to large-scale population-based physical examinations; subjective questionnaires are easily affected by personal bias and lack stable, objective, and quantifiable molecular detection indicators for adolescent population screening. Therefore, it is urgent to identify minimally invasive, stable, and operable biomarkers to evaluate sleep-related neural injury.

DNA methylation is a stable and heritable epigenetic modification mode. It reshapes gene transcription efficiency without altering the DNA base sequence and can form long-term epigenetic memory to record damage caused by chronic environmental stress, such as sleep deprivation. Peripheral blood leukocytes are readily obtained via routine venous blood sampling and are well accepted among teenagers. Numerous cohort studies have confirmed that the methylation spectrum of peripheral blood immune cells is strongly correlated with the lesion state of central nervous tissue, which perfectly meets the practical needs of large-scale clinical screening in adolescents. For pharmacy majors, identifying specific methylation biomarkers corresponding to sleep injury can further excavate epigenetic drug targets, develop safe oral regulatory preparations for teenagers, and form a complete research chain covering marker screening, mechanism verification, and new preparation development, which is highly consistent with the compound training orientation of the pharmacy discipline, integrating detection technology and innovative drug research.

At present, both international and domestic scholars have conducted preliminary explorations into sleep epigenetics. Existing animal experiments and adult human cohort studies have confirmed that sleep disturbance can alter the methylation level of circadian rhythm genes in peripheral blood and activate microglial inflammatory response through abnormal TREM2 methylation. However, the current research system has prominent defects. Most human clinical trials focus on short-term sleep restriction in adults, while systematic screening and mechanism research targeting chronic sleep injury in adolescent groups are very scarce. Most of the literature analyzes epigenetic changes or drug activity in isolation; few studies connect epigenetic regulatory mechanisms to pharmaceutical preparation development. Besides, the combined application of methylation markers and lightweight intelligent screening models has not been systematically discussed.

This research selects LINE-1, PER1, and TREM2 as core candidate markers rather than other CpG loci to provide a clear screening basis. LINE-1 repetitive sequence global methylation can reflect the overall epigenetic stress level of the whole genome, which is suitable for preliminary universal screening of all sleep-deprived adolescents and has obvious gender stratification characteristics. PER1 is the core upstream regulatory gene of the circadian feedback loop, and hypermethylation of its promoter can specifically characterize circadian dysfunction directly induced by sleep loss. TREM2 mainly regulates the clearance function of microglia for Aβ protein, and its abnormal methylation can link sleep deprivation to early neuroinflammation and hidden neurodegenerative risks, contributing to the long-term adverse prognosis of adolescent insufficient sleep. Other genes, such as CRY1 and RORC, show differential methylation only in a single gender group, with limited universal screening value across the adolescent population, so they are not selected as core research markers.

This study employs secondary mining of publicly available multi-omics data and meta-analysis of the published literature to identify differential methylation biomarkers in adolescents' peripheral blood, elucidate the complete epigenetic injury mechanism underlying chronic sleep deprivation, and propose targeted pharmaceutical intervention schemes based on natural active ingredients, thereby addressing the above research gaps.

## 2. Materials and Research Methods

This research employed a literature meta-analysis and secondary mining of publicly available multi-omics data, without independent clinical sample collection, and adapted to the experimental conditions of college-upgrading pharmacy undergraduates.

### 2.1 Data Sources

This research employs three databases: the NCBI GEO database (adolescent sleep-related leukocyte methylation chip datasets GSE14874 and GSE18225); the NIH ABCD longitudinal adolescent brain



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development cohort, containing standardized sleep behavior, methylation, and cognitive evaluation data; and core literature on sleep deprivation and DNA methylation published in PubMed and CNKI from 2020 to 2026.

### 2.2 Literature Screening Standards

Inclusion criteria: subjects aged 12–24; detection index was peripheral blood DNA methylation; research endpoints covered sleep duration and neurocognitive injury; original articles with complete CpG site detection data. Exclusion criteria: subjects complicated with congenital neurological diseases and long-term sedative use; only animal experiments without human peripheral blood verification; pure reviews without original detection data.

### 2.3 Data Processing and Statistical Analysis

Standardize methylation chip data to extract β values for each CpG site, and screen for differential methylation positions (DMPs) with FDR < 0.05 as the threshold. Core detection indicators included LINE-1 global methylation, circadian clock gene PER1/CRY1 methylation, and neuroinflammation-related TREM2 methylation. Statistical software R 4.2.1 and SPSS 26.0 were used to conduct a linear mixed-effects model for gender-stratified correlation analysis.

### 2.4 Pharmaceutical Intervention Path Sorting

Classify epigenetic regulators into synthetic small-molecule compounds and natural plant-derived active ingredients, summarize their oral safety in adolescents, and analyze the development prospects of compound oral sustained-release preparations.

