Review Article

Methylation Risk Scores Incorporating Epigenetic Biomarkers: A Systematic Review of Diagnostic Accuracy and Clinical Utility Across Multiple Diseases

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Abstract

Methylation Risk Scores (MRS) that incorporate DNA methylation-derived Epigenetic Biomarkers (EBPs) offer a promising route to improved diagnosis, risk prediction and personalised disease management. This systematic review synthesises evidence from 12 clinical studies (January 2020-May 2024) identified via PubMed, ScienceDirect, bioRxiv and medRxiv using PRISMA-compliant screening, with studies selected on the basis of primary human data assessing MRS/EBP performance across a range of non-cancer conditions. Data extraction captured study design, sample size, analytic approach and key performance metrics (hazard ratios, odds ratios, C-Index, AUC, ICC, sensitivity, specificity, Pearson r^2). A narrative synthesis was performed owing to heterogeneity in cohorts, outcomes and analytic pipelines.

Across studies, MRS demonstrated consistent and moderate-to-strong predictive power: hazard ratios ranged from 1.4-2.3 and odds ratios from 1.7-2.2; C-Index values were 0.70-0.80 and AUC values reached as high as 0.89 (type 2 diabetes complications), with typical sensitivity 78-88% and specificity 82-89%. Reliability metrics were favourable (ICC 0.77-0.85; r^2 0.65-0.74). Disease contexts included metabolic syndrome, cardiovascular events, type 2 diabetes, kidney disease, frailty, cognitive outcomes and osteoarthritis; several studies reported overlapping CpG loci and biologically plausible gene associations (e.g. CPT1A, ABCG1), supporting mechanistic relevance [35,12,86].

Methodologically, studies employed EWAS, penalised regression (notably elastic net), machine-learning classifiers and Multi-Omics Integration (OMICmAge), reflecting rapid methodological innovation [16,66]. Principal barriers to clinical translation include lack of standardised DNAm measurement and analysis protocols, limited cohort diversity, high initial costs and regulatory uncertainty [85,87]. Emerging opportunities lie in AI/ML enhancement, multi-omics and single-cell epigenomics, and targeted epigenetic therapeutics. In summary, current evidence indicates that MRS incorporating EBPs can meaningfully improve disease prediction and prognostication across multiple conditions, but widespread clinical adoption will require standardisation, broader validation in diverse populations and resolution of economic and regulatory challenges.

Scientific-Theoretical Agenda

This section sets out the theoretical foundations, methodological principles and an applied research agenda for Methylation Risk Scores (MRS) that incorporate DNA methylation-derived Epigenetic Biomarkers (EBPs). It synthesises mechanistic knowledge of epigenetics with current practice in MRS development, identifies methodological and translational bottlenecks, and proposes priority directions for research that would enable robust clinical deployment.

Epigenetic Foundations and Mechanistic Rationale

Epigenetics denotes heritable changes in gene expression that occur without alteration of the DNA sequence [47,7]. DNA Methylation (DNAm) the covalent addition of a methyl group to cytosine residues within CpG dinucleotides has been the most intensively characterised mechanism because of its relative stability in peripheral tissues and its functional impact on transcriptional regulation *Schmits, et al.,* (2019) [36]. Methylation of promoter-

proximal CpG islands commonly reduces transcription factor binding and represses gene expression, while demethylation may permit transcriptional activation Shuai, et al., (2020) [91]. CpG sites are non-uniformly distributed across the genome and cluster in islands frequently associated with gene promoters; methylation status at these loci therefore has outsized regulatory importance [23,97,40].

From a pathophysiological perspective, DNAm integrates genetic predisposition and environmental exposures (diet, smoking, psychosocial stress, pollutants), providing a molecular record of cumulative and recent exposures that can influence disease onset and progression [67,64]. This dual sensitivity to genotype and environment is the central theoretical justification for MRS: where Polygenic Risk Scores (PRS) represent inherited genetic liability, MRS can capture dynamic, exposure-responsive elements of risk and thus potentially improve prediction for complex diseases whose aetiology is strongly modulated by environment and lifestyle *Wattacherill, et al.,* (2023) [65,43].

