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Archivos de la Sociedad Española de Oftalmología

journal homepage: www.elsevier.es/oftalmologia

Review

The potential of DNA methylation as a biomarker for age-related macular degeneration: A systematic review

El potencial de la metilación del ADN como biomarcador de la degeneración macular asociada a la edad: una revisión sistemática

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ARTICLE INFO

Keywords:

Epigenetics
Age-related macular degeneration
DNA methylation
Methylation patterns
Age-related macular degeneration biomarkers

Palabras clave:

Epigenética
Degeneración macular asociada a la edad
Metilación del ADN
Patrones de metilación
Biomarcadores de la degeneración macular asociada a la edad.

ABSTRACT

Age-related macular degeneration (AMD) is a multifactorial disease influenced by genetic and environmental factors, yet its pathogenesis remains incompletely understood. DNA methylation, increasingly recognized as a disease indicator, has been linked to AMD and may represent a promising biomarker or therapeutic target. This systematic review, conducted according to PRISMA 2020 guidelines, analyzed 13 studies addressing DNA methylation in AMD populations (2012–2025). Results revealed that 25% reported hypermethylation, 8% hypomethylation, and 41% both patterns, while 15% found no significant differences. Notably, one study described downregulation of DNA methyltransferases in advanced stages compared with early or intermediate AMD. Despite the limited evidence, findings support the relevance of methylation in AMD prognosis and therapy. Further research with robust methodologies is essential to clarify the role of epigenetic mechanisms in disease progression and to explore their potential for guiding targeted therapeutic strategies

RESUMEN

La degeneración macular asociada a la edad (DMAE) es una enfermedad multifactorial influenciada por factores genéticos y ambientales, cuya patogénesis aún no se comprende completamente. La metilación del ADN, reconocida como un indicador de enfermedad, se ha vinculado con la DMAE y podría representar un biomarcador o un objetivo terapéutico prometedor. Esta revisión sistemática, realizada según las directrices PRISMA 2020, analizó 13 estudios publicados entre 2012 y 2025. Los resultados mostraron que el 25% informó hipermetilación, el 8% hipometilación y el 41% ambos patrones, mientras que el 15% no observó diferencias significativas. Un estudio destacó la disminución de ADN metiltransferasas en fases avanzadas en comparación con las etapas tempranas o intermedias. Aunque la evidencia sigue siendo limitada, estos hallazgos respaldan la relevancia de la metilación en el pronóstico y el tratamiento de la DMAE. Se requieren investigaciones más sólidas para comprender mejor su papel epigenético.

Introduction

Age-related macular degeneration (AMD) is a progressive degenerative disease affecting the central retina, primarily impacting individuals aged 50 and above.¹ It is the leading cause of irreversible vision loss globally, with prevalence expected to increase by 15% by 2050 due to

population aging.^{1–3} The pathophysiology of AMD remains incompletely understood, though it is characterized by hallmark features such as drusen - yellow deposits of lipids and proteins - and the degeneration of retinal pigmented epithelium cells, leading to photoreceptor loss.^{4,5}

AMD has substantial economic and social consequences, including medical expenses, visual impairment, reduced productivity, and increased caregiver burden.^{2,6} Risk factors like genetics, age, smoking, and diet have been investigated for their roles in AMD onset and progression.^{5,7,8}

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<https://doi.org/10.1016/j.oftale.2026.502521>

Received 18 October 2025; Accepted 6 December 2025

Available online 23 February 2026

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Advanced AMD manifests in two primary forms: atrophic AMD (aAMD), marked by progressive macular atrophy, and neovascular AMD (nAMD), involving abnormal blood vessel growth beneath the macula and leading to rapid vision loss.¹ The lack of effective therapies for early/intermediate AMD stages and advanced aAMD,⁹ along with the need for frequent monitoring of nAMD due to persistent disease activity in nearly 40% of patients,¹⁰ underscores the importance of identifying reliable biomarkers to enhance public health strategies and deepen understanding of AMD.¹¹

Current research has targeted various biological pathways in AMD, including drusen composition (e.g., vitronectin, clusterin, apolipoprotein E, and β -amyloid),¹ cellular apoptosis, oxidative and inflammatory stress,¹² lipid metabolism, and immune regulation.^{13–15} Genetic variants linked to complement factor H pathways, ARMS2/HTRA1,^{16,17} lipid,^{17,18} energy metabolism,¹⁹ and inflammatory pathways (e.g., TIMP3)¹⁷ have also been implicated. However, genetics alone explains only 40–60% of AMD risk,²⁰ and the association with non-invasive imaging biomarkers remains limited.

Ocular fundus imaging techniques^{21,22} have been fundamental in supporting findings related to oxidative stress, mainly through the accumulation of free radicals and consequent toxicity,²³ as well as the accumulation of lipofuscin in the RPE, resulting from degraded photoreceptor outer segments.²⁴ Changes in the retina and choroid observed in human eyes²⁵ or in inner neural layers²⁶ have also been explored as potential responses to inflammation in animal models of oxidative stress using imaging techniques.²² Moreover, novel therapeutic approaches such as anti-Ang-2 therapy in nAMD^{27,28} or complement factor 3 (C3) modulation in aAMD^{29,30} have shown promise in slowing disease progression.

In recent years, epigenetics has emerged as essential for deciphering previous and new mechanisms in AMD, offering the potential for early detection and optimization of therapeutic approaches. Epigenetics involves changes in gene expression that occur without alterations in DNA sequence, influenced by factors like environment and lifestyle.^{31,32} By turning genes “on” or “off” or modulating their activity levels, these changes can have an impact on how genes are expressed.³³ Key mechanisms include DNA methylation, non-coding RNA, and histone modifications, which can regulate gene activity and influence developmental processes, cellular differentiation, and environmental responses.^{31,32,34}

DNA methylation, one of the most extensively studied epigenetic processes, involves adding a methyl group to cytosine residues, typically at CpG sites, potentially silencing or reducing gene expression without altering the underlying DNA sequence.^{31,32,35} This process is catalyzed by DNA methyltransferases, including maintenance methyltransferase DNMT1 and *de novo* methyltransferases DNMT3A and DNMT3B.³⁶ DNMT1 is responsible for maintaining methylation patterns during DNA replication and reflects the epigenetic state of a disease, whereas DNMT3A and DNMT3B are involved in establishing new methylation marks, which may change in response to environmental stimuli or therapeutic interventions.^{31,32,35}

DNA methylation patterns can be influenced by factors such as aging and environmental exposures, and these patterns have been linked to various diseases, including cardiovascular, neurological, and retinal disorders.³⁷ In AMD, methylation changes may influence genes associated with key pathways in the disease-related pathways,³⁸ and these alterations seem to differ across AMD stages.³⁸

Although some studies have linked oxidative stress impairment,³⁹ lipid metabolism dysregulation,^{40,41} and inflammation processes⁴² to specific epigenetic modifications, a significant knowledge gap remains regarding how these methylation changes contribute to AMD progression and influence therapeutic responses.^{27,28} Furthermore, comprehensive assessments still lack the relationship between methylation patterns and key pathological processes in AMD, including neurodegenerative changes, vascular dysfunction, and retinal histological markers.^{21,22}

This systematic review aims to synthesize existing evidence on differential DNA methylation in AMD, examine key epigenetic pathways involved in its pathogenesis, and identify distinct methylation patterns across disease stages. Additionally, it seeks to explore potential therapeutic opportunities, particularly those targeting epigenetic modifications to mitigate or prevent disease progression, by addressing gaps in the current understanding of AMD’s epigenetic landscape.

Materials and methods

The present systematic review was developed considering the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA 2020) guidelines. Additionally, the protocol of this review was previously registered in the International Prospective Register of Systematic Reviews - PROSPERO (CRD42024581510).

