

Epigenetic clocks in preterm neonates: Bridging developmental biology and precision neonatal research (Review)

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Abstract. The abrupt disruption of intrauterine epigenetic programming caused by preterm birth exposes the developing neonate to the physiological stressors of the neonatal intensive care unit (NICU). Birth weight and gestational age are the two main factors used in current clinical risk stratification. The biological heterogeneity and differing developmental outcomes observed among infants with identical clinical profiles, however, are not taken into consideration by these traditional parameters. DNA methylation-based epigenetic clocks estimate tissue- and model-specific age and may support research into developmental timing and associations with NICU exposures, including inflammation, hyperoxia and pain. The present review summarizes the principal epigenetic clocks relevant to preterm neonates, critically examines their tissue specificity, performance and validation, and discusses methodological, ethical and translational priorities. Neonatal clocks estimate developmental timing rather than cellular senescence; developmental velocity requires longitudinal measurements. The confounding effects of postnatal clinical interventions and tissue-specific cellular heterogeneity render the interpretation of these metrics difficult. In the present review, neonatal epigenetic clocks should be used as research tools to improve neonatal phenotyping, rather than as predictive diagnostic biomarkers at the bedside. Establishing standardized, tissue-specific bioinformatic pipelines, integrating multi-omics data and validating models across various populations are all necessary to translate these tools into clinical practice. High assay costs, lengthy turnaround times, a lack of organized parental counseling frameworks, and unresolved data governance and ownership protocols are additional obstacles to clinical implementation. Neonatal

epigenetic clocks ultimately provide a research framework for future precision neonatology by illuminating how early-life biological programming influences long-term health trajectories.

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1. Introduction

Preterm birth remains one of the leading causes of neonatal mortality and long-term neurodevelopmental disabilities worldwide, affecting ~13 million infants annually. Although notable advances in neonatal intensive care have substantially improved survival, clinicians continue to encounter marked heterogeneity in outcomes among infants with similar gestational ages, birth weights and clinical characteristics. Some extremely preterm infants achieve near-normal neurodevelopment, whereas others develop significant neurological, respiratory, cardiovascular or metabolic complications, despite apparently comparable neonatal courses. This persistent variability suggests that conventional clinical indicators do not fully reflect the underlying biological maturity and developmental resilience of individual infants (1-5).

Current neonatal risk assessments rely predominantly on chronological parameters, such as gestational age, birth weight

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and postnatal age. While these measures remain indispensable in clinical practice, they provide limited information regarding the biological state of developing organs or the cumulative impact of prenatal and postnatal environmental exposure. Increasing evidence indicates that developmental maturity cannot always be inferred from chronological age alone, as fetal programming is shaped by complex interactions among genetic susceptibility, epigenetic regulation, intrauterine conditions, inflammation, oxidative stress, nutrition and neonatal intensive care exposure (1,3,6-8). Consequently, two infants born at the same gestational age may exhibit substantially different developmental trajectories at the molecular level despite similar clinical presentations.

DNA methylation-based epigenetic clocks have emerged as promising tools for quantifying biological age. Unlike chronological age, biological age reflects cumulative molecular changes that occur during development and may provide insight into the pace of biological maturation. However, a key conceptual distinction needs to be made here. In adults, epigenetic age acceleration generally reflects accelerated biological aging and increased susceptibility to disease. By contrast, neonatal epigenetic clocks primarily measure developmental timing rather than age. Therefore, the interpretation of epigenetic age acceleration or deceleration in preterm infants fundamentally differs from that in adults and should not be viewed as evidence of accelerated aging (1,2,6,9).

This distinction represents a major unresolved conceptual challenge in neonatal epigenetics. The current literature frequently applies terminology derived from adult aging research to neonatal populations, despite profound biological differences between developmental methylation and aging-associated methylation. Such extrapolation risks oversimplifying developmental biology and may lead to inappropriate interpretations of biological age measurements in early life. A clear differentiation between developmental maturity and pathological biological aging is essential for the appropriate interpretation of neonatal epigenetic-clock measurements (1,2,6).

Observational studies have reported associations between neonatal DNA methylation, serious neonatal morbidities and later brain growth or neurodevelopmental outcomes (10,11). These associations do not establish individual prognostic utility. Selecting and interpreting a clock also requires evidence about tissue specificity, the prediction target and performance in the relevant developmental range (12,13). The gestational-age clocks developed by Knight *et al* and Bohlin *et al*, the pediatric buccal epigenetic (PedBE) clock, NEOage, placental clocks and the Horvath pan-tissue clock were developed using different tissues, CpG signatures, training populations and analytical assumptions (1,4,5,14-16). Consequently, these models are not interchangeable, and inappropriate application may generate misleading biological interpretations (13).

Framed against these gaps, the present review sets out an operational, rather than an aspirational agenda. The present review argues that the principal value of neonatal epigenetic clocks lies not in immediate bedside implementation, but in their capacity to redefine developmental phenotyping in neonatal research. Rather than functioning as diagnostic tests, epigenetic clocks should be regarded as integrative research biomarkers capable of linking prenatal programming,

neonatal intensive care unit (NICU) environmental exposures, molecular adaptation and long-term developmental outcomes. Therefore, their greatest contribution may lie in identifying biologically meaningful subgroups of preterm infants that are not recognized using conventional clinical classifications alone (3,6,12,13).

It is proposed that future progress requires a shift from isolated methylation studies to integrated developmental biology. Epigenetic clocks should be interpreted alongside transcriptomics, proteomics, metabolomics, microbiome profiling, neuroimaging and artificial intelligence-based predictive modelling, rather than as independent biomarkers. Such multi-omics integration may improve the understanding of developmental plasticity, while avoiding oversimplified interpretations based solely on biological-age estimates (7,8,12,17).

However, significant barriers continue to impede routine clinical implementation. Tissue specificity, dynamic postnatal methylation changes, assay cost, laboratory infrastructure, bioinformatics expertise, analytical standardization, longitudinal reproducibility, and uncertain clinical thresholds remain major obstacles. Furthermore, ethical concerns, including parental anxiety, data privacy, epigenetic discrimination, informed consent for genomic information and inequitable access in low-resource settings, need to be addressed before translation into precision neonatal care can be responsibly considered (7,12,18).