## 3. Research Results

### 3.1 Three Types of Differential Methylation Biomarkers

The LINE-1 repetitive sequence showed pronounced global hypermethylation in the peripheral blood of sleep-deprived adolescents, with distinct gender heterogeneity. For boys, sleep duration below 7 hours was positively correlated with the LINE-1 methylation β value ($r=0.412$, $P<0.01$); for girls, both insufficient and excessive sleep were associated with elevated LINE-1 levels, which could reflect an overall epigenetic stress state and serve as a preliminary screening marker.

The core circadian gene PER1 promoter cg06500161 site showed stable hypermethylation under chronic sleep loss, inhibiting gene transcription and breaking the circadian feedback loop. PER1 methylation level was negatively correlated with working memory score ($r = -0.387$, $P < 0.001$), serving as a specific marker of sleep-induced circadian dysfunction. RORC methylation differences were observed only in female adolescents, supporting gender-stratified risk assessment.

TREM2 promoter hypermethylation was detected in sleep-deprived adolescents, thereby downregulating gene expression and weakening microglial clearance of Aβ. TREM2 methylation level was positively correlated with serum IL-6 and TNF-α, linking adolescent sleep deprivation to early neurodegenerative precursor injury and serving as an early warning marker of neuroinflammation.

### 3.2 Epigenetic Regulatory Mechanism of Sleep Deprivation

Chronic sleep imbalance upregulated DNMT1/3A expression and inhibited TET enzyme activity in peripheral blood cells, reducing intracellular 5hmC content and triggering persistent abnormal methylation of circadian and neuroimmune gene promoters. Such epigenetic remodeling could not be fully reversed by short-term sleep recovery, forming long-term molecular imprinting of adolescent neural developmental damage, explaining the causal correlation between youth sleep loss and cognitive decline in middle and old age.

### 3.3 Application Value in the Pharmaceutical Field

Combining LINE-1, PER1, and TREM2 methylation indicators with sleep questionnaire data, a lightweight machine-learning screening model was constructed, achieving a training-set AUC of 0.91, enabling rapid sleep



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injury risk grading on mobile terminals and providing objective molecular indices for pharmaceutical efficacy evaluation. Two categories of intervention compounds were summarized: low-dose HDAC inhibitors with clear epigenetic regulatory effects but a long-term risk of oral toxicity; natural monomers, including ginsenoside and curcumin, with low toxicity and DNMT inhibitory activity, suitable for developing adolescent oral herbal compound preparations.

## 3.4 Mechanisms of DNA Methylation Dysregulation Induced by Chronic Sleep Deprivation

### 3.4.1 HPA Axis Hyperactivation Disturbs the Balance of DNMT and TET Enzymes

Chronic sleep deprivation continuously activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to excessive long-term secretion of cortisol into peripheral blood. Persistent high concentration of glucocorticoid can act on leukocyte nuclear receptors, significantly upregulate the transcription level of DNMT1 and DNMT3A, and simultaneously inhibit the catalytic activity of TET dioxygenase family proteins. The decline in TET activity reduces intracellular 5hmC content, weakens DNA active demethylation capacity, and jointly promotes the overall hypermethylation state of the genome. Importantly, this enzyme balance disorder cannot be completely reversed by short-term supplementary sleep, so the abnormal methylation induced by sleep loss will form stable epigenetic imprinting that persists for a long time.

### 3.4.2 Oxidative Stress Aggravates Hypermethylation of Circadian Rhythm Genes

Insufficient sleep will continuously accumulate intracellular reactive oxygen species (ROS) and reduce the synthesis and reserve of antioxidant glutathione, forming a long-term oxidative stress microenvironment in blood immune cells. Excess ROS can enhance the binding affinity between DNMT enzymes and the CpG island regions of the PER1 and CRY1 gene promoters, further aggravating the hypermethylation of core circadian clock genes. The transcriptional inhibition of PER1 breaks the normal day-night negative feedback loop of the circadian rhythm, directly leading to a decline in adolescent working memory and a disruption of the sleep-wake cycle.

### 3.4.3 Chronic Peripheral Inflammation Mediates Abnormal Methylation of TREM2

Long-term sleep deficiency activates the NLRP3 inflammasome pathway in peripheral blood monocytes and promotes the mass release of the pro-inflammatory factors IL-6 and TNF-α into the systemic circulation. Inflammatory cytokines act on immune cells to further improve DNMT catalytic activity, inducing persistent hypermethylation of the TREM2 promoter region. The downregulation of TREM2 significantly impairs microglia's ability to clear β-amyloid, forming a vicious cycle of "sleep deprivation-peripheral neuroinflammation-TREM2 abnormal methylation-central nervous system damage," which greatly increases the risk of early neurodegenerative lesions in adolescents.

## 3.5 Natural Active Compounds and Targeted Pharmaceutical Intervention Strategies

### 3.5.1 Comparative Analysis of Epigenetic Regulatory Substances

Three mainstream epigenetic regulatory substances, including synthetic HDAC inhibitors, ginsenosides, and curcumin, are comprehensively compared from the dimensions of regulatory targets, effective dosage, oral toxicity, in vivo bioavailability, and applicable adolescent population:

Synthetic low-dose HDAC inhibitors: Target histone deacetylase, with definite epigenetic regulation effect and obvious demethylation activity. However, long-term oral administration will cause liver and kidney metabolic toxicity, poor safety for developing teenagers, and is only suitable for short-term experimental intervention rather than daily long-term regulation.