Constructing Methylation Risk Scores: Data, Pipelines and Algorithms

Data Generation and Preprocessing

MRS construction typically begins with genome-wide DNAm data generated on microarray platforms (e.g. Illumina 450K or EPIC arrays) or by sequencing approaches [13,77]. Raw data require rigorous preprocessing probe-level quality control, normalisation, batch-effect correction and, where necessary, deconvolution to account for cellular composition to reduce technical artefact and improve comparability across cohorts [63,25]. Standardised pipelines for these steps remain incomplete across the field, producing one of the principal barriers to reproducibility [85].

Feature Selection and Model Training

Feature selection for MRS draws on several complementary strategies. Epigenome-Wide Association Studies (EWAS) identify CpG sites statistically associated with outcomes of interest, while candidate-gene and functional genomic approaches prioritise sites with demonstrated regulatory impact [83,62]. Machine-learning methods (elastic net, random forest, penalised Cox models) are then used for variable selection and model building; elastic net has been particularly prominent because it manages multicollinearity and high-dimensional predictor spaces common to methylation data [30,14]. Cross-validation and independent cohort validation remain essential to avoid overfitting and to estimate generalisable performance [25,90].

Performance Metrics and Evaluation

Robust evaluation of MRS uses multiple complementary metrics: discrimination (AUC, C-Index), calibration, effect size (hazard ratios, odds ratios), and reliability (intra-class correlation, Pearson r^2). Sensitivity and specificity characterise classification performance where thresholds are applied [1-10,11-29]. Reporting across these metrics, alongside transparent methods for feature selection and weighting, is crucial for cross-study comparison and clinical interpretation.

Biological Plausibility and Cross-Disease Signal

A compelling theoretical prerequisite for MRS is biological plausibility: the CpG sites that contribute substantially to a score should map to genes and pathways mechanistically connected to the phenotype. Across the reviewed literature, recurring loci (for example, CPT1A and ABCG1) and loci associated with lipid metabolism, inflammation and cellular ageing support mechanistic interpretation for metabolic and cardiovascular endpoints [30-35,12]. The identification of overlapping CpGs across different disease endpoints suggests that certain epigenetic signatures reflect shared pathophysiological axes (inflammation, metabolic dysfunction, biological ageing) and may underpin pleiotropic predictive utility across conditions such as metabolic syndrome, cardiovascular disease and frailty *Marioni, et al.,* (2015); *Li, et al.,* (2022).

Epigenetic Clocks, Ebps and Conceptual Nomenclature

Epigenetic clocks (Horvath's clock, Hannum, PhenoAge and more recent integrative measures such as OMICmAge) provide quantitative indices of biological age derived from DNAm patterns and have proven predictive of mortality and age-related morbidity [36-53,8,17]. These clocks form a subset of EBPs and illustrate how methylation patterns can summarise latent biological processes. However, the field suffers from inconsistent terminology (EBP, EpiSigns, EpiScores), hindering comparability and translation. A theoretical agenda must therefore prioritise consensus definitions and taxonomies to ensure clarity in how different classes of methylation-derived markers are described and validated [54-92].

Methodological Limitations and Sources of Bias

Cohort Heterogeneity and Population Generalisability

A recurrent methodological concern is cohort composition. Many studies to date are drawn from relatively homogeneous populations which reduces power to detect population-specific effects and limits external validity [92]. Meta-analytic integration is hampered by heterogeneity of cohorts, phenotype definitions and assay platforms.

Technical Variability

Differences in array platforms, laboratory procedures, and preprocessing choices introduce batch effects and technical noise

that can masquerade as biological signal [21]. Lack of standardised pipelines for preprocessing and normalisation therefore threatens reproducibility [85].

Cellular Heterogeneity and Sample Type

Peripheral blood is commonly used for convenience but comprises mixed cell types whose proportions vary with age, health status and acute exposures. Without adequate deconvolution, observed DNAm differences may reflect shifts in cell composition rather than locus-specific regulation [63].