Despite these advances, three unresolved gaps continue to limit the field: The absence of standardized quantitative benchmarks for reporting biological-age deviation and its incremental predictive value over gestational age and birth weight; the inconsistent application of clock platforms to research questions for which they were not developed; and the lack of pragmatic governance, counseling, and equity frameworks for translating methylation-derived biological age into clinical or family-facing contexts. Addressing these gaps is the primary motivation for the present review. Accordingly, the present review advances a specific and testable perspective: Neonatal epigenetic clocks should be regarded as developmental research instruments rather than clinical biomarkers. It is proposed that their greatest scientific value lies in improving the biological characterization of preterm infants, identifying mechanistic pathways linking NICU exposure to long-term outcomes, and informing future precision-neonatal research through standardized, longitudinal, multicenter and multi-omics investigations.

2. Why the molecular architecture of epigenetic clocks matters in preterm neonates

The successful application of epigenetic clocks in neonatal medicine fundamentally depends on understanding what these clocks measure. Although commonly described as estimators of 'biological age', DNA methylation clocks are mathematical models derived from specific sets of CpG methylation sites whose methylation levels correlate with developmental or chronological time. Notably, these models do not directly measure aging, but instead infer biological timing through predictable methylation changes that occur during human development. Consequently, the biological interpretation of epigenetic age depends on the molecular architecture of the

clock, the tissue analyzed, the developmental stage and the computational algorithm used to generate predictions (1-5).

DNA methylation predominantly occurs at cytosine residues followed by guanine in CpG dinucleotides, where methyl groups regulate chromatin accessibility and gene expression without altering the underlying DNA sequence. During fetal development, DNA methylation undergoes extensive dynamic remodeling as pluripotent cells differentiate into tissue-specific lineages. Thousands of CpG sites exhibit predictable developmental changes throughout gestation, rendering them attractive biomarkers for estimating biological maturity. However, only a relatively small subset of these CpG loci consistently correlate with developmental timing and therefore contribute to epigenetic-clock construction (1,2,6).

CpG selection is one of the most critical determinants of clock performance. Rather than selecting CpG sites solely as they exhibit differential methylation, investigators typically identify loci that demonstrate reproducible quantitative associations with gestational or chronological age across training cohorts. Statistical approaches, such as elastic-net regression, LASSO regression, ridge regression and other machine-learning algorithms, reduce thousands of candidate CpG sites to parsimonious predictive models, while minimizing overfitting (1,2,4,5,7). Consequently, different epigenetic clocks contain different numbers of CpG sites, reflecting distinct optimization strategies rather than universal biological signatures. This methodological diversity explains why clocks developed for one biological question can perform poorly when applied to others.

The computational algorithm underlying the epigenetic clock is equally critical. The majority of first-generation clocks were designed to predict chronological age with maximal statistical accuracy by identifying methylation patterns that are strongly associated with age. The resulting biological-age estimate represents a weighted mathematical combination of methylation values across selected CpG loci. The applicability of these models depends on their training age range and calibration. The Horvath model incorporates a non-linear age transformation before adulthood; nevertheless, it was not designed to resolve gestational age. Chronological-age accuracy across the life course does not establish gestational-age performance (1,2,7,8).

Second-generation epigenetic clocks extend beyond chronological prediction by incorporating biological and clinical outcomes into model development. Instead of simply estimating age, these clocks aim to capture physiological integrity, disease susceptibility and mortality risk. Although this strategy has substantially improved the prediction of adult health outcomes, its application in neonatal medicine remains uncertain, as developmental maturation and biological aging represent fundamentally different biological processes. Therefore, extrapolating adult biological-aging paradigms to preterm infants risks conceptual misinterpretation and may overestimate the clinical significance of neonatal epigenetic-age acceleration (7,8,12).

For neonatal clock studies, performance should be reported using standardized metrics appropriate to the intended prediction task, including mean absolute error or root-mean-square error for age estimation, association with the target age, calibration slope and intercept, and performance in prespecified

subgroups defined by gestational age, sex, ancestry, tissue and clinical severity. Development-study performance should be clearly distinguished from independent external validation; accuracy achieved in a derivation cohort does not establish clinical validity (12,13,15).

An equally critical distinction exists between gestational-age and biological-age clocks. Gestational-age clocks, including the Knight and Bohlin models, estimate fetal developmental maturity using cord-blood DNA methylation profiles and generally demonstrate close agreement with clinically determined gestational age. Their principal purpose is developmental dating rather than prediction of disease risk. Conversely, biological-aging clocks, such as the Horvath clock were developed predominantly using non-neonatal tissues to estimate chronological age across the life course. Because the prediction target and age scale differ from those required for neonatal developmental dating, their utility for characterizing preterm developmental maturity requires separate evaluation (1,2,4,5).

This distinction is not merely technical, but represents one of the central conceptual arguments of the present review. It is proposed that neonatal epigenetic clocks should be interpreted primarily as indicators of developmental tempo rather than biological-aging processes. In preterm infants, epigenetic-age acceleration should not automatically be interpreted as pathological accelerated aging, nor should epigenetic-age deceleration invariably indicate impaired development. Instead, these observations may reflect adaptive or maladaptive developmental responses to prenatal adversity, inflammation, oxidative stress, nutritional status and exposure to neonatal intensive care. Consequently, meaningful biological interpretation requires integration with clinical context, rather than reliance on methylation-derived age estimates alone (6,9,19).

The marked developmental plasticity of early life further complicates interpretation. Unlike adult methylation profiles, neonatal DNA methylation patterns change rapidly during the weeks immediately before and after birth. Environmental exposures, including hyperoxia, mechanical ventilation, infection, pain, medications, nutritional interventions and parental caregiving, may modify methylation trajectories during this sensitive developmental window. Accordingly, the same epigenetic-age estimate may reflect distinct biological mechanisms depending on the timing of sampling, the tissue analyzed, and clinical circumstances. This dynamic nature of neonatal methylation underscores why longitudinal studies are more informative than single cross-sectional measurements (6,10,19). Earlier work comparing neonatal and later-life methylation also found that many birth-associated differences attenuated, whereas a subset persisted, emphasizing that persistence cannot be assumed for every locus (20).