Literature search, eligibility criteria, and information sources

This systematic review focused explicitly on AMD and its different stages. Studies investigating other diseases alongside AMD or lacking a precise AMD diagnosis were excluded. Articles were identified through searches conducted in three databases: PubMed, Scopus, and Web of Science. The database search was performed on 30 January using the PICO(s) framework to develop search terms (Table 1) and to establish the eligibility criteria for this systematic review:

- (P)articipants: Included data from diagnosed AMD patients aged 50 years and older.
- (I)ntervention: Studies assessing DNA methyltransferase (DNMT) levels in peripheral blood, cell lines, or retinal pigmented epithelium tissues from human donors.
- (C)omparison: Studies with similar assessments conducted on a control group.
- (O)utcome: Focused on AMD stage classification, including non-advanced AMD (initial and intermediate AMD), advanced AMD (atrophic and neovascular AMD), hypomethylation/hypermethylation expression levels, and associated AMD pathways such as oxidative stress, chronic inflammation, and immune dysregulation.
- (S)tudy design: Randomized clinical trials, cohort studies, case-control studies, and cross-sectional studies.

Using the combination of the five axes, the research question was developed: Is the DNA methylation profile associated with AMD stages, and could it be linked to disease mechanisms, serving as a biomarker or potential therapeutic target for AMD?

Studies that assessed potential biomarkers for disease severity, risk evaluation, or treatment response and were published in English were eligible for inclusion. Additional structural and functional changes were considered whenever applicable.

Exclusion criteria included secondary analyses, reviews, editorials, book chapters, systematic reviews, and incomplete studies. Studies focusing on other retinal diseases, macular dystrophies, or neovascularization of any non-AMD origin, as well as studies without control groups, were excluded.

The search terms were initially developed for PubMed and subsequently adapted for Scopus and Web of Science (see Supplementary Table S1).

Study selection, data extraction, and critical appraisal

In January 2025, two independent personnel conducted searches and data extraction in duplicate. Because the articles identified on Scopus and Web of Science were identical to those found on PubMed, the results from both databases were removed before screening to reduce duplicates. Afterward, acquired articles from PUBMED were uploaded

Table 1
Search terms developed using the PICO Framework.

PICO framework	PUBMED search terms
Population: AMD patients aged 50 and above	((“Macular Degeneration”[MeSH Terms] OR “Retinal Drusen”[MeSH Terms] OR “Retina”[MeSH Terms] OR “Retinal Neovascularization”[MeSH Terms] OR “Geographic Atrophy”[MeSH Terms] OR (“choroidal neovascularisation”[All Fields] OR “choroidal neovascularization”[MeSH Terms] OR (“choroidal”[All Fields] AND “neovascularization”[All Fields]) OR “choroidal neovascularization”[All Fields]) OR (“intermediate”[All Fields] OR “intermediated”[All Fields] OR “intermediately”[All Fields] OR “intermediates”[All Fields]) AND (“arch med deporte”[Journal] OR “amd”[All Fields])) OR (“advance”[All Fields] OR “advanced”[All Fields] OR “advancement”[All Fields] OR “advancements”[All Fields] OR “advances”[All Fields] OR “advancing”[All Fields]) AND (“arch med deporte”[Journal] OR “amd”[All Fields])) OR (“Macular Degeneration”[MeSH Terms] OR (“macular”[All Fields] AND “degeneration”[All Fields]) OR “Macular Degeneration”[All Fields] OR (“age”[All Fields] AND “related”[All Fields] AND “macular”[All Fields] AND “degeneration”[All Fields]) OR “age related macular degeneration”[All Fields]))
Intervention: levels of DNA methylation expression in peripheral blood, cellular line, and/or retinal pigmented epithelium tissues	(“DNA Methylation”[MeSH Terms] OR (“dna”[All Fields] AND “methylation”[All Fields]) OR “DNA Methylation”[All Fields] OR (“Epigenomics”[MeSH Terms] OR (“Epigenetic Memory”[MeSH Terms] OR “Epigenetic Repression”[MeSH Terms]) OR “DNA Methylation”[MeSH Terms]) OR (“Epigenomics”[MeSH Terms] OR “epigenesis, genetic”[MeSH Terms] OR “DNA Methylation”[MeSH Terms] OR “dmap1 protein human”[Supplementary Concept] OR “dnmt3a protein human”[Supplementary Concept] OR “DNA methyltransferase 3B”[Supplementary Concept]))
Comparator: control groups with no AMD	“Twins”[MeSH Terms] OR “Control Groups”[MeSH Terms] OR “diseases in twins/blood”[MeSH Terms] OR “diseases in twins/diagnosis”[MeSH Terms]
Outcome: the relationship between DNA methylation and AMD and its role as a biomarker.	((“biomarker s”[All Fields] AND (“macular degeneration”[MeSH Terms] OR (“macular”[All Fields] AND “degeneration”[All Fields]) OR “macular degeneration”[All Fields] OR (“age”[All Fields] AND “related”[All Fields] AND “macular”[All Fields] AND “degeneration”[All Fields]) OR “age related macular degeneration”[All Fields]) AND (“rna”[MeSH Terms] OR “rna”[All Fields]) AND (“methyl”[All Fields] OR “methylate”[All Fields] OR “methylated”[All Fields] OR “methylates”[All Fields] OR “methylating”[All Fields] OR “methylation”[MeSH Terms] OR “methylation”[All Fields] OR “methylations”[All Fields] OR “methylational”[All Fields] OR “methylator”[All Fields] OR “methylators”[All Fields] OR “methyls”[All Fields]) AND (“dna”[MeSH Terms] OR “dna”[All Fields]) OR (“methyl”[All Fields] OR “methylate”[All Fields] OR “methylated”[All Fields] OR “methylates”[All Fields] OR “methylating”[All Fields] OR “methylation”[MeSH Terms] OR “methylation”[All Fields] OR “methylations”[All Fields] OR “methylational”[All Fields] OR “methylator”[All Fields] OR “methylators”[All Fields] OR “methyls”[All Fields]) AND (“rna”[MeSH Terms] OR “rna”[All Fields]))))

to the Rayyan Systematic Review Screening Software (QCRI). Before the screening process, duplicates were detected and removed automatically by Rayyan software. The rest was then screened by two masked reviewers (SG and PC) independently, based on information on publication type, title, abstract, and keywords, to identify relevant studies. Duplicates that the software missed and articles that do not fit the criteria for the publication type needed for this systematic review have been filtered out manually. The remaining publications were reviewed by reading the complete text to choose which to include. The studies selected underwent a quality assessment utilizing the Joanna Briggs Institute critical appraisal tools checklist for cross-sectional and case-control studies (Supplementary Tables S2 and S3). Methodological rigor was assessed by scoring weak, moderate, and strong decisions based on factors such as sample size, appropriate matching of cases to controls, use of experimental controls, and consideration of confounding factors. All comprehensive biomarkers and abbreviations are provided in the supplementary material (Supplementary Table S5).

Results

The three databases yielded a total of 1213 articles: PubMed (n = 990), Scopus (n = 128), and Web of Science (n = 95). Before the screening phase, articles from Scopus and Web of Science (n = 52) were eliminated, as they were identical to the ones acquired from PUBMED. Automation tool Rayyan also removed two duplicates before screening. After screening the remaining papers (n = 988), two duplicates were manually removed, and 884 were deemed unrelated to this systematic review’s topic by title and abstract analysis. This left 102 publications for complete text evaluation, of which 89 were deleted, leaving 13 papers that fulfilled the criteria for inclusion and exclusion. Fig. 1 summarizes this screening and selection process. Although the included studies are relevant as an initial step in exploratory epigenetic research, their

overall methodological rigor was considered suboptimal due to small sample sizes, limited consideration of confounding factors, insufficient reporting of mitigation strategies, and the absence of appropriately matched experimental controls.