Confounding, Causality and Temporality

DNAm is both a potential mediator and a consequence of disease and exposure. Distinguishing causal methylation changes from epiphenomena requires longitudinal designs, repeated measures and methods such as Mendelian randomisation leveraging Methylation Quantitative Trait Loci (mQTLs). Cross-sectional associations alone cannot establish causality [38].

Integration With Other Data Modalities and Advanced Technologies

Multi-Omics and Single-Cell Epigenomics

Combining DNAm with transcriptomics, proteomics and metabolomics (multi-omics) promises richer, mechanistically informed risk models and is exemplified by integrative efforts such as OMICmAge [18]. Single-cell bisulfite sequencing and other single-cell epigenomic methods resolve cellular heterogeneity and can pinpoint cell-type-specific methylation changes relevant to disease [37,26]. The theoretical value is clear: models built with multi-layered molecular data should better capture disease biology and may improve both sensitivity and specificity.

Artificial Intelligence, Explainability and Model Robustness

AI and machine learning including deep learning can extract complex, non-linear patterns from high-dimensional methylation data [66,45]. Yet clinical translation demands interpretable models that clinicians can interrogate. The agenda must therefore stress development of explainable AI approaches, techniques for model calibration, external validation and assessment of clinical net benefit (decision-curve analysis) rather than sole reliance on statistical discrimination metrics.

Clinical Translation: Regulatory, Economic and Workflow Considerations

The utility of MRS will be determined by clinical validity and clinical utility. Beyond accuracy, MRS must demonstrate that their use changes clinical decisions in ways that improve outcomes and are cost-effective. Regulatory approval will require standardised assays, reproducible pipelines, prospective validation and clinical trials demonstrating impact on care pathways [87,84].

Health-system integration implies interoperability with electronic health records, decision-support tools that synthesise MRS with other risk factors, and training for clinicians to interpret and communicate epigenetic risk [87,55]. Economic assessments should consider both the upfront costs of assay implementation and potential downstream savings from earlier diagnosis, reduced invasive testing and better-targeted therapies (Impact Statement).

Ethical, Social and Equity Imperatives

Epigenetic markers are sensitive to social and environmental determinants of health. If development datasets under-represent marginalised groups, MRS risk perpetuating or exacerbating health disparities [92]. The agenda must prioritise inclusive cohort recruitment, transparent reporting of sociodemographic covariates, and embedding equity considerations in model development and deployment. Consent procedures must also reflect the potential for epigenetic data to reveal sensitive life-course exposures.

Priority Research Agenda and Recommended Study Designs

To progress from promising research instruments to clinically useful tools, the following research priorities are proposed:

Standardisation Initiatives

Develop community consensus on preprocessing pipelines, normalisation strategies, reporting standards and nomenclature for EBPs and MRS [85,38].

Large, Diverse, Longitudinal Cohorts

Invest in multi-centre longitudinal studies with repeated DNAm measures and rich phenotyping to resolve temporality, measure change over time and test prognostic utility across populations [20,92].

Independent External Validation

Require independent cohort validation as a standard for any proposed MRS before claims of clinical readiness [25].

Causal Inference and Functional Follow-Up

Use mQTL-based Mendelian randomisation, perturbation experiments and single-cell assays to distinguish causal from associative methylation signals and to identify actionable targets for intervention [38].

Clinical Impact Studies

Design pragmatic clinical trials and implementation studies that measure whether MRS-guided interventions improve patient

outcomes and are cost-effective compared with standard care [87].

Multi-Omics and Integrative Modelling

Prioritise studies that combine DNAm with transcriptomic, proteomic and metabolomic data to improve mechanistic understanding and predictive performance [18].

Explainable AI And Interoperability

Develop interpretable ML frameworks and EHR-integrated decision support that clinicians can use confidently [66].

Equity-Centred Design

Explicitly recruit under-represented populations, report sociodemographic variables in publications and assess performance stratified by demographic subgroups to prevent widening disparities [92].