3. Molecular mechanisms linking NICU exposures and epigenetic remodeling

NICU exposures may influence neonatal methylation patterns through biologically plausible, interconnected pathways; however, evidence in preterm infants remains predominantly observational, and these pathways should not be interpreted as proven causes of epigenetic-age change. Inflammation and infection can activate cytokine-responsive signaling pathways,

whereas oxidative stress related to hyperoxia, hypoxia-reoxygenation, or ventilation-associated lung injury may affect redox-sensitive chromatin regulation. Mechanical ventilation and oxygen fluctuations can also contribute to systemic inflammation and mitochondrial stress (9,10,21).

DNA methyltransferases are responsible for establishing and maintaining methylation patterns, while ten-eleven translocation enzymes participate in active demethylation. One-carbon metabolism influences the availability of methyl-group donors, including S-adenosylmethionine, for methylation reactions. Reactive oxygen species, mitochondrial dysfunction, inflammatory signaling, nutritional transitions and pharmacological exposures may therefore influence methylation regulation during a period of rapid cellular differentiation. Nevertheless, observed methylation differences may also arise from changing cell composition, disease severity, treatment exposure, or developmental adaptation; mechanistic causality requires cell-specific and longitudinal confirmation (6,9,10,12,21).

Tissue-specific epigenetic kinetics and longitudinal implications. The biological interpretation of neonatal epigenetic clocks is fundamentally constrained by tissue specificity, as DNA methylation patterns vary dynamically across neonatal biospecimens.

Umbilical cord blood provides a perinatal molecular baseline that reflects fetal exposure and developmental timing at delivery; however, it is available only once. Peripheral blood enables longitudinal assessment, although serial blood collection in extremely preterm infants must be minimized due to limited blood volume, procedural burden and the risk of iatrogenic anemia. In addition, postnatal changes in leukocyte composition can alter blood-based methylation estimates independently of a true change in developmental tempo (4-6,13).

Buccal epithelial sampling is less invasive and may be feasible for serial follow-up, including following NICU discharge. However, buccal samples require standardized collection, processing and quality-control procedures as epithelial-cell proportion, oral contamination, DNA quantity and sample handling can influence methylation measurements. Buccal-derived biological-age estimates should not be interpreted as interchangeable with blood-derived estimates (13-15,22).

Placental tissue provides complementary information about prenatal programming, maternal-fetal exposures and placental function. It cannot, however, be used for postnatal longitudinal monitoring of the infant, and its methylation architecture is distinct from that of fetal blood or buccal tissue. Therefore, methylation-derived age estimates from placenta, cord blood, peripheral blood and buccal cells should not be compared as though they represent one directly interchangeable biological quantity (3,13,16,19).

Tissue-specific standardization is essential for longitudinal research. Studies should prespecify the tissue, sampling time, sample-processing method, methylation platform, cell-composition adjustment strategy and bioinformatic pipeline. Changes in tissue source between time points should not be interpreted as epigenetic-age change without careful validation (12,13,22).

Taken together, the molecular architecture of epigenetic clocks demonstrates that no single clock can address every research question in neonatal medicine. Rather than questioning which clock is universally superior, investigators should first define the biological question of interest and then select the clock whose molecular design, tissue specificity, developmental range and validation cohort best align with that objective. This hypothesis-driven approach may improve reproducibility, facilitate meaningful biological interpretation, and accelerate responsible translation of epigenetic biomarkers into precision-neonatal research.

4. Selecting the appropriate epigenetic clock: A critical perspective for neonatal research

The expanding number of available epigenetic clocks presents both opportunities and challenges for neonatal investigators. Although these models are frequently discussed collectively as epigenetic clocks, they differ substantially in biological objectives, training populations, analytical methods, tissue specificity and intended applications. Consequently, no single clock should be regarded as universally superior. Instead, clock selection should be guided by the underlying biological question rather than by familiarity or popularity.

Horvath clock: A landmark adult clock with limited neonatal utility. The Horvath clock was the first widely validated pan-tissue epigenetic clock and remains one of the most influential advances in aging research. Developed using DNA methylation profiles from multiple tissues across the life course, it accurately predicts chronological age across diverse organs and has transformed research into biological aging and age-related disease (1). However, its application to neonatal populations is considerably more limited.

A principal limitation of applying the Horvath clock to preterm developmental dating is the mismatch between its chronological-age prediction target and the neonatal question. Its age transformation accounts for more rapid changes before adulthood, but the model was not trained to distinguish gestational ages at birth (1,4). During fetal life and early infancy, DNA methylation undergoes remodeling at a markedly different pace and involves distinct regulatory pathways. Consequently, Horvath-derived biological-age estimates should not be used as primary indicators of neonatal developmental maturity (1,4,13).

Hannum clock: Valuable for adult aging, limited for developmental biology. As with the Horvath model, the Hannum clock was developed using adult peripheral-blood samples and accurately predicts chronological age in adult populations (2). Since it relies on blood-specific methylation signatures associated with adult aging, its biological interpretation in preterm infants remains uncertain. As the original model was developed in adults aged 19-101 years, its adult chronological-age performance does not validate its use for preterm developmental dating; a clock matched to the neonatal prediction target and tissue is preferable (2,13,15).

Knight and Bohlin clocks: Preferred for gestational-age estimation. The Knight and Bohlin clocks represent

first-generation gestational-age-specific epigenetic clocks and remain appropriate tools for estimating fetal developmental maturity at birth (4,5). Both models were developed using cord-blood methylation profiles and demonstrate strong agreement with clinically determined gestational age. Their principal strength lies in developmental dating rather than prediction of long-term outcomes.

The Knight clock was developed in a multiethnic cohort and used 148 CpG sites, whereas the Bohlin clock was developed in a Scandinavian birth cohort using 96 CpG sites. Both should be considered principally for research questions concerning gestational-age estimation, fetal developmental timing, or discordance between obstetric and methylation-based gestational age at birth. Neither clock should be assumed to provide a validated longitudinal measure of postnatal biological development during NICU hospitalization (4,5,13).