Information extracted included the year and country of publication, study types, and sample details (Table 2). Among the thirteen studies, eleven were cross-sectional, and two were case-control. Countries of publication origin were primarily the USA (5/13) and China (4/13), with additional studies from France (2/13), Italy (2/13), Australia (1/13), the UK (1/13), the Netherlands (1/13), and Portugal (1/13). Table 2 also indicates a marked increase in research on methylation and AMD in recent years, with nearly 40% of selected studies published between 2020 and 2025. The articles primarily examined samples from chorioretinal tissues (5/13), whole blood (7/13), and retinal pigment epithelium (5/13), focusing on key genes such as IL17RC, SIRT1, SKI, and GTF2H4, which play roles in immune response, cellular aging, and structural integrity. The most recent study investigated plasma DNA methyltransferase expression (DNMT1, DNMT3A, and DNMT3B) in patients across different AMD stages. Additional data extracted included methylation measurement techniques, AMD classification methods, and summarized study findings (Table 3).

The studies in this systematic review reveal distinct DNA methylation patterns associated with AMD, reflecting variations across different genes, sample types, and methodologies (Table 3). Of the thirteen studies reviewed, eleven identified significant methylation changes in AMD patients, with findings categorized into hypermethylation, hypomethylation, or both.

Approximately 25% of studies reported hypermethylation, particularly affecting oxidative stress and epigenetic regulation genes, such as *GSTM1*⁴³ and *LINE-1*.⁴⁴ In *GSTM1*, hypermethylation correlated with reduced gene expression, suggesting a possible link between oxidative stress-related gene regulation and AMD pathogenesis. Additionally, in-

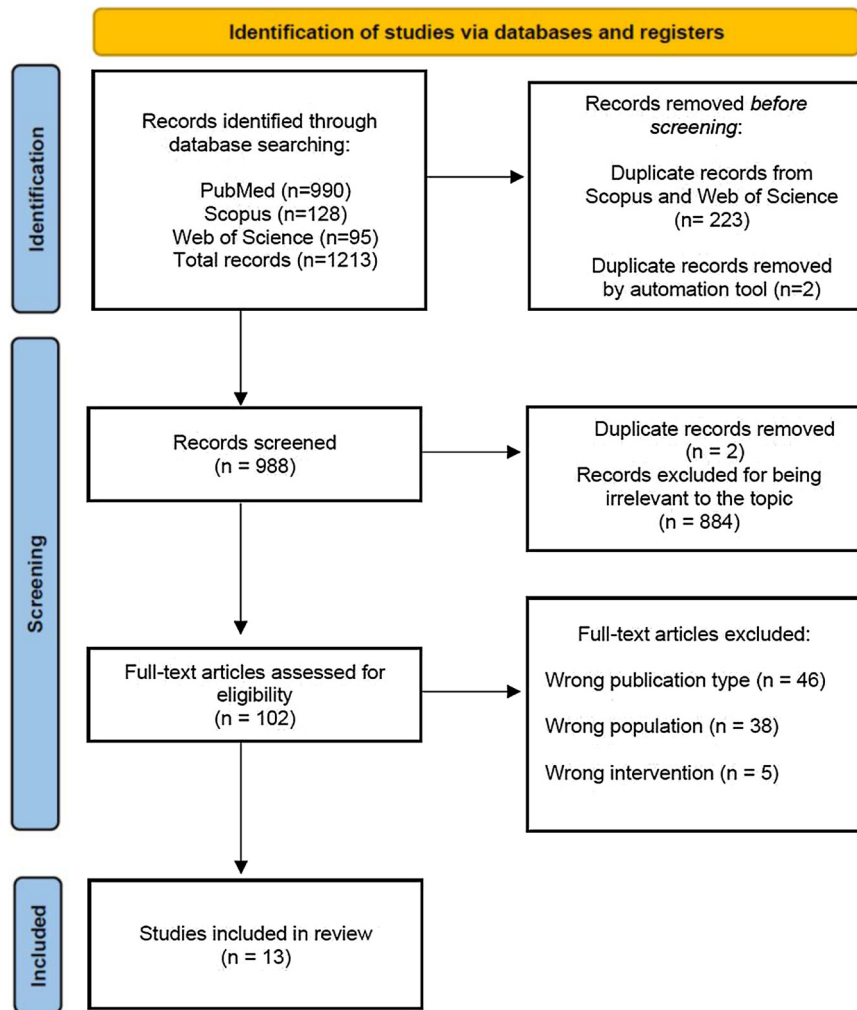


Figure 1. PRISMA 2020 flow diagram for new systematic reviews, which included searches of databases and registers only.

Figure adapted from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71

creased *LINE-1* methylation was observed alongside elevated activity of DNA methyltransferases (*DNMT1* and *DNMT3B*), hinting at broader global methylation changes within AMD patient samples.⁴⁴ Camacho et al. examined DNA methyltransferase expression (*DNMT1*, *DNMT3A*, and *DNMT3B*) across patients with different AMD stages, reporting divergent expression patterns and highlighting the potential role of differential DNMT expression in AMD pathogenesis and staging.³⁸

Hypomethylation patterns were noted in about 8% of the studies, most notably involving the immune-related *IL17RC* gene, which displayed reduced methylation and increased gene expression in AMD tissues.⁴⁵ This finding suggests a potential role for immune pathway dysregulation in AMD and aligns with the observed downregulation of *DNMT1* and *DNMT3A* in atrophic AMD compared to intermediate and neovascular AMD.³⁸ Interestingly, 41% of the studies detected hypermethylation and hypomethylation across various gene targets, indicating complex methylation dynamics within AMD disease. Notable genes showing differential methylation included *SKI*, *GTF2H4*, and *TNXB*,⁴⁶ which are linked to cellular structure, DNA repair, and immune function—key processes implicated in AMD pathology.

Only two studies using peripheral blood samples reported no significant methylation changes^{47,48} suggesting that tissue type may influence detectable methylation patterns and their potential as biomarkers. Notably, some studies, such as Saptarshi,⁴⁹ reported more pronounced

epigenetic changes in whole blood samples than retinal tissue, underscoring the importance of sample selection when investigating AMD methylation.

While methylation profiles varied significantly by tissue type and gene target, this review indicates that genes involved in oxidative stress, immune response, and cellular structure regulation may undergo epigenetic changes in AMD, contributing to its complex molecular landscape. The pathways identified in the selected studies, and the sample types are detailed in Supplementary Table (Table S4).

Discussion

Genome-wide association studies have identified various genetic variants that increase susceptibility to AMD. However, the late-onset nature of AMD suggests that genetic predisposition alone does not fully explain its pathogenesis.^{50,51} Environmental factors, such as smoking and reduced vitamin D levels, have also been linked to advanced AMD.⁷ These influences may interact with epigenetic mechanisms, particularly DNA methylation, which was the specific focus of this systematic review. The current findings offer an integrative synthesis of available evidence on DNA methylation in AMD, highlighting potential candidate biomarkers and therapeutic targets organized as follows: (1) AMD development, progression, and treatment relevance of methylation changes, and (2) key genes and molecular pathways identified through

Table 2
Summary of studies included in the systematic review.