Concluding Synthesis of The Theoretical Agenda

The theoretical case for MRS is strong: DNAm bridges genetic predisposition and environmental exposure, yielding biomarkers that are both mechanistically plausible and practically informative for disease prediction. Methodological advances (elastic net and other penalised models, ML techniques, single-cell technologies) and multi-omics integration have markedly increased predictive capacity. However, for MRS to fulfil their translational promise the field must confront standardisation deficits, expand cohort diversity, demonstrate causal relationships where possible, and validate clinical utility through prospective implementation studies. Addressing these priorities alongside careful attention to ethical and equity considerations will be central to embedding MRS and EBPs into responsible, effective clinical practice.

Discussion / Review

This expanded Discussion synthesises and critically appraises the evidence from the 12 studies included in the systematic review and situates that evidence within methodological, clinical, regulatory and ethical contexts. Its aim is to state clearly what the present evidence supports, to identify where further work is essential, and to outline realistic translational pathways for Methylation Risk Scores (MRS) that incorporate DNA methylation-derived Epigenetic Biomarkers (EBPs).

Recap of Principal Empirical Findings

Across the twelve studies reviewed, MRS incorporating EBPs delivered consistent and generally moderate-to-strong predictive performance across a heterogeneous set of non-cancer conditions. Effect sizes (hazard ratios 1.4-2.3; odds ratios 1.7-2.2) and discrimination metrics (C-Index ~0.70-0.80; AUCs up to 0.86-0.89 in selected studies) indicate that MRS capture signal that is relevant for

disease prediction and prognostication in conditions including type 2 diabetes, cardiovascular disease, kidney disease, frailty, cognitive decline and osteoarthritis [35,12,86,48]. Reliability measures (ICC 0.77-0.85; r^2 0.65-0.74) support the reproducibility of methylation-derived metrics within cohorts when standardised pipelines are used [20]. The recurring identification of biologically meaningful loci (for example, CPT1A, ABCG1, PHOSPHO1, SREBF1) strengthens confidence that MRS are not purely statistical artefacts but reflect pathophysiological processes [35,12]. Nonetheless, heterogeneity of study designs, platforms, cohorts and preprocessing approaches produces a complex evidence base in which the promise is clear but the route to reliable, generalisable clinical tools remains contingent upon addressing multiple methodological, infrastructural and social challenges.

Disease-Specific Considerations and Interpretation

Cardiovascular Disease: The cardiovascular applications reported relatively high discrimination (AUCs ≈ 0.85 in some studies) and high sensitivity/specificity in short-term event prediction [12,18]. Mechanistically, DNAm loci associated with lipid handling and inflammation (e.g. ABCG1, SREBF1) are plausibly linked to atherothrombotic risk. Clinically, the most realistic near-term use is improved risk stratification to guide the intensity of preventive therapies (statin initiation, antihypertensives, behavioural interventions). However, clinical utility depends on demonstrating that reclassification by MRS changes management and improves hard endpoints beyond what existing calculators (e.g. QRISK, SCORE) accomplish.

Metabolic Disease and Type 2 Diabetes: Type 2 diabetes produced some of the largest AUC values and hazard ratios (e.g. AUC up to 0.89; HRs ≈2.1-2.3) in reviewed [86,20]. CpGs mapping to genes governing lipid metabolism and hepatic function (CPT1A) provide mechanistic plausibility. MRS could be used to identify high-risk individuals for intensive lifestyle or pharmacologic prevention; yet, as with cardiovascular disease, the imperative is prospective testing of whether MRS-guided prevention reduces incidence or complications versus standard risk stratification. **Kidney Disease and Diabetic Nephropathy:** DNAm panels were predictive of kidney function decline and progression of diabetic kidney disease in several studies [50], with HRs approaching 1.9. Given the high clinical and economic burden of end-stage kidney disease, MRS that reliably predict progression might support earlier nephrology referral, intensified glycaemic/blood-pressure control, or therapeutic prioritisation. The challenge will be integrating MRS predictions with well-established biomarkers (eGFR, albuminuria) and demonstrating incremental prognostic value and actionable thresholds.

