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# Analysis of genetic insights and molecular pathways in age-related macular degeneration: A functional enrichment

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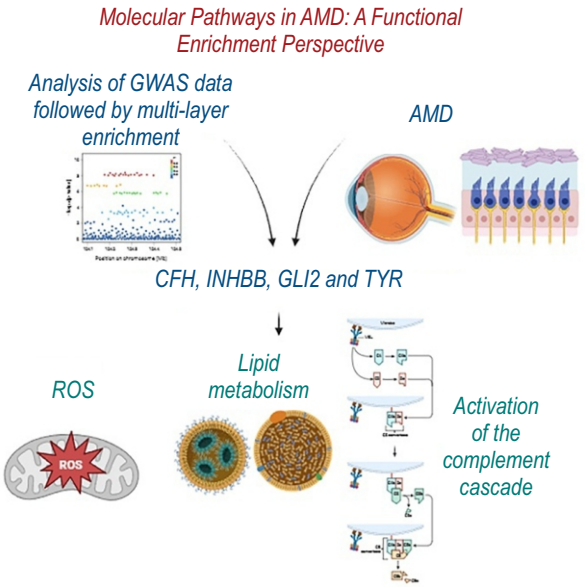
## Abstract

**Aim:** Age-related macular degeneration (AMD) is a leading cause of vision loss in older adults, with genetic factors strongly influencing onset and progression. This study aimed to analyse GWAS data to identify AMD-associated genetic variants and explore their biological and regulatory significance.

**Methodology:** Publicly available GWAS datasets were analysed using Gene Ontology (GO), KEGG, and Reactome databases to identify functional pathways. MicroRNA enrichment analysis assessed regulatory control, whilst MetaboAnalyst linked genetic variation to metabolic alterations. Unsupervised machine learning approaches, including principal component analysis (PCA), K-means, and hierarchical clustering, identified biological patterns among single nucleotide polymorphisms (SNPs).

**Results:** Strong associations were observed at known susceptibility loci, particularly CFH, with additional genes including INHBB, GLI2, and TYR implicated in immune regulation and retinal development. Enrichment analyses highlighted complement activation, lipid metabolism, and oxidative stress as key biological processes. MicroRNA analysis revealed regulators influencing inflammatory and immune pathways, whilst clustering identified three distinct SNP groups supported by hierarchical patterns.

**Interpretation:** By integrating genetic, functional and regulatory data, this study advances molecular understanding of AMD, identifying complement dysregulation, immune dysfunction, and metabolic imbalance as central contributors, alongside novel candidate biomarkers and therapeutic targets with potential implications for precision medicine in AMD management.




**Key words:** Age-related macular degeneration, Functional enrichment, Genome wide association study, Genetic variants, Molecular pathways

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## Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among the elderlies in many countries. The disease progresses through multiple stages (Flores *et al.,* 2021) . Early AMD shows retinal pigment epithelium (RPE) abnormalities with accumulation of drusen deposits between Bruch's membrane and RPE (Somasundaran *et al.,* 2020). As it advances, larger drusen (>125µm) may diminish, leaving retinal depigmentation (Murari *et al.,* 2024). The presence of reticular drusen and extensive large, soft drusen increases progression risk to advanced stages (Dutheil *et al.,* 2020). Late-stage AMD has two forms: exudative AMD and geographic atrophy (GA) (von der Emde *et al.,* 2020). Exudative AMD involves abnormal blood vessels beneath the retina, causing retinal/ RPE detachment, hemorrhage and scarring (Somasundaran *et al.,* 2020). Geographic atrophy is characterized by RPE and photoreceptor cell loss in the macula, causing loss of vision (Pfau *et al.,* 2020).