PedBE clock: A pediatric buccal-cell clock. The pediatric buccal epigenetic clock was designed for infants and children using buccal epithelial cells rather than blood (14). As buccal epithelial cells originate from embryonic ectoderm, they may be informative in developmental research, including longer-term pediatric follow-up. The PedBE clock is therefore more appropriate for buccal-cell studies in infancy and childhood than adult pan-tissue clocks.

Nevertheless, buccal methylation profiles cannot substitute directly for blood-based measurements. Tissue-specific methylation patterns differ substantially, and biological-age estimates derived from one tissue should not be directly compared with those derived from another.

NEOage clock: A neonatal-specific tool for very preterm infants. The NEOage platform represents a critical methodological advance as it was developed for estimating postmenstrual and postnatal age in very preterm infants using buccal epithelial-cell samples (15). Unlike cord-blood gestational-age clocks, NEOage was designed to model the rapid extrauterine developmental period experienced by very preterm infants.

NEOage is relevant to developmental-age research in very preterm infants within its training age range; longitudinal applications and extrapolation beyond NICU discharge require additional validation. Its advantages include neonatal specificity and separate postmenstrual-age and postnatal-age prediction models. Its limitations include dependence on buccal sampling, the need for rigorous sample-quality and cell-composition control and the limited scope of external assessment across diverse settings. In the original NOVI derivation cohort of 542 very preterm infants, performance was evaluated by leave-one-out cross-validation within the derivation cohort; this was internal validation, not independent external validation. The original NEOage study by Graw *et al* (15) reported correlations of 0.93-0.94 and root-mean-square errors (RMSEs) of 1.28 weeks for the postmenstrual-age models, 1.63 weeks for the postnatal-age 450k model and 1.55 weeks for the postnatal-age EPIC model. Mean absolute error and median absolute error were not reported. The 450k-compatible models were subsequently assessed in the independent GSE72120 saliva dataset. Among the 34 preterm infants in that external dataset, postmenstrual-age prediction

had $r=0.61$ and $RMSE=1.09$ weeks, whereas postnatal-age prediction had $r=0.76$ and $RMSE=1.55$ weeks. These values are RMSEs, not MAEs, and derive from the external saliva assessment rather than NOVI internal cross-validation; the external assessment was limited by its small preterm sample and cross-tissue application. NEOage results should therefore be interpreted as research measures rather than clinical decision tools (15).

5. Placental epigenetic clocks: Understanding prenatal programming

Placental epigenetic clocks provide complementary rather than competing information. As the placenta records intrauterine environmental exposures, including maternal inflammation, nutritional status, hypoxia, endocrine signaling and vascular dysfunction, placental methylation patterns may provide valuable insight into fetal programming before birth (3,19). Therefore, placental analyses may be valuable for investigating mechanisms underlying spontaneous preterm birth, fetal growth restriction, preeclampsia, and maternal disease. However, placental clocks should not be interpreted as direct indicators of neonatal biological age because placental tissue possesses a unique methylation architecture distinct from fetal tissues. The robust placental clock was developed for placental gestational-age estimation and is therefore most appropriately interpreted as a placental developmental-timing measure, not a longitudinal postnatal infant clock (16).

6. Artificial intelligence and machine learning, and practical barriers

Recent advances in artificial intelligence have expanded beyond conventional elastic-net regression to include deep learning, random forests, gradient boosting, and ensemble machine-learning approaches capable of integrating high-dimensional methylation data with transcriptomic, proteomic, metabolomic, imaging and clinical datasets. Rather than simply predicting age, future models may estimate developmental trajectories, identify biologically meaningful neonatal subphenotypes and improve prediction of long-term outcomes (7,12,17).

At present, neonatal applications of integrated multi-omics clocks, single-cell epigenomic methods, transcriptomic or proteomic clocks and artificial intelligence-based prediction models remain emerging research areas. Bulk-tissue methylation estimates can be affected by changing cell mixtures; single-cell and multi-omics approaches may help resolve this limitation; however, they require independent validation, transparent algorithms and longitudinal outcome assessment prior to clinical translation (7,12,17).

Hypothetical research scenario. To illustrate how epigenetic-clock data may inform future research design, consider a multicenter observational cohort of infants born before 28 weeks of gestation. Cord blood is collected at birth and standardized buccal samples are obtained at term-equivalent age. Of note, two infants with the same gestational age and similar clinical characteristics may exhibit different age-prediction residuals at term-equivalent age. This finding would not justify changing the clinical management of either infant. Instead,

investigators could prespecify analyses assessing whether the residual is associated with later neurodevelopment following adjustment for illness severity, inflammation, nutrition, medications, tissue type and cell composition. If replicated in independent cohorts, such a measure could be evaluated as a research stratification variable; it should not be used as an individual prognostic label or criterion for treatment allocation (11-13,15).

Practical barriers to the clinical implementation of epigenetic clocks

Infrastructure, cost and turnaround time. The routine clinical application of neonatal epigenetic clocks remains restricted by substantial infrastructural and financial barriers. The majority of NICUs do not possess advanced molecular laboratories, dedicated methylation platforms, or specialized bioinformatics expertise required to process and interpret genome-wide DNA methylation data efficiently (12,15). In addition, high assay costs limit feasibility for repeated clinical use. Existing methylation workflows require DNA extraction, bisulfite conversion or array preparation, quality control, preprocessing, normalization, cell-composition adjustment and bioinformatic analysis, preventing rapid turnaround for time-sensitive bedside decision-making.

Repeated sampling. Longitudinal assessment requires repeated biological sampling because methylation patterns change rapidly during the neonatal period, particularly in preterm infants (6,15,22). However, repeated blood sampling increases procedural burden and may contribute to anemia in vulnerable neonates. Buccal epithelial sampling provides a less invasive alternative; however, sample quality, contamination, cellular composition and collection procedures require careful standardization (13-15,22).

7. Quality control, reproducibility and standard operating procedures

Internationally harmonized quality-control procedures are required before epigenetic-clock testing can be compared across laboratories. Standard operating procedures should define the sample type, sampling time, storage conditions, DNA-extraction method, assay platform, batch-control procedures, preprocessing pipeline, cell-composition adjustment, quality thresholds, reporting format and handling of failed samples. Assay reproducibility should be demonstrated using blinded replicates, reference materials where available, version-controlled bioinformatic pipelines and independent interlaboratory validation (12,13).