Frailty and Ageing Phenotypes: Epigenetic clocks and frailty risk scores demonstrated useful discrimination for age-related decline [48,16]. Because epigenetic clocks measure accumulated biological ageing, they may be particularly suited to population-

level stratification for preventive geriatric interventions. However, translating clock deviations into discrete clinical actions requires development of evidence-based interventions proven to alter clock measures and clinical endpoints.

Cognitive Outcomes and Neurodegeneration: DNAm associations with cognitive ability and brain health are emerging [86]. While predictive performance is presently moderate, epigenetic measures that reflect lifelong exposures or biological ageing could complement imaging and fluid biomarkers in risk stratification for neurodegenerative disease. Key gaps include tissue specificity (blood vs brain), the causal relevance of peripheral methylation marks, and the long lead times to clinical outcomes requiring very large longitudinal cohorts.

Osteoarthritis and Musculoskeletal Disease: Studies predicting knee osteoarthritis progression reported AUCs in the mid-0.70s to low-0.80s [27]. Here, MRS might identify patients at high risk of structural progression who could be prioritised for disease-modifying interventions as they become available. Again, demonstration of clinical impact such as reducing pain, improving mobility, or deferring joint replacement will be critical.

Psychiatric Outcomes: Associations between childhood trauma-linked methylation signatures and later psychiatric disorders show HRs around 1.8 [89]. Such predictive markers raise both clinical possibilities (early psychosocial support) and ethical challenges (stigmatisation, sensitive information); any clinical application must be accompanied by robust consent processes and supportive clinical pathways.

Methodological Critique Deeper Analysis

Assay Platforms and Probe Chemistry

Different studies used Illumina 450K arrays, EPIC arrays or sequencing [13,77]. Each platform has different coverage, probe chemistries and susceptibility to cross-hybridisation. Platform choice influences which CpGs are available for analyses and complicates pooling. Theoretical and practical solutions require cross-platform harmonisation strategies, probe-level mapping tables and, where feasible, replication using sequencing-based approaches that avoid array probe limitations.

Preprocessing, Normalisation and Batch Correction

The lack of consensus on preprocessing (quantile normalisation, functional normalisation, Noob, SWAN etc.) produces divergent methylation beta distributions and affects downstream feature selection [63]. Batch effects introduced at DNA extraction, bisulfite conversion or array processing can generate spurious associations if cases and controls are processed in different batches. Rigorous study design (randomisation of samples across plates), comprehensive batch covariate modelling and use of established normalisation pipelines are necessary but not uniformly applied.

The field would benefit from a community-adopted "best practice" pipeline and pre-registration of preprocessing choices.

Cellular Composition and Deconvolution

Peripheral blood is a composite tissue; cell proportions vary with infection, inflammation and ageing. Deconvolution algorithms (Houseman, reference-based/non-reference) mitigate this issue but depend on accurate reference methylomes and assume linearity. Single-cell methylation technologies remove ambiguity but are currently resource intensive. Analytic strategies should consistently report whether and how cell-type effects were controlled.

Feature Selection, Penalisation and Model Stability

Elastic net has been widely used due to its capacity to manage multicollinearity and perform variable selection [14]. However, elastic net solutions can vary with tuning parameters and the composition of training data; bootstrap stability analyses, reporting of selected CpG lists with weights, and publication of model coefficients are needed to enable replication and meta-analytic synthesis. Methods to assess and report model calibration (e.g. calibration plots, Brier scores) are underused but crucial for clinical reliability.

Overfitting, Optimism and Reproducibility

With high predictor:sample ratios, even penalised models can overfit. Independent external validation in truly distinct cohorts is the gold standard for establishing generalisability but was not universal. Where external validation occurred, it increased confidence; where it was absent or limited, conclusions should be more tentative [25]. Transparent code and data sharing, within ethical constraints, are essential to mitigate selective reporting and publication bias.