First Author, publication year, and reference number	Title	Year	Country	Study type	Sample information
Hunter, 2012 ⁴³	DNA methylation is associated with altered gene expression in AMD	2012	USA	Cross-sectional	Postmortem RPE/choroid from patients with AMD (n = 10) and age-matched controls (n = 11). The average postmortem tissue harvest time was 5.2 h (2.5–9.0 hours). The postmortem specimens were flash-frozen upon tissue harvest and stored at –70 °C. A foveal horizontal section was then performed, and the neurosensory retina and RPE/choroid were separated.
Wei, 2012 ⁴⁴	Hypomethylation of the IL17RC promoter associates with age-related macular degeneration	2012	USA	Cross-sectional	Chorio-retinal tissues and peripheral blood mononuclear cells (PBMCs) were sampled from 6 patients with AMD and six without AMD. PBMCs from AMD patients and healthy controls were cultured in RPMI-1640 medium. ARPE-19 cells and primary human RPE cells were then cultured in complete Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 2 mM L-glutamine, and 1X penicillin-streptomycin antibiotics with or without IL-17A (20 ng/mL), or IL-17F (20 ng/mL) overnight.
Oliver, 2013 ⁴⁵	Hypomethylation of the IL17RC promoter in peripheral blood leukocytes is not a hallmark of age related macular degeneration	2013	Australia & USA & France	Cross-sectional	DNA samples from PBMCs of 298 age and sex-matched samples (100 bilateral geographic atrophy, 99 bilateral neovascularization, and 99 controls). PBMCs and granulocytes were isolated from whole blood collected using Ficoll-Plaque Premium.
Oliver 2015 ⁴⁶	Differential DNA methylation was identified in the blood and retina of AMD patients.	2015	USA	Cross-sectional	DNA samples from PBMCs of 298 age and sex-matched samples (100 bilateral geographic atrophy, 99 bilateral neovascularization, and 99 controls). Sample extraction information was not included in the article.
Pinna, 2015 ⁴⁷	Plasma Homocysteine and Asymmetrical Dimethyl-L-Arginine (ADMA) and Whole Blood DNA Methylation in Early and Neovascular Age-Related Macular Degeneration: a Pilot Study.	2015	Italy	Case-control	DNA samples from whole blood of 39 early AMD, 27 neovascular AMD, and 132 sex and age-matched controls without maculopathy. Blood samples were collected from each participant after an overnight fast.
Maugeri 2019 ⁴⁸	Characterization of SIRT1/DNMTs Functions and LINE-1 Methylation in Patients with Age-Related Macular Degeneration.	2019	Italy	Cross-sectional	PBMCs samples from 40 AMD patients and 10 age and sex matched controls. The samples were collected into Ethylenediaminetetraacetic acid (EDTA) tubes for molecular analysis. The nuclear Extraction Kit isolated nuclear proteins from peripheral blood samples to measure DNMTs and SIRT1 activity. Peripheral blood samples were centrifuged at 2500 rpm for 15 min to analyze DNMTs and SIRT1 expression. The puffy coat portion was transferred to a cryovial and stored at –20 °C until use.
Xu, 2019 ⁴⁹	Identification of aberrantly methylated differentially expressed genes in age-related macular degeneration.	2019	China	Cross-sectional	Gene expression datasets containing 118 samples from the retina were downloaded from the Gene Expression Omnibus (GEO). The data series contains 63 samples from donors with preclinical AMD or AMD and 55 from donors without ocular disease. Gene methylation datasets were also downloaded from GEO, which included eight samples from donors with preclinical AMD and 9 samples from donors without ocular disease.

Table 2 (Continued)

First Author, publication year, and reference number	Title	Year	Country	Study type	Sample information
Porter, 2019 ⁵⁰	Whole-genome methylation profiling of the retinal pigment epithelium of individuals with age-related macular degeneration reveals differential SKI, GTF2H4, and TNXB gene methylation.	2019	USA/ UK/Netherlands	Cross-sectional	RPE cells from 44 human donor eyes (25 AMD and 19 controls) and 55 RPE samples (30 AMD and 25 controls). Dissection was performed under a GT Vision GXM XTL3TV6 stereomicroscope, and RPE cells were mechanically isolated from Bruch's.
Shen, 2020 ⁵¹	Integrated bioinformatics analysis of aberrantly-methylated differentially expressed genes and pathways in age-related macular degeneration	2020	China	Cross-sectional	Genome-wide DNA methylation profiling of RPE-choroid and retinal tissue of 41 patients with AMD and 42 controls. Methylation profile data from Oliver et al. Genome-wide DNA methylation profiling of peripheral blood from 9 patients with AMD and nine controls was also included in this analysis. RPE from 25 AMD human donors and 19 regular control donors. Whole blood samples from 14 AMD patients and 16 controls.
Saptarshi, 2021 ⁵²	Epigenetic Age Acceleration Is Not Associated with Age-Related Macular Degeneration	2021	France	Case-control	RPE from 25 AMD human donors and 19 regular control donors. Whole blood samples from 14 AMD patients and 16 controls.
Wang, 2021 ⁵³	Integrated analysis of DNA methylation and transcriptome profile to identify key features of age-related macular degeneration	2021	China	Cross-sectional	RPE samples were from 25 AMD donors (levels 2 and 3) and 19 regular control donors (level 1). Anterior segments are removed with a coronal incision. The vitreous was removed to obtain an unobstructed view of the posterior segment with the neurosensory retina intact. The neurosensory retina was then carefully dissected from the underlying RPE layer. DNA methylation profiles were retrieved from the GEO database, which includes nine control retina tissues and 9 AMD retina tissues.
Liang, 2022 ⁵⁴	Identification of differentially expressed and methylated genes and construction of a co-expression network in age-related macular degeneration	2022	China	Cross-sectional	DNA methylation profiles were retrieved from the GEO database, which includes nine control retina tissues and 9 AMD retina tissues.
Camacho, 2025 ³⁸	DNA Methyltransferase Expression (DNMT1, DNMT3a, and DNMT3b) as a Potential Biomarker in Age-Related Macular Degeneration	2025	Portugal	Cross-sectional	Expression of genes DNMT1, DNMT3A, DNMT3B was evaluated using samples from PBMCs of 38 AMD patients with different stages of the disease.

Legend: AMD = Age-related macular degeneration; ARPE-19 = human retinal pigment epithelial; EDTA = Ethylenediaminetetraacetic acid; DNMTs = methyltransferases; FBS = fetal bovine serum; GEO = Gene Expression Omnibus; RPE = retinal pigment epithelium; PBMCs = peripheral blood mononuclear cells.

methylation analyses. Fig. 2 summarizes the main pathways and genes implicated via methylation profiling in different AMD stages.

AMD development, progression, and potential treatment targets

The following subsections reflect the key biological processes implicated in AMD pathogenesis, as identified through DNA methylation studies included in this review. This organization intends to discuss in a focused evaluation of how methylation patterns may contribute to disease onset, progression, and potential therapeutic intervention

a) AMD Development: Oxidative Stress

Oxidative stress plays a critical role in the pathogenesis of AMD, primarily due to the retina's high metabolic activity, lipid-rich composition, and continuous exposure to light.^{52,53} DNA methylation studies have shown that hypermethylation of GSTM1 and GSTM5, two key antioxidant genes, is associated with reduced expression, increasing the retina's susceptibility to oxidative damage.⁴³ These findings suggest

that specific methylation alterations are related to the oxidative stress pathway in AMD, underlining their potential as early-stage methylation biomarkers. Similarly, others⁵⁴ highlighted hypomethylation in key regulatory genes, including *CDKN1C*, *EZR*, *IGF2*, and *SLC2A1*, which are involved in glucose transport and metabolic processes. Impairment of *SLC2A1* may disrupt the homeostasis of outer segment photoreceptors and the RPE, contributing to AMD-related lesions such as drusen formation and RPE atrophy. Dysregulation in these genes may exacerbate oxidative stress, making them potential early biomarkers of AMD.