Regulatory, accreditation and cost-effectiveness considerations. Epigenetic clocks are not established diagnostic tests for neonatal care. Any future clinical implementation would require a clearly defined intended use, analytical validation, clinical validation, laboratory quality-management processes, compliance with applicable national accreditation requirements and appropriate regulatory oversight. Cost-effectiveness should also be demonstrated: A clock-derived result would need to improve a patient-important outcome or a research decision beyond established predictors, such as gestational age, birth weight, illness severity and standard developmental

follow-up, while justifying assay, personnel, infrastructure and opportunity costs. These requirements have not yet been met for neonatal epigenetic-clock testing (12,18).

8. Ethical, governance and equity considerations in neonatal epigenetic testing

As neonatal epigenetic testing produces molecular information that may remain informative across the course of life, its governance requires safeguards beyond those used for routine laboratory testing. Consent processes should specify the data custodian, storage location, retention period, permitted secondary analysis or reanalysis, controlled-access data-sharing procedures and options for withdrawal where feasible. As parents provide permission at birth, governance plans should also consider whether re-contact or re-consent is appropriate as the child matures. Individual-level methylation data should be securely stored, access should be restricted to authorized users and audit trails should be maintained (18).

Insurance and educational implications require specific attention. A molecular label, such as ‘accelerated epigenetic age’ could be misinterpreted by third parties as a prediction of future disease, disability, cognitive ability, or developmental potential. Until valid individual-level clinical interpretation and legal safeguards exist, neonatal epigenetic results should not be disclosed to insurers, schools, employers, adoption agencies, or other non-clinical third parties (18).

Practical family counseling should avoid deterministic language. When epigenetic testing is undertaken in research, families should be informed that methylation-derived age is a research measurement rather than a diagnosis; that deviations from chronological age do not currently justify a change in clinical management; that estimates are influenced by tissue, cell composition, illness and technical factors; and that uncertainty remains substantial. Written information in the preferred language of the family and an opportunity for follow-up discussion with an appropriately trained clinician or genetic counselor should be available when individual results are discussed (18).

Hauskeller and Shore (18) emphasize that the ethical significance of epigenetic-age testing lies not only in the numerical estimate, but also in how it shapes understandings of vulnerability, resilience and future risk. Applied to neonatal practice, this framework supports three safeguards: The use of developmentally appropriate language such as ‘developmental timing’ rather than ‘aging’; avoiding placing unvalidated individual prognostic interpretations in the medical record; and establishing multidisciplinary oversight involving neonatology, laboratory medicine, ethics, data governance, genetic counseling and parent representation before testing is offered outside approved research (18).

Disparities between high-resource and low-resource settings should also be considered. High-throughput methylation profiling, secure data infrastructure and specialist bioinformatics expertise are concentrated in high-income academic centers, while a number of low- and middle-income settings face competing priorities for essential newborn care. In addition, clock-development cohorts may not adequately represent all ancestral and geographic populations. Future studies are warranted to include diverse populations in development and validation, report subgroup-specific performance,

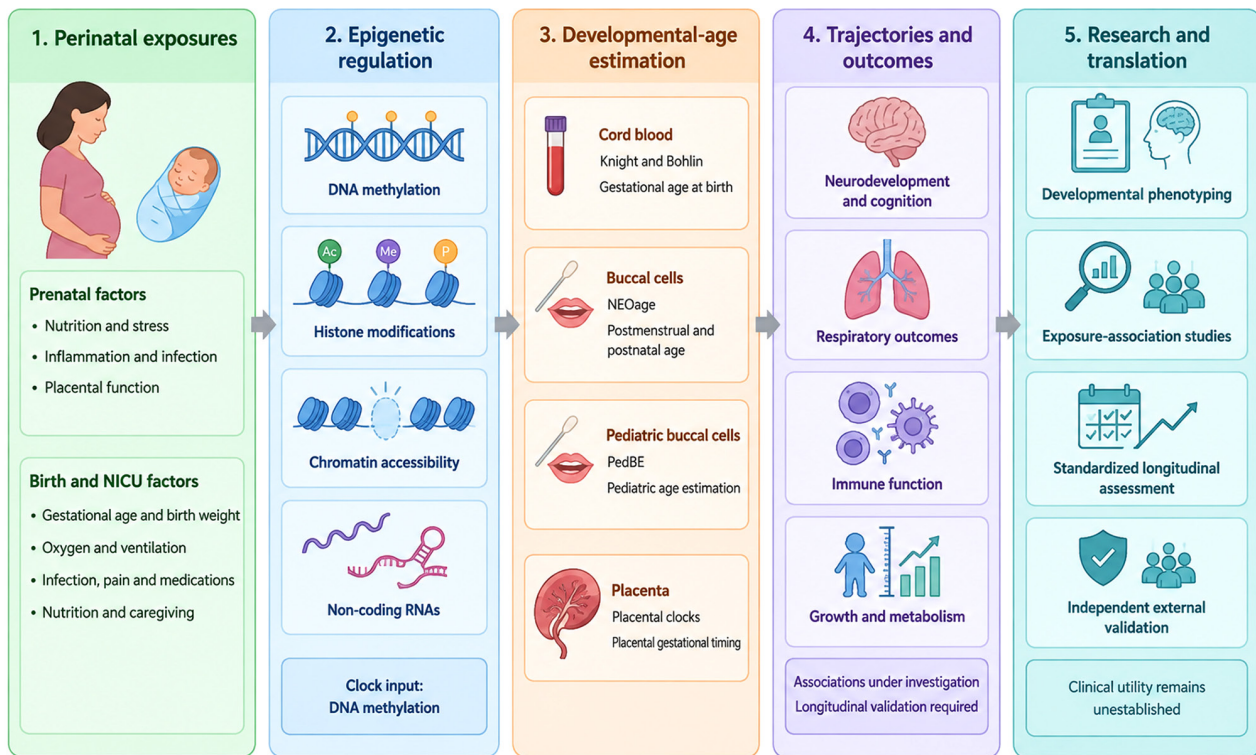


Figure 1. Conceptual framework for developmental epigenetic-clock research in preterm infants. Maternal, perinatal and neonatal intensive care unit exposures may influence epigenetic regulation through DNA methylation, histone modifications, chromatin accessibility and non-coding RNAs. The clocks illustrated use DNA methylation to estimate tissue- and model-specific developmental timing: Knight and Bohlin clocks estimate gestational age at birth using cord blood; NEOage estimates postmenstrual and postnatal age using buccal samples from very preterm infants; PedBE estimates pediatric age using buccal cells; and placental clocks estimate placental gestational timing. Potential associations with neurodevelopment, respiratory outcomes, immune function, growth and metabolism require longitudinal investigation. Proposed research applications include developmental phenotyping, exposure-association studies and standardized longitudinal assessment, supported by independent external validation. Arrows indicate conceptual associations rather than established causality. Clock-derived age deviations should not automatically be interpreted as pathological aging, and these measures are not established for individual prognosis or treatment decisions. NICU, neonatal intensive care unit; PedBE, pediatric buccal epigenetic.