AMD development involves genetic and environmental factors. While aging is primary, smoking, high body mass index, hypertension and cardiovascular disease also contribute (Seddon *et al.,* 2020; Kuan *et al.,* 2021). Genetic predisposition is crucial in late AMD stages (Bhumika *et al.,* 2024). Twin studies have shown heritability of early AMD at 45% and late AMD at 71% (Cruickshanks *et al.,* 2017). GWAS on late AMD identified high-impact variants in *CFH, ARMS2, C2/CFB* and *C3*, with odds ratios of 1.7-4.5. Lower-impact variants have been found in *CFI, FRK/COL10A1, TNFRSF10A, LIPC, CETP, TIMP3, REST* and *VEGFA* (odds ratios 1.15-1.4). These findings highlight the role of immune responses, inflammation, lipid metabolism, angiogenesis, and other processes in late AMD (Klein *et al.,* 2013; Acar *et al.,* 2023; Hayman *et al.,* 2024). A GWAS meta-analysis of early-stage AMD (4,089 cases, 20,453 controls) (Klein *et al.,* 2013) confirmed CFH and ARMS2 associations and identified ApoE's role in early onset. Variants in GLI3, GLI2 and TYR suggested involvement in retinal regeneration and melanin biosynthesis (Brombin and Patton, 2024). Genetic risk factors for early AMD showed weaker effects than late-stage disease.

In an another study Landowski *et al.* (2019) identified a strong genetic association between a *CFH* polymorphism and AMD. A genome-wide screening of 96 cases and 50 controls revealed that individuals homozygous for a specific risk allele had a 7.4-fold increased likelihood of developing AMD. The polymorphism, located at amino acid 402 (Y402H), lies in a region of *CFH* that binds heparin and C-reactive protein, indicating a potential mechanism for its influence on AMD pathogenesis. The findings reinforce the role of *CFH* in immune system regulation and the inflammatory processes underlying AMD progression. Another study examined gene-environment interactions, analyzing how smoking affects genetic susceptibility to AMD in 1,207 cases and 686 controls (Seddon *et al.,* 2020). The study confirmed genetic associations at *CFH, ARMS2* and *CFB/C2* loci, and found a novel SNP (rs17073641) in SERPINB8-CDH7 that was protective in non-smokers but increased AMD risk in smokers. These findings indicate that environmental factors may alter genetic risk variants' effects in AMD pathogenesis. The initial genome-wide association studies (GWAS) on age-related macular degeneration (AMD) identified genetic variants by analyzing SNPs across populations (Seddon *et al.,* 2020; Osterman *et al.,* 2022; Kwong *et al.,* 2024). These studies focused on discovering risk loci, validating known associations (*CFH, ARMS2* and *C3*), and evaluating disease susceptibility through statistical association analyses. This study uses deposited GWAS data and functional enrichment analysis to understand the biological significance of genetic variants. By integrating GO analysis, KEGG pathway mapping, miRNA regulation analysis and metabolomic associations, this study explores molecular mechanisms underlying AMD, examining CFH and ARMS2's roles in complement system dysregulation, inflammatory responses, and RPE integrity.

## Materials and Methods

The study utilized publicly available genome-wide association study (GWAS) datasets with clear AMD diagnostic criteria, appropriate control groups, and robust genotyping methods were selected to ensure reliability. Only datasets with defined phenotypes, population uniformity, and stringent quality checks were included. As this is a bioinformatics analysis, participant recruitment was not applicable. Data were sourced from the GWAS Catalog. Ethical clearance was not required since the secondary data,which is publicly accessible,was used in the study. All datasets were properly referenced, and the work adhered to the Declaration of Helsinki and related ethical guidelines. GWAS data were processed to identify AMD-associated genes, which were curated and assessed for statistical robustness. Gene Ontology analysis classified these genes by biological processes, molecular functions, and cellular components relevant to retinal degeneration. Pathway enrichment using KEGG (Kanehisa *et al.,* 2017) and Reactome (Vastrik *et al.,* 2007) highlighted mechanisms such as inflammation, oxidative stress, angiogenesis, and extracellular matrix remodelling. miRNA enrichment (miRTarBase, TargetScan) revealed regulatory interactions, while MetaboAnalyst linked genetic findings to metabolic changes, suggesting potential biomarkers. Unsupervised methods, including K-means, hierarchical clustering, and principal component analysis, grouped SNPs by genomic location, allele frequency, and significance to visualise key contributors to AMD pathogenesis.