One of the earliest clinical signs of AMD is lipofuscin accumulation in RPE cells, resulting from incomplete degradation of photoreceptor outer segments. This buildup generates free radicals, contributing to oxidative stress and inducing cellular toxicity, triggering RPE dysfunction.^{23,24}

Recent investigations into ferroptosis—a form of regulated cell death driven by iron-dependent lipid peroxidation—have also underscored the significance of *SLC2A1* in AMD pathology. As a gene regulating glucose availability in RPE cells, *SLC2A1* has been identified as a promising diagnostic marker and a potential therapeutic target in AMD related to oxidative stress.³⁹ Furthermore, oxidative imbalance has been linked to the dysregulation of vascular endothelial growth factor (VEGF) through

Table 3
Study characteristics summary of the articles included in the systematic review.

First Author, publication year, and reference number	DNA Methylation measurement method	AMD classification method	Outcomes
Hunter, 2012 ⁴³	Illumina Infinium HumanMethylation27 microarray of bisulfate treated DNA	Classified by medical history and confirmed by gross examination of the retina	The mRNA levels of <i>GSTM1</i> and <i>GSTM5</i> were significantly reduced in AMD versus age-matched controls in RPE/choroid and NSR, corresponding to hypermethylation of the <i>GSTM1</i> promoter with increased susceptibility to oxidative Stress. Reduced methylation of the <i>IL17RC</i> gene promoter in AMD patients results in increased protein and mRNA expression in the retina, choroid, and peripheral blood, promoting proinflammatory events. There is no evidence of differential methylation between AMD cases and age-matched controls. Hypomethylation within the <i>IL17RC</i> gene promoter in peripheral blood is unsuitable as a clinical biomarker of AMD.
Wei, 2012 ⁴⁴	DNA Methylation Microarray (MeDIP-chip) analysis using NimbleGen Human DNA Methylation 2.1 M Deluxe Promoter Array.	Not included in the article.	
Oliver, 2013 ⁴⁵	Illumina Infinium HumanMethylation450 Bead array of bisulfate treated DNA	Baltimore cohort: Diagnosis of advanced AMD was based on the presence of GA or CNV (equivalent to AREDS category 4 or 5). Controls were identified as > 60 years of age, having fewer than five small drusen (< 63 μm), and no RPE abnormalities. Australian Cohort: The clinical criteria for the diagnosis of AMD included the presence of extensive drusen and associated pigmentary abnormalities of the RPE layer or evidence of advanced disease (GA or CNV). All control subjects underwent a comprehensive optical coherence tomography and colour fundus imaging examination. To be included as a control, subjects required no evidence of ophthalmic pathology or no prior or known family history of ocular disease.	
Oliver, 2015 ⁴⁶	Illumina Infinium HumanMethylation450 Bead array of bisulfate treated DNA.	Donor eyes obtained from the National Disease Research Interchange had limited clinical history, and Macular photos were taken before the isolation of macular calottes from each eye. Macular tissue was passed through a sucrose gradient and frozen. Sections were stained with hematoxylin and eosin and reviewed by an ocular pathologist for histologic evidence of AMD. As for the sample obtained from the Baltimore cohort, diagnosis of advanced AMD was based on the presence of GA or CNV (equivalent to AREDS category 4 or 5). Controls were identified as > 60 y of age, having fewer than five small drusen (< 63 μm), and no RPE abnormalities.	Significant DNA methylation differences in the blood of neovascular AMD patients near the ARMS2 gene.
Pinna, 2015 ⁴⁷	Capillary Electrophoresis	All patients with early AMD underwent a complete ophthalmic evaluation, including fundus photography with digitally captured 350 stereoscopic pairs centered on the optic disc and the macula. All wet AMD patients underwent a complete ophthalmic evaluation, including fluorescein angiography and macular OCT scans (3D OCT-1000 Mark II, Topcon Co, Tokyo, Japan). Patients with clinical evidence of geographic atrophy in the fellow eye were excluded.	There are no significant differences in the degree of whole blood DNA methylation between patients with early and neovascular AMD and controls.

Table 3 (Continued)

First Author, publication year, and reference number	DNA Methylation measurement method	AMD classification method	Outcomes
Maugeri, 2019 ⁴⁸	LINE-1 methylation level was measured by pyrosequencing-based methylation analysis of three CpG islands using the PyroMark Q24 instrument.	A dilated retinal exam, optical coherence tomography, and fluorescein angiography were performed for AMD cases.	LINE-1 methylation level was higher in AMD patients compared with controls. AMD patients exhibited increased total DNMTs activity. Contrary to what was observed in RPE cells. The LINE-1 methylation level was consistently positively associated with total DNMTs activity, DNMT1, and DNMT3B expression in AMD patients.
Xu, 2019 ⁴⁹	Immunoprecipitation of Methylated DNA (MeDIP)	175 samples from extramacular retinal pigmented epithelium, choroid, and 118 samples from the retina (Gene Expression Omnibus)	For DMGs in gene methylation datasets, 6537 hypermethylated genes and 3805 hypomethylated genes were found. The results indicate abnormal methylation of many genes associated with AMD development (NOP56, EZR, IGF2, SLC2A1, CDKN1C).
Porter, 2019 ⁵⁰	Illumina 450k BeadChip array and validating findings using bisulfite pyrosequencing	The tissue was subdivided into no AMD (no drusen or a few small drusen < 63 µm) and AMD, based upon the criteria for intermediate drusen (> 63 µm and < 125 µm), or the presence of one or more large drusen (> 125 µm), and the presence of pigmentary changes or geographic atrophy. Samples with CNV were deliberately excluded from the study.	Genome-wide DNA methylation profiling identified differential methylation of multiple loci, including the SKI, GTF2H, and TNXB genes in AMD. Bisulfite pyrosequencing validated the differentially methylated locus cg18934822 in the SKI gene and cg22508626 within the GTF2H4 gene and excluded global DNA methylation changes in the RPE in AMD.
Shen, 2020 ⁵¹	Illumina HumanMethylation 450 Bead array And Limma package in R software to analyze and identify differentially methylated genes.	The Iowa eyes were selected from a well-characterized repository of more than 3900 donors. The Oregon eyes were generally classified as AMD based on medical histories confirmed by ophthalmological records.	A total of 4,117 hypermethylated and 511 hypomethylated genes were identified. Among the aberrantly methylated and differentially expressed genes, PPP3CA, TGFB2, and SOCS2 were implicated in various pathways, including sphingolipid metabolism.
Saptarshi, 2021 ⁵²	The Illumina Infinium HumanMethylation450K BeadChip array was used to obtain RPE DNA methylation data, and the Illumina Infinium MethylationEPIC BeadChip array was used to obtain whole blood-derived DNA methylation data.	According to the Age-Related Eye Disease Study (AREDS) classification, samples were obtained from individuals' phenotypes.	Poor performance of epigenetic clocks was observed in RPE tissues. However, it improved when analyzing whole blood-derived gDNA data. Epigenetic age acceleration was not observed in AMD but was observed in the blood of smokers with and without AMD.
Wang, 2021 ⁵³	Illumina Infinium HumanMethylation450 Bead array	The Minnesota Grading System of eye bank eyes for AMD was used for classification.	A total of 456 DEGs and 4827 DMCs were identified between AMD and controls.
Liang, 2022 ⁵⁴	Illumina Infinium Methylation 450K	Not included in the article	16 hypermethylated and 15 hypomethylated genes were identified for extramacular AMD. Furthermore, 13 hypermethylated and 31 hypomethylated were identified for macular AMD.