Statistical Reporting Standards

Beyond AUC or HRs, useful statistics include net reclassification improvement (NRI), Integrated Discrimination Improvement (IDI), decision-curve analysis and clinical utility metrics. Few studies consistently reported these. For clinical adoption, the field should standardise reporting to include such measures to evaluate whether MRS change clinical decisions and yield net patient benefit [6].

Causal Inference and Functional Validation

DNAm can be both cause and consequence. Distinguishing causality requires triangulation: repeated measures to establish temporal precedence; mQTL analyses for instrumental variable inference; and functional follow-up (CRISPR-based epigenome editing, in vitro perturbation) to demonstrate phenotypic effects. [38] *Hüls & Czamara, et al.,* (2020) emphasised this point; yet, most included studies remain associative. Greater investment in causal inference and functional assays is needed before treating particular methylation changes as therapeutic targets.

Translational Readiness and Clinical Utility

From Prediction to Practice

Predictive performance alone does not guarantee clinical utility. The essential next steps are pragmatic trials that test MRS-guided care pathways. For example, a randomised implementation trial might evaluate whether MRS-based stratification for pre-diabetes prevention reduces incident diabetes compared with guideline-based care. Such trials should capture patient-centred outcomes, cost-effectiveness, acceptability and unintended harms.

Interoperability, EHR Integration and Decision Support

MRS must be packaged for clinical workflows: standardised laboratory reporting, integration into electronic health records and decision-support systems that translate a score into clear management options. Clinician training materials and patient-facing explanations will be necessary to avoid misinterpretation.

Thresholds, Reclassification and Actionability

Clinical thresholds for MRS need calibration and consensus. Reclassification tables and decision-curve analysis should inform whether moving someone from low to high risk by MRS should alter management. Without such thresholds tied to evidence-based interventions, scores will be of limited use.

Economic, Regulatory and Commercial Considerations

Cost-Benefit Calculus

Upfront investment in laboratory platforms, bioinformatics infrastructure and workforce training is substantial; however, modelling studies should evaluate long-term savings from earlier intervention, reduced hospital admissions and targeted therapeutics. Health economic evaluations require realistic assumptions about assay costs, disease prevalence and intervention efficacy; these analyses remain sparse.

Regulatory Pathways

Regulatory approval will require analytical and clinical validation. Regulators expect reproducible assays, demonstration of clinical validity and data on clinical utility. Currently, guidance for epigenetic diagnostics is evolving. Early engagement with regulators and alignment on validation standards will streamline translation [84,87].

Commercialisation And Stewardship

Commercial offerings (e.g. Cardio Diagnostics, Dionysus Health) illustrate private-sector interest. Commercial players can accelerate scale-up but must adhere to transparency, independent validation and equitable access principles. Public-private partnerships could facilitate large-scale cohort creation and broader validation if governed to protect public interest.

Ethical, Social and Equity Implications

Epigenetic profiles encode environmental exposures and social determinants; this raises the risk that MRS could act as proxies for disadvantage. Ethical deployment requires:

a) **Representative recruitment** to avoid models that perform poorly in under-represented groups [92].

b) **Transparent communication** about the probabilistic and modifiable nature of epigenetic risk, avoiding deterministic framing.

c) **Protection against misuse** by insurers or employers; policy frameworks must restrict discriminatory use of epigenetic data.

d) **Informed consent** that addresses the potential sensitivity of detected exposures (e.g. childhood trauma) and downstream implications.

Patient and public engagement should be prioritised to shape acceptable uses of MRS and to co-design communication materials that contextualise risk.

Practical Roadmap and Recommendations

To move from robust science to responsible clinical application, the field should pursue a phased roadmap:

Immediate (0-2 Years)

Consolidate best-practice pipelines for preprocessing and reporting; establish community standards for cell deconvolution and benchmark datasets; require external validation for publication claims.

Short Term (2-5 Years)

Create multi-centre consortia for diverse longitudinal cohorts with repeated DNAm measures; publish calibration and reclassification analyses; begin small pragmatic trials of MRS-guided interventions in high-yield use cases (e.g. diabetes prevention).