## Results and Discussion

The genetic analysis of age-related macular degeneration (AMD) highlights key chromosomal regions associated with the disease.On chromosome 1, significant variants were identified in the CFH gene, a well-established genetic risk factor for AMD. Similarly, chromosome 2 harbored risk variants near INHBB and GLI2, suggesting their potential involvement in disease susceptibility. Chromosome 4 featured an

**Table 1:** Representation of genes as reported by different GWAS studies

| REGION | CHR_ID | CHR_POS | REPORTED GENE(S) | MAPPED_GENE |
|---|---|---|---|---|
| 1q31.3 | 1 | 1.97E+08 | *CFH* | *CFH* |
| 1q31.3 | 1 | 1.97E+08 | *CFH* | *CFH* |
| 2q14.2 | 2 | 1.21E+08 | *INHBB, GLI2* | *LINC01101 - Y_RNA* |
| 2q14.2 | 2 | 1.21E+08 | *INHBB, GLI2* | *LINC01101 - Y_RNA* |
| 4q13.1 | 4 | 62476552 | *Intergenic* | *RPL21P47 - HMGN1P11* |
| 4q26 | 4 | 1.16E+08 | *Intergenic* | *KRT18P21 - EIF3KP3* |
| 5p15.2 | 5 | 9978972 | *Intergenic* | *LINC02112 - ATPSCKMT* |
| 6p21.1 | 6 | 45917324 | Intergenic | *CLIC5* |
| 6q21 | 6 | 1.06E+08 | *Intergenic* | *LINC02836 - RN7SKP211* |
| 7p14.1 | 7 | 42136683 | *GLI3* | *GLI3* |
| 8q24.21 | 8 | 1.26E+08 | *FAM84B* | *LINC00861 - RNU6-869P* |
| 8q24.21 | 8 | 1.26E+08 | *Intergenic* | *LINC00861 - RNU6-869P* |
| 10q21.1 | 10 | 52815236 | *MBL2* | *MBL2 - Y_RNA* |
| 10q26.13 | 10 | 1.22E+08 | *ARMS2, HTRA1* | *HTRA1* |
| 11q14.3 | 11 | 89157946 | *TYR* | *GRM5 - TYR* |
| 11q14.1 | 11 | 83110340 | *PCF11, RAB30* | *RAB30-DT* |
| 13q13.3 | 13 | 37491309 | *POSTN, TRPC4* | *LINC01048* |
| 13q13.3 | 13 | 37491309 | *POSTN, TRPC4* | *LINC01048* |
| 17q22 | 17 | 57031373 | *Intergenic* | *SCPEP1 - RNF126P1* |
| 19p13.3 | 19 | 3944242 | *DAPK3, ITGB1BP3* | *NMRK2 - DAPK3* |
| 19p13.3 | 19 | 3944242 | *DAPK3, ITGB1BP3* | *NMRK2 - DAPK3* |

intergenic region mapped to RPL21P47 - HMGN1P11, which may play a regulatory role (Table 1). The chromosomal distribution of AMD-associated variants shown in Table 1 reveals a disease architecture that spans multiple genomic regions, with both established and potentially novel loci contributing to susceptibility. The repeated identification of CFH on chromosome 1q31.3 across multiple GWAS records strongly validates its central role in AMD—the Y402H variant in CFH impairs the ability of complement factor H to regulate C3b-mediated opsonisation on retinal surfaces, thereby amplifying chronic complement activation (Veuskens *et al.,* 2025).

The chromosome 2 loci mapping near INHBB and GLI2 are particularly interesting. INHBB encodes inhibin B, a TGF-beta superfamily member involved in retinal cell survival signalling, while GLI2 is a transcription factor in the Hedgehog pathway with roles in photoreceptor development (Wachten and Christensen, 2025). Their appearance in AMD GWAS data points towards developmental and regenerative defects in the retina as contributors to disease susceptibility, a dimension often underappreciated in the predominantly complement-focused AMD literature. The intergenic hits on chromosomes 4, 5, 6 and 8 are likely regulatory variants whose effects on gene expression in retinal cell types require further functional validation through eQTL analyses. The GO Biological Process table summarizes the enrichment analysis performed on the gene set from your Age-related macular degeneration (AMD) GWAS. Several biological processes emerged as significantly enriched, suggesting that the risk loci for AMD are not randomly distributed but tend to cluster in specific functional pathways. Notably, terms such as Positive Regulation of T Cell Differentiation appear, with key genes like CD46 and members of the GLI family (GLI2 and GLI3) contributing to this signal (Table 2).