Table 3 (Continued)

First Author, publication year, and reference number	DNA Methylation measurement method	AMD classification method	Outcomes
Camacho, 2025 ³⁸	Total RNA was extracted using Quick-RNA™ Whole Blood (Zymo Research, Orange, CA, USA). One-step NZY Speedy RT-qPCR Green kit (NZYtech, Lisbon, Portugal) CFX Connect™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA)	Patients were recruited and classified according to the Age-Related Eye Disease Study (AREDS) classification.	The study identified distinct expression patterns associated with different AMD stages. <i>DNMT1</i> , <i>DNMT3A</i> , and <i>DNMT3B</i> were downregulated in late AMD compared to early and intermediate stages. Additionally, <i>DNMT1</i> and <i>DNMT3A</i> expression were reduced in atrophic AMD compared to intermediate and neovascular AMD.

Legend: AMD = Age-related macular degeneration; NSR = neurosensory retina; KEC = Kellogg Eye Center; AREDS = Age-related Eye Disease Studies; CNV = Choroidal Neovascularization; GA = Geographic Atrophy; OCT = Optical Coherence Tomography; GSTM1 = Glutathione S-Transferase M1; DNMTs = methyltransferases; IL17RC = Interleukin-17 receptor C; LINE-1 = Long Interspersed nuclear elements; RPE = retinal pigment epithelium; DMGs = Differentially Methylated Genes; SKI = SKI proto-oncogene; DEGs = Differentially expressed genes; DMCs = intragenic differentially methylated CpGs; GTF2H = general transcription factor IIH subunit H4; TNXB = Tenascin X.

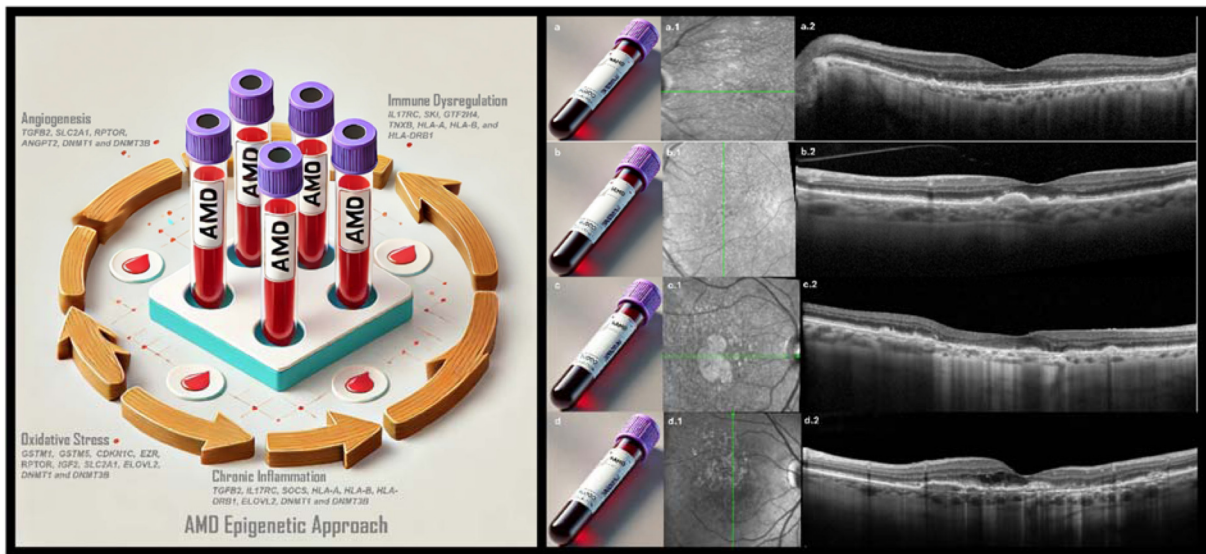


Figure 2. Key pathways and genes associated with different AMD stages assessed through Optical Coherence Tomography imaging. **Legend:** All images were acquired using a Spectralis device (Heidelberg Engineering, Heidelberg, Germany). (a) Example of a blood sample collected from a patient with early AMD. (a.1) Near-infrared SLO image of the left eye showing multiple pinpoint drusen-like structures, compatible with small drusen and superficial cuticular drusen, without signs of RPE changes or atrophy. (a.2) Horizontal B-scan revealing predominantly small drusen, preserved foveal bouquet integrity, no external limiting membrane or ellipsoid zone disruption, and an intact RPE band without hypertransmission. (b) Example of a blood sample collected from a patient with intermediate AMD. (b.1) Near-infrared SLO image of the left eye demonstrating numerous intermediate and large drusen, along with dark-limited spots corresponding to subretinal drusenoid deposits located temporal to the fovea. (b.2) Vertical B-scan showing hyperreflective foci over drusen and reflective material beneath the RPE. (c) Example of a blood sample collected from a patient with atrophic AMD. (c.1) Near-infrared SLO image of the right eye illustrating a well-demarcated area of atrophy temporal to the foveola, with multiple medium and large drusen present but no apparent RPE tear. (c.2) Horizontal B-scan revealing complete RPE and outer retinal atrophy (cRORA). (d) Example of a blood sample collected from a patient with neovascular AMD. (d.1) Near-infrared SLO image of the right eye showing marked reflectance changes in the inferior macular region, compatible with macular edema, scattered drusen-like deposits, and RPE alterations. (d.2) Vertical B-scan depicting an irregular RPE detachment, subretinal and intraretinal fluid, and subretinal hyperreflective material, suggestive of neovascular exudation associated with Type 1 macular neovascularization.

hypoxia-inducible factor (HIF)- α , which impairs choroidal vascularization.⁵⁵ Ocular fundus imaging can monitor these vascular changes, offering a non-invasive method to assess AMD progression.^{21,22,55}

b) AMD Progression: Chronic Inflammation and Immune Dysregulation

Chronic inflammation is a critical factor in the progression of AMD, contributing to the formation of drusen, disruption of Bruch's membrane, degeneration of the RPE, and the subsequent development of choroidal neovascularization.^{56,57} Epigenetic changes in immune-

related genes further underscore the role of immune dysregulation in AMD. Wei et al.⁴⁵ identified hypomethylation of *IL17RC* in AMD patients, which increases *IL17RC* expression and promotes inflammatory responses in the macula. Similarly, Porter et al.⁴⁶ observed hypermethylation in genes such as *SKI*, *GTF2H4*, and *TNXB*, which are associated with immune regulation and DNA repair, highlighting the significance of these pathways in AMD progression.

Shen et al.⁵⁸ reported hypermethylation of *TGFB2*, a gene implicated in inflammation and angiogenesis under hypoxic conditions. Elevated *TGFB2* expression in RPE cells undergoing hypoxia-induced epithelial-

mesenchymal transition (EMT) results in the development of fibrotic tissue resembling scar tissue in advanced AMD.⁵⁹ Consequently, targeting the *TGFB2* signaling pathway could represent a novel therapeutic approach to mitigating AMD progression.

Indeed, signs of inflammation have been observed in animal models of oxidative stress, with retinal layer alterations detected through imaging techniques.²² Suppressors of cytokine signaling (SOCS) proteins also play a crucial role in regulating inflammatory pathways. Dysregulated SOCS expression may disrupt the cytokine balance in retinal tissues, contributing to chronic inflammation and AMD pathology.⁶⁰ Notably, Liu et al.⁶¹ demonstrated that SOCS2 influences the ubiquitin-autophagy-lysosomal pathway, essential for cellular homeostasis in retinal cells. These findings suggest that modulating SOCS proteins could present new therapeutic avenues for AMD.