Medium Term (5-10 Years)

Scale assays to clinical laboratory standards; integrate MRS into EHR decision support in pilot centres; perform health economic analyses and engage regulators for pathway definition.

Long Term (>10 Years)

Routine clinical use where proven effective and equitable; development of targeted epigenetic therapeutics informed by causal CpG identification; international governance frameworks for data use and anti-discrimination protections.

Concrete methodological steps include routine publication of model coefficients and CpG lists, cross-platform replication efforts, mandatory subgroup performance reporting and pre-registration of analytical plans.

Limitations of the Present Review and Inference Boundaries

The review intentionally excluded cancer, narrowing its scope but also excluding a widely studied domain of epigenetics that could inform methods. The limited number of studies per disease and heterogeneity in methods constrain the ability to perform quantitative synthesis; a narrative synthesis provides interpretive clarity but lacks pooled effect estimates. The reliance on published performance metrics invites publication bias. Finally, because several reviewed studies used overlapping cohorts or similar population sources, independence of evidence must be interpreted cautiously.

Concluding Assessment

The assembled evidence indicates that MRS incorporating EBPs are a maturing class of biomarkers with genuine potential to improve disease prediction and stratification across a range of clinically important non-cancer conditions. Their strengths lie in dynamic sensitivity to exposures, mechanistic plausibility of key CpG associations and demonstrable incremental predictive value in several contexts. Yet the path to routine clinical use is nontrivial: methodological standardisation, broader population validation, causal and functional validation of key loci, demonstration of clinical utility in prospective trials, and careful ethical governance are all prerequisites.

If these conditions are addressed, MRS could shift preventive medicine and personalised care toward earlier, more precisely targeted interventions. Achieving that future will require coordinated research consortia, method standardisation, health economic evidence and regulatory engagement all pursued with an explicit equity mandate. In sum, MRS are a powerful and promising tool in the biomarker repertoire; realising their clinical promise demands rigorous, deliberate and ethically informed translation.

Conclusion

This systematic review demonstrates that Methylation Risk Scores (MRS) incorporating DNA methylation-derived Epigenetic Biomarkers (EBPs) are a promising addition to the biomarker landscape for non-cancer conditions. Across twelve studies (January 2020-May 2024), MRS consistently showed moderate-to-strong predictive performance (HRs 1.4-2.3; ORs 1.7-2.2; C-Index/AUC typically 0.70-0.89) and acceptable reliability (ICC 0.77-0.85; r^2 0.65-0.74), with recurring biologically plausible loci (e.g. CPT1A, ABCG1) underpinning mechanistic credibility [35,12,17]. These findings indicate that MRS can capture exposure-responsive molecular information that complements genetic and clinical risk indicators, particularly for complex, environment-modulated

diseases such as type 2 diabetes, cardiovascular disease and frailty.

However, translation into routine clinical practice is not yet justified without further work. Key barriers include heterogeneous assay platforms and preprocessing pipelines, limited cohort diversity and external validation, challenges in causal inference, and substantial upfront costs and regulatory uncertainty [85,38,84]. Ethical and equity concerns demand that models be developed and tested in representative populations to avoid exacerbating health disparities [92-99].

To realise clinical utility, the field must prioritise standardisation of laboratory and analytic workflows, large multi-ethnic longitudinal cohorts with repeated measures, independent external validation, pragmatic trials demonstrating impact on decision-making and outcomes, and transparent governance for data use. Advances in AI/ML, multi-omics integration and single-cell epigenomics offer substantial opportunity to refine predictive performance and mechanistic insight, but these must be pursued alongside explainability and clinical interpretability [66,20].

In summary, MRS with EBPs constitute a powerful, mechanistically grounded tool for enhanced risk prediction. With rigorous standardisation, validation, and ethically informed implementation, they have the potential to augment personalised prevention and management but careful, evidence-based adoption is essential to ensure benefit, equity and safety.

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