This table provide insights into the subcellular localizations associated with the genes implicated in AMD. The enrichment for components such as the Endopeptidase Complex and Serine-Type Endopeptidase Complex underscores the potential importance of protein degradation and processing systems in the disease. Notably, the appearance of Melanosome and Melanosome Membrane terms is especially relevant, given that melanosomes are pigment-containing organelles within the retinal pigment epithelium (RPE) (Table 3). The GO Biological Process enrichment results carry meaningful interpretive weight when read alongside the clinical phenotype of AMD. The top enriched term — Positive Regulation of T Cell Differentiation (OR = 33.76, $p < 0.05$) — implicates CD46 and the GLI family in immune cell fate decisions within the retinal microenvironment. CD46 is a complement regulatory protein that not only protects host cells from complement damage but also serves as a co-stimulatory molecule in T cell activation (Alibrandi *et al.,* 2025); its enrichment here directly bridges complement biology and adaptive immune dysregulation in AMD.

The Smoothened Signalling Pathway and its negative regulator terms confirm that Hedgehog pathway activity, mediated through GLI2 and GLI3, is a relevant biological process in AMD—a finding that aligns with the emerging literature on Hedgehog-mediated RPE survival and photoreceptor neuroprotection. The Maintenance of Blood-Brain Barrier term (ITGB1, MBL2) is particularly noteworthy in the retinal context, as the inner blood-retinal barrier shares functional and structural

**Table 2:** GO Biological Process Enrichment. The table lists each term with the number of overlapping genes (Overlap), statistical significance (P-value and Adjusted P-value), the strength of association (Odds Ratio), the combined enrichment score (Combined Score), and the corresponding genes

| Term | Overlap | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|
| Positive regulation of T Cell differentiation (GO:0045582) | 3/64 | p<0.05 | 0.07079 | 33.75975 | 298.38067 | *CD46; GLI3; GLI2* |
| Embryonic digestive tract development (GO:0048566) | 2/19 | p<0.05 | 0.08979 | 78.23922 | 608.90186 | *GLI3; GLI2* |
| Negative regulation of smoothened signalling pathway (GO:0045879) | 2/25 | p<0.05 | 0.08979 | 57.81159 | 417.77181 | *GLI3; GLI2* |
| Maintenance of blood-brain barrier (GO:0035633) | 2/29 | p<0.05 | 0.08979 | 49.23704 | 341.10718 | *ITGB1; MBL2* |
| Smoothened signalling pathway (GO:0007224) | 2/34 | p<0.05 | 0.08979 | 41.53333 | 274.51366 | *GLI3; GLI2* |

**Table 3:** GO Cellular Component Enrichment. Term' refers to the functional category or pathway. 'Overlap' indicates the number of genes shared between the dataset and the term. 'P-value' represents the significance of enrichment, while 'Adjusted P-value' accounts for multiple testing. 'Odds Ratio' quantifies the likelihood of gene overlap compared to random expectation. 'Combined Score' integrates the P-value and odds ratio. 'Genes' lists all genes contributing to the enrichment

| Term | Overlap | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|
| Endopeptidase complex (GO:1905369) | 2/14 | p<0.05 | 0.01583 | 110.86667 | 932.20884 | *CFH; MBL2* |
| Melanosome (GO:0042470) | 2/31 | p<0.05 | 0.03977 | 45.83678 | 311.42283 | *APOE; TYR* |
| Multivesicular body (GO:0005771) | 2/50 | p<0.05 | 0.06854 | 27.66667 | 161.69651 | *APOE; MBL2* |
| Low-Density Lipoprotein particle (GO:0034362) | 1/6 | p<0.05 | 0.12040 | 128.79355 | 598.87424 | *APOE* |
| Chylomicron (GO:0042627) | 1/8 | p<0.05 | 0.12040 | 91.98618 | 401.40404 | *APOE* |

characteristics with the blood-brain barrier, and its disruption is an early and critical event in AMD-associated retinal oedema and neovascularisation (Veuskens *et al.,* 2025).