Furthermore, immune-related genes such as *HLA-A*, *HLA-B*, and *HLA-DRB1* have been implicated in persistent inflammation in AMD progression.⁶² The importance of immune dysregulation and Complement activity has long been addressed, and its markers are found in the retina and choroid in human eyes²⁵ and the inner neural layers.²⁶ Recent studies have shown that immune cell activity within the retina, regulated by genes like *HERC3*, contributes to neurodegeneration, retinal thinning, and inflammation, further emphasizing the role of immune dysregulation in AMD.^{25,26,42} Complement factor dysregulation has been identified as a central mechanism in AMD progression.⁶³ For instance, the relationship between complement component C5a and *DNMT1* upregulation has been demonstrated in kidney tissue,⁶⁴ and this interaction has been associated with various retinal diseases, including AMD. Understanding the cross-talk between complement activation and epigenetic modifications offers valuable insights into AMD's complex mechanisms and may help identify novel therapeutic targets.

c) AMD Treatment: DNA Methylation-Based Therapeutic Targets

Epigenetic modifications have emerged as promising therapeutic targets in the management of AMD. Saptarshi et al.⁴⁹ observed accelerated epigenetic aging in smokers with AMD, highlighting the combined impact of environmental factors and epigenetic alterations on disease progression. This study identified RPTOR as a hypomethylated regulator in the mTOR pathway, implicated in inflammatory and oxidative stress. Inhibiting RPTOR could reduce inflammation and abnormal angiogenesis, offering a potential treatment strategy for AMD.⁶⁵ Evidence linking RPE function to photoreceptor outer segment integrity and *DNMT1* expression further supports epigenetic-based intervention.⁶⁶ Additionally, dysregulation of the WNT signaling pathway has been implicated in AMD progression, with canonical WNT pathway underactivation associated with aAMD and overactivation contributing to nAMD angiogenic processes.^{67–69}

Porter et al.⁴⁶ proposed that epigenetic changes in genes such as *SKI* and *TGFB2* may influence complement regulation, a pathway central to AMD pathology. Modulating TGF- β signaling could reduce inflammation and complement deposition in the RPE, slowing AMD progression. Shen et al.⁵⁸ similarly identified *TGFB2* as a potential therapeutic target, with interventions aimed at inhibiting angiogenesis and inflammation, two critical processes driving AMD.

Targeting immunological genes presents another potential strategy for AMD treatment. Genes such as *HLA-A*, *HLA-B*, and *HLA-DRB1*⁶² have been implicated in immune response modulation, which may alleviate chronic inflammation in AMD. Maugeri et al.⁴⁴ found increased *DNMT* activity in AMD patients, suggesting that *DNMT* inhibitors could be employed to modify global methylation profiles and reduce disease progression. Elevated *LINE-1* methylation levels further support the relevance of DNMTs as therapeutic targets in AMD.

In cases of nAMD resistant to conventional therapies, dual targeting of VEGF and Ang-2 pathways has shown promise. Functional and structural improvements have been reported using combined anti-VEGF and anti-Ang-2 therapies, such as Faricimab.²⁷ Ang-2, by competi-

tively inhibiting Ang1 binding to the Tie2 receptor, plays a crucial role in maintaining vascular stability and regulating angiogenesis.²⁸ Notably, low *ANGPT2* methylation status has been associated with poor prognostic markers in angiogenic diseases, including cancer.⁷⁰ In situations of analogous angiogenesis, the hypomethylation of *ANGPT2*, which destabilizes blood vessels and promotes VEGF-driven angiogenesis, supports the therapeutic rationale for combining anti-VEGF and anti-Ang-2 agents to achieve better vascular stabilization in resistant nAMD cases.^{27,28}

Complement factor modulation has also gained attention for aAMD progression. Recent studies have demonstrated structural and functional improvements through interventions targeting complement factor 3 (C3), which plays a critical role in maintaining the integrity of the RPE, photoreceptors, and glial cells.²⁹ Initial findings indicate that C3-targeted therapies may delay atrophy in both RPE and photoreceptors, providing a promising strategy to slow AMD progression.³⁰

Key genes and pathways affected by DNA methylation in AMD

In line with our objective, this section synthesizes the most consistently identified genes and pathways affected by DNA methylation in AMD, highlighting their potential as biomarkers or therapeutic targets.

a) Complement Pathway and Immune Regulation (Genes: *SKI*, *TGFB2*, *HLA-A*, *HLA-B*, *HLA-DRB1*)

Target Mechanism: Modulating *SKI* and *TGFB2* expressions could reduce chronic inflammation and immune dysregulation by minimizing complement accumulation in the RPE. *HLA* genes also play a pivotal role in immune responses, and targeting their epigenetic regulation may help counter persistent inflammation in AMD.^{46,58,62}

These findings suggest that controlling complement activity and immune pathways through epigenetic modification could slow AMD progression, particularly in its advanced stages.

b) Oxidative Stress Response (Genes: *GSTM1*, *GSTM5*, *SLC2A1*, *RPTOR*)

Target Mechanism: Hyper- and hypomethylation in these genes are linked to oxidative stress. Targeting *GSTM1* and *GSTM5* methylation could restore antioxidant function, while modulating *SLC2A1* and *RPTOR* could improve glucose transport and reduce oxidative stress, potentially slowing AMD progression.^{43,49,54} Hunter et al.⁴³ explored the role of epigenetic regulation in antioxidant genes relevant to AMD. They found that the mRNA levels of glutathione S-transferase isoforms *GSTM1* and *GSTM5* were significantly reduced in AMD patients' RPE/choroid and neurosensory retina compared to controls.

c) mTOR Pathway Regulation (Gene: *RPTOR*)

Target Mechanism: Hypomethylation of *RPTOR* impacts mTOR pathway activity, contributing to oxidative and inflammatory stress. Targeting *RPTOR* to inhibit mTOR activation may mitigate retinal damage from oxidative stress.⁴⁹

d) DNA Methyltransferase Inhibition (Genes: *DNMT1*, *DNMT3B*, *LINE-1*)

Target Mechanism: Increased DNMT activity in AMD suggests that DNMT inhibitors could alter global methylation patterns, reducing AMD risk. Elevated *LINE-1* methylation highlights the role of global methylation changes in AMD's pathogenesis, supporting DNMTs as therapeutic targets.⁴⁴ The most recent study reported distinct expression patterns across different AMD stages, with *DNMT1*, *DNMT3A*, and *DNMT3B* downregulated in late AMD compared to early and intermediate stages.

Additionally, *DNMT1* and *DNMT3A* exhibited differential expression in atrophic AMD compared to intermediate and neovascular AMD.³⁸

Study gaps and future research directions

The limitations identified in this systematic review start with the variability in how DNA methylation has been assessed across studies and the absence of standardized AMD classification systems in several included articles. Nevertheless, despite these heterogeneities, the synthesis of findings confirms that methylation alterations—particularly in genes related to inflammation, oxidative stress, and lipid metabolism—play a consistent and significant role in AMD pathogenesis.⁷¹

Many included studies have not adopted the most recent AMD classification frameworks and display methodological inconsistencies in assessing epigenetic markers. However, recent research provides valuable insights into AMD susceptibility and progression, particularly by emphasizing the potential of methylation-based approaches.⁷² Experimental studies using animal models have further reinforced the role of mitochondrial dysfunction and energy imbalance in AMD pathogenesis, highlighting their importance in RPE homeostasis.⁷³ Moreover, methylation of the regulatory region of the *ELOVL2* gene has been shown to impair RPE function, and RPE contributes to the formation of drusen, a key biomarker of AMD. We acknowledge that some evidence derives from harvested retinal tissues, which may not fulfil the criteria for clinically accessible clinical biomarkers. However, these findings are essential to identify mechanistically relevant methylation alterations that can later be validated in peripheral samples. Notably, two of the included studies (Wei et al., 2012⁴⁵ and Shen et al., 2020⁵⁸) directly analysed both retinal tissues and peripheral blood, identifying congruent methylation patterns in genes associated with inflammation, such as *IL17RC*, *TGFB2*, and *SOCS2*. These findings support the potential of peripheral blood to reflect disease-relevant epigenetic changes and to serve as a surrogate for retinal tissue in clinical contexts.