support equitable data governance and local analytic capacity, and avoid diverting resources from proven neonatal interventions (12,13,18).

9. Current limitations and concepts

Despite substantial advances, several important limitations continue to restrict the clinical application of neonatal epigenetic clocks. The majority of available clocks have been developed using relatively small cohorts and require validation in larger, ethnically diverse populations. Differences in tissue sources, DNA methylation platforms, bioinformatic pipelines, and analytical algorithms limit inter-study comparability and reproducibility. Furthermore, longitudinal data linking neonatal epigenetic-age trajectories to long-term neurodevelopmental outcomes are limited. However, the high cost of methylation profiling, the lack of standardized laboratory protocols and the need for specialized computational expertise currently preclude routine clinical implementation. Consequently, epigenetic clocks should be regarded as potent research tools for developmental biology and precision-neonatal research rather than as established bedside biomarkers.

The conceptual associations among perinatal exposures, epigenetic regulation, tissue-specific developmental-age estimation, developmental outcomes and proposed neonatal research applications are summarized in Fig. 1.

It is proposed that future neonatal studies should abandon the search for a universally ‘best’ epigenetic clock. Instead, investigators should adopt a hypothesis-driven strategy in which clock selection is determined by the developmental question of interest. Gestational-age clocks are most appropriate for estimating fetal developmental maturity; the PedBE clock is appropriate for buccal-cell studies during pediatric developmental follow-up; the NEOage clock is relevant to longitudinal developmental research in very preterm infants; placental clocks are suited to studying prenatal programming; whereas Horvath and Hannum clocks should primarily serve as reference tools for comparative aging research rather than as primary neonatal biomarkers. This approach is likely to improve reproducibility, facilitate meaningful biological interpretation and accelerate responsible translation of epigenetic biomarkers into precision-neonatal research.

In summary, the argument advanced herein is deliberately narrow. It is not suggested that neonatal epigenetic clocks are ready for the bedside, nor that any single platform is universally preferable. Instead, it is suggested that their value in research is sufficient to justify their use as a standardized phenotyping layer in neonatal cohort studies, provided that use proceeds alongside explicit data governance, transparent reporting of tissue- and population-specific performance and structured communication with families. When these conditions are met, neonatal methylation-derived biological age may provide a defensible

route toward more biologically informed research stratification without overstating what current evidence supports.

10. Current and future perspectives in neonatal epigenetics

Single-cell epigenomic approaches. Single-cell epigenomic methods may help distinguish cell-type-specific methylation signals from changes caused by tissue heterogeneity. Their neonatal use remains technically demanding and currently investigational; however, such methods may eventually clarify whether methylation differences observed in bulk blood reflect altered cell composition, developmental programming, or both.

For the purposes of the present review, single-cell epigenomic approaches are discussed as methodological opportunities rather than as established preterm developmental-age clocks. Developmental observations in fetal, pediatric or adult tissues should not be represented as validation in preterm neonates. A future single-cell clock would require a defined developmental-age target, reproducible cell-specific measurements and independent assessment in the intended neonatal population (3,12,13,15).

Multi-omics data integration. Future neonatal cohorts should evaluate methylation findings alongside transcriptomic, proteomic, metabolomic, microbiome, imaging, and clinical data when biologically justified. The objective should not be to create increasingly complex models alone, but to determine whether integrated models improve biological interpretation and predictive performance beyond established clinical measures.

In the present review, integrated multi-omics developmental-age models are considered a future research direction, not an established neonatal test. Multi-omics association studies and clinical-outcome prediction models should not be described as developmental-age clocks unless developmental or biological age is their explicit prediction target. Any proposed preterm model should be evaluated in an independent cohort before its generalizability is claimed (7,12,13,15).

Artificial intelligence and predictive modelling. AI and machine-learning approaches may help analyze multidimensional neonatal datasets, but model interpretability, external validation, calibration, reproducibility, fairness across populations and clinical utility should be demonstrated before any implementation is considered (12,13).

The artificial intelligence prediction of neonatal clinical outcomes is not equivalent to an epigenetic clock. For any future machine-learning model, investigators should distinguish age/developmental-age estimation from clinical-outcome prediction and report the input features, target outcome, training sample, internal-validation approach, independent external-validation cohort, calibration, discrimination or regression error, subgroup performance, and incremental value beyond gestational age, birth weight, illness severity and established clinical predictors.

Harmonized global reporting standards. Future multicenter studies are required to adopt harmonized reporting standards that include tissue type, sampling time, laboratory methods, CpG platform, preprocessing pipeline, cell-composition adjustment, clock version, calibration, error metrics, subgroup

performance, missing-data methods and external validation. Such transparency is necessary for meaningful comparison across cohorts and healthcare settings.

Quantitative performance and validation of major clocks. Table I separates derivation performance from independent validation. For the Horvath and Hannum clocks, reported metrics arise from life-course and adult chronological-age models and should not be interpreted as neonatal developmental-age performance. Validation designs differ between clocks. Knight *et al* evaluated independent testing cohorts, whereas Bohlin *et al* evaluated a separately processed sample from the same MoBa parent cohort. NEOage used internal leave-one-out cross-validation and an independent saliva dataset. These designs should be described explicitly rather than classified solely by whether the results appeared in the original publication (4,5,15). Where a published article did not report mean absolute error, root-mean-square error, correlation, or a genuinely independent external-validation metric, this is stated as ‘not reported’ or ‘not identified’ rather than inferred.