The GO Molecular Function analysis demonstrated the biochemical activities of AMD-associated gene proteins. The table reveals enrichment for complement system functions, including Complement Component C3b Binding, consistent with complement dysregulation's role in AMD pathogenesis through retinal inflammation. The data also showed enrichment for Oxidoreductase Activity functions critical in managing cellular oxidative stress—a known AMD factor (Table 4). The GO Molecular Function enrichment results directly link the biochemical activities of AMD genes to known disease mechanisms. The highest enrichment for Complement Component C3b Binding (OR = 166.33, p < 0.05) driven by CFHR4 and CFHR5 is mechanistically critical — complement factor H-related proteins 4 and 5 compete with CFH for C3b binding on retinal surfaces, and their overrepresentation in AMD GWAS data suggests that dysregulation of C3b clearance in the retina is a primary driver of the chronic inflammatory milieu in which drusen form and expand. The Oxidoreductase Activity enrichment via TYR (tyrosinase) connects AMD to melanin biosynthesis and oxidative metabolism in RPE melanosomes — melanin granules serve as antioxidant reservoirs in RPE cells, and their progressive loss with ageing contributes to oxidative stress-mediated photoreceptor damage (Kaufmann and Han, 2024). The Phosphatidylcholine-Sterol O-acyltransferase Activator Activity term (APOE) links lipid esterification to AMD, consistent with the well-established role of APOE isoforms in modulating drusen lipid composition and retinal lipoprotein metabolism. The KEGG pathways table provides a systems-level analysis of molecular networks perturbed in age - related macular degeneration. The most significant enrichment was observed for Complement and coagulation cascades, with a highly significant P-value (4.08E–9). This reinforces the complement system's central role in AMD pathogenesis, as its dysregulation contributes to chronic inflammation and retinal degeneration. Other immune-related KEGG pathways like *Staphylococcus aureus* infection appeared enriched, sharing common inflammatory mediators with AMD (Table 5).

The KEGG enrichment results provide the strongest pathway-level signal in this entire analysis. The Complement and Coagulation Cascades pathway ($p = 4.08\times10^{-9}$, OR = 58.10) with six contributing genes — CFH, CFHR4, CFHR5, CD46, CFB, MBL2—represents a remarkably concentrated genetic signal around a single molecular system, underscoring that complement

**Table 4:** GO Molecular Function Enrichment. Gene enrichment statistics. Each term is accompanied by the number of overlapping genes (Overlap), statistical significance (P-value), multiple-testing corrected significance (Adjusted P-value), effect size (Odds Ratio), combined enrichment metric (Combined Score), and the list of genes involved

| Term | Overlap | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|
| Complement C3b Binding (GO:0001851) | 2/10 | p<0.05 | 0.00775 | 166.33333 | 1515.06076 | *CFHR4; CFHR5* |
| Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, another compound as one donor, and incorporation of one atom of oxygen (GO:0016716) | 1/5 | p<0.05 | 0.11122 | 161.0 | 777.85948 | *TYR* |
| Phosphatidylcholine-Sterol O-acyltransferase Activator Activity (GO:0060228) | 1/5 | p<0.05 | 0.11122 | 161.0 | 777.85948 | *APOE* |
| Cell-Matrix adhesion mediator activity (GO:0098634) | 1/6 | p<0.05 | 0.11122 | 128.79355 | 598.87448 | *ITGB1* |
| Leucine zipper domain binding (GO:0043522) | 1/8 | p<0.05 | 0.11122 | 91.98618 | 401.40404 | *DAPK3* |