Although peripheral samples may not capture the full complexity of retinal epigenetics, they offer a clinically accessible means of detecting AMD-related methylation signatures, notably when correlated with retinal imaging biomarkers. Further studies are needed to strengthen this translational link.

Despite methodological variations, the findings consistently support a link between AMD and epigenetic mechanisms, suggesting potential therapeutic targets within these pathways,^{40,41} which were previously discussed in the AREDS reports.⁷⁴

Despite the limitations discussed, the studies included in this review underscore the relevance of epigenetic mechanisms in AMD. Two key aspects emerge as critical considerations for future basic and clinical research:

a) Different mechanisms may contribute to the distinct phenotypes observed in AMD progression. Including cases with different late-stage AMD phenotypes, such as atrophic AMD and exudative AMD, several studies may have introduced overlapping epigenetic mechanisms, potentially underestimating the role of *DNMT* expression and overall methylation levels. Nonetheless, biological evidence indicates that *DNMT1* is essential for maintaining RPE function and the integrity of the photoreceptor outer segment,⁶⁶ and recent downregulation has been associated with advanced aAMD.³⁸ A recent study that examined AMD from a biological perspective highlighted epigenetic differences that accumulate during disease progression, mainly focusing on WNT signaling, *FRZB*, and *TLE2*.⁶⁷ Clinically, the underactivation of canonical WNT signaling appears relevant to atrophic AMD, as it compromises RPE integrity. In contrast, overactivation of this pathway is associated with increased VEGF expression, promoting the angiogenesis characteristic of nAMD. Notably, increased CpG methylation of *FRZB* has been observed, correlating with higher expression in macular RPE/choroid tissues. The therapeutic potential of

epigenetic interventions has also been explored. For example, treatment with 5-Aza-2'-deoxycytidine (5-aza-dC), a DNA methylation inhibitor, was found to attenuate choroidal neovascularization by inhibiting the WNT/ β -catenin signaling pathway through promoter demethylation of the WNT antagonist Notum.⁶⁸ Similarly, another study demonstrated that 5-aza-dC treatment reduced VEGF expression, further supporting the relevance of epigenetic regulation in angiogenesis, a central process in exudative AMD.⁶⁹

b) Clinically, it should be emphasized that despite the differences between the previously discussed studies, mitochondrial dysfunction, oxidative stress, and altered methylation patterns in mitochondrial-related genes may be linked to key imaging biomarkers and distinct AMD phenotypes. The activity of the *ELOVL2* gene, which regulates the elongation of polyunsaturated fatty acids (PUFAs), has been shown to influence aging in the mouse retina, providing a molecular link between PUFA metabolism and visual function.⁴⁰ These fatty acids play a key role in maintaining the health of the RPE and photoreceptors,⁴¹ which are essential for preventing the accumulation of drusen, the primary biomarker of AMD.^{75,76} The impairment of energy pathways may also explain recent findings that highlight the vulnerability of the outer neuroretinal layers in AMD, suggesting patterns of early and progressive neurodegeneration.⁷⁷⁻⁷⁹

The presence of drusen and subretinal drusenoid deposits, both significant biomarkers of AMD, has been associated with accelerated neurodegeneration⁷⁸ alongside dysregulated cholesterol metabolism, tissue inflammation, and cellular senescence.⁸⁰

Interestingly, a non-small cell lung cancer study identified a 10-gene methylation signature associated with the clinical benefit of bevacizumab treatment, mainly through regulating VEGFA and VEGFR2 gene activity.⁸¹ This relationship between methylation signatures and the activity of VEGFA and VEGFR2 is highly relevant to late-stage AMD progression, given the critical role of these genes in angiogenesis and fibrosis regulation.^{82,83}

Based on these findings, several key directions for future research can be suggested. First, the characterization of *DNMT* expression across different stages of AMD could provide valuable insights into the epigenetic regulation of disease progression. Studies linking *DNMT* expression with retinal histology would help to clarify the relationship between methylation and neurodegeneration, particularly in the inner neural layers.⁷⁷⁻⁷⁹ Additionally, vascular characterization through choroidal imaging and analysis of vascular metrics, including neovascular membranes, could further our understanding of epigenetic influences on AMD.⁸⁴

Imaging studies could also benefit from investigating *NMT* expression concerning typical outer retinal layer alterations⁸⁵ and autofluorescence patterns, which may reflect the RPE integrity.⁸⁶ Finally, the potential for DNA methylation-based interventions should be explored by evaluating differences in *DNMT* expression across AMD stages and treatment responses, particularly in refractory cases, to assess the quality of therapeutic responses and the extent of functional improvements.

Conclusions

This review highlights how DNA methylation in genes related to oxidative stress, inflammation, and immune regulation shapes AMD's molecular landscape. Epigenetic markers such as *GSTM1*, *IL17RC*, *SKI*, and *TGFB2* may be valuable biomarkers and potential therapeutic targets. However, challenges in standardizing AMD classification across studies remain a significant barrier to advancing research in this area. Further investigations into these pathways, alongside consistent replication studies, are essential to validate current findings and pave the way for personalized interventions to modify gene expression to slow AMD progression.

Exploring variations in DNMT expression with therapeutic responses and retinal histological changes may provide additional insights into the role of epigenetic regulation in AMD. Although research on DNA methylation in AMD is limited, the available evidence suggests promising prospects for using methylation as both a prognostic marker and a therapeutic target. Using blood samples as a less invasive research source is particularly advantageous, with studies such as Wei et al. demonstrating concordance between blood and tissue methylation patterns. However, expression with therapeutic responses and retinal histological changes may provide additional insights into the role of epigenetic regulation in AMD. Although research on DNA methylation in AMD is limited, the available evidence suggests promising prospects for using methylation as both a prognostic marker and a therapeutic target. Using blood samples as a less invasive research source is particularly advantageous, with studies such as Wei et al. demonstrating concordance between blood and tissue methylation patterns. However, this approach remains a topic of debate, as some studies suggest that further validation is necessary to establish blood-based methylation as a reliable biomarker for AMD.^{47,48}

Additionally, the potential influence of intravitreal therapy on methylation profiles must be considered.⁴⁸ Anti-VEGF treatments, widely used in AMD management, may impact DNMT expression and methylation patterns. This potential bias warrants further investigation into the relationship between anti-VEGF therapy, epigenetic changes, and therapeutic outcomes. Future studies should explore differences in DNMT expression among patients undergoing anti-VEGF treatment to better elucidate its impact on methylation, therapeutic response, and disease progression. By addressing these gaps, researchers can advance our understanding of the epigenetic mechanisms underlying AMD and contribute to developing novel, personalized therapeutic strategies.

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CRediT authorship contribution statement

SG and PC designed the research study, SG performed the research, PC provided help and advice on methods and study selection, and SG, ER, BP, MB, and PC analyzed the data and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethical considerations

Protocol registration: PROSPERO CRD42024581510

The contents underlying the research text are included in the manuscript.

Declaration of Generative AI and AI-assisted technologies in the writing process

The author(s) declare that no artificial intelligence tools were used to generate or develop the original text of this manuscript.

Funding

This work was also partially funded by FCT/MCTES UIDB/05608/2020 (<https://doi.org/10.54499/UIDB/05608/2020>) and UIDP/05608/2020 (<https://doi.org/10.54499/UIDP/05608/2020>), IDI&CA grant IPL/2022/MetAllAMD_ESTeSL by H&TRC- Health & Technology Research Center, ESTeSL- Escola Superior de Tecnologia da Saúde and by Retina Institute of Lisbon (IRL).

Declaration of competing interest

The author(s) declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.oftal.2026.502521>.

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