For NEOage, leave-one-out cross-validation in the NOVI derivation cohort is internal validation and is not equivalent to independent external validation. The values PMA: $r=0.61$, RMSE=1.09 weeks and PNA: $r=0.76$, RMSE=1.55 weeks, arose from the independent GSE72120 saliva assessment among 34 preterm infants, not from NOVI internal cross-validation. These values are RMSEs, not MAEs (15).

Quantitative values are useful for transparent reporting, but Table I should not be interpreted as a league table. Mean absolute error, root-mean-square error, correlation and calibration depend on the target, outcome scale, tissue, age range, sampling time, methylation platform, and study population. A lower error for a gestational-age clock does not establish superiority over a pediatric chronological-age, postmenstrual-age, or placental clock estimating a different biological construct. Table I summarizes the intended targets, derivation populations, quantitative performance measures, and validation status of major epigenetic clocks relevant to neonatal and pediatric research.

Clock-specific interpretation

Horvath and Hannum clocks. Horvath's original pan-tissue model used 353 CpGs across 51 tissues and cell types. In test datasets separate from training, the correlation was 0.96 and the median absolute error was 3.6 years; these are life-course chronological-age results, not preterm developmental-age validation (1). Hannum *et al* developed a 71-CpG blood model in adults aged 19-101 years. The primary-cohort correlation was 0.96 with a reported error of 3.9 years; in an independent 174-person adult cohort, the correlation was 0.91 with a reported error of 4.9 years. The error values are retained in the terminology of the original report rather than relabelled as mean or median absolute errors (2). Neither original report established an indication for preterm developmental-age estimation (1,2,13).

Knight and Bohlin gestational-age clocks. The Knight clock used 148 CpGs selected from cord-blood and neonatal blood-spot training datasets. The training-set correlation was 0.99. In the first independent testing sample of 1,135 neonates,

Table I. Quantitative performance and validation characteristics of major epigenetic clocks relevant to neonatal and pediatric research.

Clock	Intended target	Tissue	Development/ derivation population	Derivation and original testing performance	Independent testing/ subsequent evaluation	Important limitations/ interpretation	(Refs.)
Horvath pan-tissue clock	Chronological age	Multiple tissues/cell types	8,000 samples; 51 tissues/cell types; 353 CpGs	Held-out test: $r=0.96$; median absolute error=3.6 years; RMSE not reported.	Independent neonatal developmental-age validation: Not identified.	Adult pan-tissue chronological-age clock; original test data are not neonatal performance data.	(1)
Hannum blood clock	Chronological age	Adult whole blood	656 adults aged 19-101 years; primary $n=482$, independent validation $n=174$; 71 CpGs	Primary cohort: $r=0.96$; reported error=3.9 years. Error retained as described in the original report.	Independent adult validation $n=174$; $r=0.91$; reported error=4.9 years. Not neonatal validation.	Adult blood clock; not validated as neonatal developmental-age measure.	(2)
Knight clock	Gestational age at birth	Cord blood and neonatal blood spots	Six training datasets spanning gestational ages and ancestries; 148 CpGs	Training $r=0.99$. First independent test $n=1,135$; $r=0.91$; mean absolute difference=1.49 weeks; median absolute difference=1.24 weeks.	Additional independent test $n=92$; $r=0.89$; median absolute difference=0.89 weeks. Later ECHO evaluation also reported tissue- and platform-specific performance.	Independent test cohorts were distinct from training. Two-infant serial pilot does not establish longitudinal clinical validity.	(4,13)
Bohlin clock	Gestational age at birth	Cord blood	Norwegian MoBa: training $n=1,068$; testing $n=685$ from separate batch of the same parent cohort; 96 CpGs	MoBa 1 training and MoBa 2 testing. Original regression fit and prediction-interval metrics are not MAE or RMSE; no conversion is made here.	Subsequent ECHO evaluation: for preterm EPIC blood spots ($n=272$), Spearman $r=0.67$; median absolute error=3.91 weeks (13).	Validation metrics not directly comparable with MAE/RMSE; estimates gestational age at birth.	(5,13)
PedBE clock	Pediatric chronological/developmental age	Buccal epithelial cells	Pediatric buccal-cell cohort; 94 CpGs	Original test: median absolute error=0.35 years; Pearson $r=0.98$; RMSE not reported.	ECHO preterm infant buccal samples ($n=480$): Spearman $r=0.62$; median absolute error=0.09 years (13). Results are tissue- and age-specific.	Tissue-specific pediatric clock; performance declines in non-buccal tissues.	(13,14)
NEOage PMA-EPIC and PMA-450k	Postmenstrual age	Development: buccal swabs; external: saliva	NOVI: 542 infants born <30 weeks of gestation; buccal DNA at NICU discharge	LOO cross-validation within NOVI: $r=0.93$ -0.94; RMSE=1.28 weeks for PMA-EPIC and PMA-450k. MAE and median absolute error: not reported.	PMA-450k only: independent GSE7120 saliva dataset; 34 preterm infants; $r=0.61$; RMSE=1.09 weeks. MAE and median absolute error: Not reported.	LOO is internal validation, not external. External assessment was independent but small and cross-tissue; broader same-tissue multicenter validation remains limited.	(15)
NEOage PNA-EPIC and PNA-450k	Postnatal age	Development: buccal swabs; external: saliva	NOVI: 542 infants born <30 weeks of gestation; buccal DNA at NICU discharge	LOO cross-validation within NOVI: $r=0.93$ -0.94; RMSE=1.55 weeks for PNA-EPIC and 1.63 weeks	PNA-450k only: independent GSE7120 saliva dataset; 34 preterm infants; $r=0.76$; RMSE=1.55 weeks. MAE	LOO is internal validation, not external. External assessment was independent but small and cross-tissue;	(15)

Table I. Continued.