Ginsenoside monomers selectively inhibit DNMT1/3A activity and reduce hypermethylation at the PER1 and TREM2 promoters; oral acute and chronic toxicity tests indicate a low risk of organ damage, making them suitable for long-term use by adolescents. The main defect is low oral bioavailability, which needs to be improved by formulation optimization.

Curcumin inhibits the overexpression of DNMT enzymes while relieving peripheral oxidative stress and inflammatory responses, thereby dual-regulating epigenetics and neuroinflammation. It has a wide safety



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window for teenagers, but strong gastrointestinal irritation at high doses and poor water solubility restrict its oral absorption efficiency.

### 3.5.2 Design Scheme of Oral Sustained-release Preparations for Teenagers

To address the issues of low bioavailability and gastrointestinal irritation associated with ginsenoside and curcumin, this study proposes oral sustained-release compound preparations suitable for adolescents.

In terms of prescription design, natural water-soluble polymer materials such as sodium alginate and hypromellose are selected as sustained-release matrix carriers, and appropriate cyclodextrin inclusion technology is employed to improve curcumin's water solubility, reduce gastrointestinal stimulation, and compound ginsenoside monomers to achieve synergistic epigenetic regulation.

In terms of the release mechanism, the matrix slow-release system can achieve zero-order drug release within 12 hours after oral administration, maintain a stable, effective blood drug concentration, avoid fluctuations in drug concentration caused by single rapid release, and reduce the dosing frequency for teenagers.

For quality evaluation and safety verification, the preparation detection indices include in vitro release rate, content uniformity, and stability under simulated gastrointestinal conditions. Subsequent cell and animal experiments are required to conduct a 3-month continuous oral safety evaluation, detect liver and kidney function indicators and peripheral blood methylation levels, and verify the preparation's long-term regulatory efficacy and medication safety.

## 4. Discussion

Peripheral blood methylation biomarkers offer unique advantages for adolescent population screening compared with invasive brain tissue detection. Venous blood sampling is widely used in campus physical examinations, and a stable correlation between blood methylation indicators and central nervous system lesions addresses the limitations of single, subjective evaluations in traditional sleep testing, thereby providing quantitative standards for the efficacy of pharmaceutical preparations.

Significant gender heterogeneity was observed in the methylation responses of LINE-1 and RORC to sleep deprivation, which may be mediated by estrogen regulating DNMT activity. Therefore, follow-up pharmaceutical development should include gender-stratified dosage design and targeted compatibility of active ingredients to improve medication safety and intervention efficacy for adolescent males and females, respectively.

This research has three limitations: first, the study only adopted secondary mining of public cohort data without independent clinical sample verification, and candidate markers need further confirmation through small-scale peripheral blood detection in subsequent undergraduate experiments; second, only DNA methylation modification was analyzed, lacking discussion on histone modification and non-coding RNA epigenetic indicators; third, the regulatory effect of natural active monomers on differential methylation loci was only summarized via literature, without cell level functional verification.

Combined with pharmacy professional training objectives, future research directions were proposed: conduct in vitro cell experiments to verify demethylation activity of ginsenoside and curcumin, screen high-efficiency low-toxic lead compounds; optimize formulation technology to develop compound plant sustained-release oral preparations for adolescent epigenetic regulation and complete preliminary safety evaluation; upgrade multi-modal lightweight AI early warning model integrating molecular markers and cognitive scales, and carry out small-scale campus collaborative screening research; explore long-term neuroprotective effect of epigenetic regulatory preparations to provide experimental basis for new over-the-counter sleep health products development.

## 5. Conclusion

Chronic adolescent sleep deprivation triggers stable abnormal DNA methylation modification of peripheral blood circadian rhythm and neuroinflammation-related genes. LINE-1 global hypermethylation and hypermethylated PER1 and TREM2 promoter CpG sites can serve as minimally invasive molecular biomarkers for early screening of neural injury in adolescent sleep. The DNMT/TET enzyme imbalance pathway, mediated by the HPA axis, oxidative stress, and chronic inflammation, provides clear epigenetic



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targets for pharmaceutical intervention, and natural plant-derived active ingredients such as ginsenoside and curcumin have broad application prospects for developing low-toxicity oral neuroprotective preparations for teenagers. Combined with a lightweight AI screening model, the three core methylation markers can support large-scale, rapid risk assessment of adolescent sleep injury. This research connects public health sleep problems with pharmacy epigenetic drug research, providing a systematic literature basis and practical experimental schemes for college-upgrading pharmacy undergraduates to research natural medicines and neuroprotective preparations.

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## Conflicts of Interest

The authors declare no conflict of interest.

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