**Table 5:** KEGG Pathways Enrichment. Enriched terms and associated genes. The table shows: Term name, number of overlapping genes (Overlap), significance values (P-value and Adjusted P-value), Odds Ratio, Combined Score, and the genes contributing to each term

| Term | Overlap | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|---|
| Complement and coagulation cascades | 6/85 | p<0.05 | 2.94098E-7 | 58.09834 | 1122.22871 | *CFH; CFHR4; CFHR5; Cd46; CFB; MBL2* |
| Staphylococcus aureus infection | 3/95 | p<0.05 | 0.01677513 | 22.34933 | 171.45010 | *CFH; CFB; MBL2* |
| Hedgehog signalling pathway | 2/56 | p<0.05 | 0.08203597 | 24.58519 | 138.20773 | *GLI3; GLI2* |
| Basal cell carcinoma | 2/63 | p<0.05 | 0.08203597 | 21.75628 | 117.28746 | *GLI3; GLI2* |
| Pathways in cancer | 4/531 | p<0.05 | 0.14114849 | 5.26999 | 24.37461 | *ITGB1; DAPK3; GLI3; GLI2* |



dysregulation is the dominant molecular theme in AMD pathogenesis. What is noteworthy here is the co-enrichment of Staphylococcus aureus infection pathway, which shares complement and opsonisation components with AMD (Wonfor *et al.,* 2022); this is not a coincidence but rather reflects the shared use of complement and coagulation proteins across innate immune defence and sterile retinal inflammation. The Hedgehog Signalling Pathway (GLI3, GLI2; OR = 24.59) and Basal Cell Carcinoma pathway enrichments are mechanistically coherent — both rely on GLI-mediated transcription and hint at shared molecular machinery between retinal developmental circuits and epithelial carcinogenesis, a connection that has precedent in studies linking AMD and some ocular malignancies. Pathways in Cancer enrichment (ITGB1, DAPK3, GLI3, GLI2) further reinforces the cell survival and proliferation dysregulation angle in AMD. The Reactome pathways enrichment data complements KEGG results using curated pathways from the Reactome database, which provides detailed expert-curated biological processes. The top pathway Complement Cascade shows high significance, a recurring theme in AMD research. Multiple complement-related genes—*CFH, CFHR4, CFHR5, CD46, CFB* and MBL2—highlight complement activation's critical role in disease progression. This finding is compelling as the complement system mediates chronic inflammation in the retina, contributing to tissue damage and drusen formation.

The HMDB metabolites enrichment data—although much smaller in this dataset—offers a window into the metabolic alterations associated with AMD risk. In this particular analysis, the only significantly enriched metabolite identified was I3P (HMDB01498). The detection of I3P with a noteworthy odds ratio suggests that the AMD-associated gene set may influence, or be influenced by, a specific metabolic pathway in which I3P plays a key role. The identification of I3P (indole-3-phosphate; HMDB01498) as the sole significantly enriched metabolite is an intriguing finding that warrants interpretation. Indole-related metabolites are connected to the kynurenine pathway of tryptophan metabolism, which has established links to oxidative stress, mitochondrial dysfunction, and inflammatory cytokine regulation — all processes known to be dysregulated in AMD retinal tissue (Lu *et al.,* 2025; Wang *et al.,* 2025). While a single metabolite enrichment should be interpreted cautiously given the

small gene set, the connection between AMD-susceptibility genes and tryptophan/indole metabolic pathways suggests that RPE metabolic reprogramming extends into amino acid catabolism. This is consistent with published metabolomics studies in AMD patients showing altered amino acid profiles in plasma and vitreous samples. The limited metabolite signal also reflects a known constraint of GWAS-driven metabolomics — the gene set captures primarily genetic susceptibility loci rather than all pathophysiologically active genes, and broader untargeted metabolomics in AMD patient tissue would likely reveal a much richer metabolic perturbation landscape.