Clock	Intended target	Tissue	Development/ derivation population	Derivation and original testing performance	Independent testing/ subsequent evaluation	Important limitations/ interpretation	(Refs.)
Robust placental clock	Gestational age	Placenta	Training n=1,102 placental samples; 558 CpGs; independent test n=187	for PNA-450k. MAE and median absolute error: not reported. Independent test: median absolute error=0.96 weeks; Pearson r=0.99. Original MAE abbreviation denotes median, not mean, absolute error.	and median absolute error: not reported. Original independent test n=187. Later ECHO preterm EPIC placentas (n=399); Spearman r=0.60; median absolute error=2.50 weeks (13).	broader same-tissue multicenter validation remains limited. Placental developmental- timing clock; not a postnatal infant clock.	(13,16)
RMSE, root-mean-square error; MAE, mean absolute error; PMA, postmenstrual age; PNA, postnatal age; LOO, leave-one-out. NEOage external results refer to the 450k-compatible models applied to 34 preterm infants in the independent GSE72120 saliva dataset; these are RMSEs, not MAEs, and do not derive from NOVI internal cross-validation (15). For the robust placental clock, 0.96 weeks is the median absolute error, abbreviated MAE in the original article; elsewhere in this table MAE denotes mean absolute error only when explicitly stated. ECHO evaluation of NEOage includes NOVI training participants and should not be treated as wholly independent validation (13,16).							

the correlation was 0.91, mean absolute difference was 1.49 weeks and median absolute difference was 1.24 weeks. An additional independent testing sample of 92 neonates yielded a correlation of 0.89 and a median absolute difference of 0.89 weeks. These were independent testing cohorts, not simply a random internal hold-out split. Serial sampling in two preterm infants provided preliminary longitudinal evidence, but did not establish longitudinal clinical validity (4). The Bohlin ultrasound-based clock used 96 CpGs and was developed in MoBa 1 (n=1,068), with predictions evaluated in MoBa 2 (n=685), a separately processed sample from the same parent cohort. Prediction-interval widths and regression standard errors should not be presented as MAE or RMSE. Later multi-cohort evaluation reported tissue- and platform-dependent performance for both clocks (5,13).

PedBE, NEOage and placental clocks. PedBE was derived in pediatric buccal cells using 94 CpGs. In the original testing set, the median absolute error was 0.35 years and Pearson's correlation was 0.98; these results describe the PedBE testing cohort (14). Independent pediatric multi-tissue evaluation found that PedBE performed best in pediatric buccal samples, while performance varied in non-buccal tissues (13). NEOage was developed specifically in buccal samples from 542 very preterm infants in the NOVI cohort to estimate postmenstrual age and postnatal age. Internal performance was assessed by leave-one-out cross-validation within NOVI, not by independent external validation; the original study reported correlations of 0.93-0.94, with RMSEs of 1.28 weeks for postmenstrual-age models, 1.63 weeks for the postnatal-age 450k model, and 1.55 weeks for the postnatal-age EPIC model. MAE and median absolute error were not reported. The 450k-compatible models were subsequently assessed in the independent GSE72120 saliva dataset. Among 34 preterm infants in that external dataset, postmenstrual-age prediction had r=0.61 and RMSE=1.09 weeks, whereas postnatal-age prediction had r=0.76 and RMSE=1.55 weeks. Thus, the values 1.09 weeks, 1.55 weeks, r=0.61, and r=0.76 arise from the external preterm saliva assessment and are not NOVI internal cross-validation values. The external assessment was independent of model development but was limited by small sample size and use of saliva rather than buccal tissue. For placental gestational-age estimation, Lee *et al* reported a median absolute error of 0.96 weeks and a correlation of 0.99 in independent test datasets (n=187). The original article abbreviated median absolute error as MAE; it should not be reinterpreted as mean absolute error (16). Placental clocks characterize placental gestational timing and should not be interpreted as postnatal infant clocks (13-16).

Recent empirical evidence and remaining gaps

Neonatal methylation association evidence. Recent primary studies provide neonatal evidence that is distinct from clock development. In a 2024 study on preterm infants, Nist *et al* (21) examined DNA methylation, inflammation and neurobehavior, reporting empirical associations rather than an age-clock model. Hodge *et al* (22) examined methylation associations with neonatal age among infants born very preterm and identified age-associated loci, including loci in genes relevant to neurodevelopment. Nist *et al* measured saliva methylation at one time point, and Hodge *et al* analyzed buccal

samples at NICU discharge. These studies strengthen the biological rationale for future serial developmental epigenetic research but do not constitute external validation of a clinical epigenetic clock. Earlier longitudinal work also demonstrated dynamic methylation change during the first postnatal year after preterm birth (6). Observational associations cannot establish that NICU exposures cause later methylation or neurodevelopmental differences (6,21,22).

Single-cell, multi-omic, transcriptomic, proteomic and artificial intelligence evidence. In the present review, these approaches are discussed as research priorities rather than established preterm developmental-age tests. Neonatal methylation association studies provide empirical evidence, but do not by themselves validate single-cell, transcriptomic, proteomic or integrated multi-omics age models (21,22). Adult clock findings should not be extrapolated to preterm infants without evaluation of the tissue, developmental target, age range and independent validation population (7,12,13,15).

Single-cell genomic and chromatin approaches have generated developmental evidence in fetal and other non-neonatal tissues; however, they are not yet established as validated age-estimation tools in preterm neonatal cohorts. Their principal contribution to neonatal research would be to separate true cell-intrinsic methylation change from shifts in leukocyte or epithelial composition, resolve cell-specific developmental trajectories, and identify rare populations that bulk assays obscure. Similarly, neonatal transcriptomic and proteomic studies may identify biomarkers of inflammation, sepsis, lung disease, or neurodevelopmental risk, but a biomarker association or a clinical-outcome prediction model is not a transcriptomic or proteomic epigenetic clock (3,12,13).

Machine-learning methods are already used in neonatal outcome prediction, but prediction of a clinical outcome is not equivalent to developmental-age estimation. Any future neonatal multi-omics or artificial intelligence model should state the input features, target, training sample, internal-resampling method, external-validation cohort, calibration, discrimination or regression error, subgroup performance, and incremental value beyond gestational age, birth weight and established clinical predictors. Until these criteria are met, AI-based neonatal omics models should remain research tools (12,13,15).

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RK conceptualized the manuscript, conducted the literature review, drafted the manuscript and approved the final version. Data authentication is not applicable.

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Competing interests

The author declares that he has no competing interests.

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