The miRTarBase table compiles experimentally validated microRNA–target interactions for the genes derived from the AMD GWAS analysis. miRTarBase is a curated resource that documents interactions confirmed through experimental methods such as reporter assays, Western blot or qRT-PCR, lending high confidence to the regulatory relationships listed. In this dataset, several microRNAs were noted (for example, hsa-miR-6853-3p, hsa-miR-29c-3p, hsa-miR-93-5p, among others) along with their corresponding target genes. Target Scan predicts microRNA-target interactions based on sequence complementarity and evolutionary conservation, unlike miRTarBase which uses experimental evidence. In this dataset, human microRNAs like hsa-miR-1973, hsa-miR-1469, hsa-miR-662 were predicted to target genes from the AMD GWAS data (Rooda *et al.,* 2020). Many predicted targets overlapped with miRTarBase findings, reinforcing key regulatory nodes, with genes like *ITGB1, APOE* and *GLI* family members appearing as targets. This overlap between predicted and experimental datasets strengthens evidence for these microRNAs' roles in AMD. The predicted interactions suggest microRNA regulation could influence complement activation, lipid metabolism, cell adhesion, and developmental signaling—all relevant to AMD pathophysiology.

The convergence of miRTarBase experimental evidence and TargetScan predictions on a common set of AMD-related target genes substantially strengthens the case for miRNA-mediated regulation as a functional layer in AMD pathogenesis. hsa-miR-29c-3p targeting ITGB1 and RAB30 is particularly meaningful — miR-29c is a known regulator of extracellular matrix remodelling and fibrosis, and its suppression of ITGB1 (integrin beta-1) would impair RPE-Bruch's membrane adhesion, a defect directly relevant to drusen biogenesis and RPE detachment in AMD (Hammadi *et al.,* 2023). hsa-miR-93-5p is a member of the miR-17~92 cluster widely implicated in angiogenesis regulation — its presence in this AMD-linked miRNA set raises the possibility that genetic variants affecting miR-93-5p target sites could influence neovascular AMD progression, a hypothesis worth testing in wet AMD cohorts. The targeting of APOE and GLI family members by multiple miRNAs creates a regulatory convergence point where post-transcriptional control intersects with lipid metabolism and developmental signalling simultaneously, suggesting that miRNA dysregulation in AMD is not random, but operates through coherent biological modules. Age-related macular degeneration (AMD) shows marked genetic complexity, involving known and novel loci influencing disease progression (Ding *et al.,* 2017; Bhumika *et al.,* 2024). Gene Ontology enrichment highlights immune regulation, notably T-cell fate mechanisms, underscoring inflammation's critical role (Zhang *et al.,* 2014). Developmental terms such as Embryonic Digestive Tract Development and Negative Regulation of Smoothened Signalling Pathway suggest overlap between developmental networks and retinal disease. Smoothened-mediated Hedgehog signalling indicates potential developmental cues in adult retina (Le *et al.,* 2021). Enrichment for Negative Regulation of Protein Metabolic Process and Gene Expression points to altered protein turnover and transcriptional control contributing to degeneration.

Retinal pigment epithelium (RPE) maintains photoreceptors and homeostasis; pigment granule abnormalities and lipoprotein enrichment link AMD to lipid metabolism, consistent with drusen deposition (Somasundaran *et al.,* 2020; Maurya *et al.,* 2023). Cellular component enrichment—Multivesicular Body, endoplasmic reticulum, and Golgi apparatus—indicates intracellular trafficking defects influencing protein and lipid handling (Gallo *et al.,* 2016). Other enriched activities include Phosphatidylcholine-Sterol O-acyltransferase Activator, cell–matrix adhesion mediators, and cadherin binding, implicating lipid modification, matrix interactions, and transcriptional regulation. KEGG analysis reveals Hedgehog signalling, Basal cell carcinoma, cell adhesion molecules, phagosome, axon guidance, and cholesterol metabolism pathways, integrating immune dysfunction, developmental signalling, and lipid dysregulation. Reactome shows complement cascade disruption, GLI-mediated transcription, RUNX2 regulation, and fibronectin matrix formation, supporting immune and extracellular matrix alterations. I3P enrichment suggests oxidative and metabolic stress relevant to AMD pathology.

MicroRNA interactions indicate post-transcriptional regulation: hsa-miR-29c-3p targets ITGB1 and RAB30, linking integrin signalling with inflammation; *GLI3, GLI2* and *APOE* connect developmental and lipid pathways. miRTarBase and TargetScan predict redundant microRNA control, highlighting biomarker potential.Key variants in *CFH, ARMS2, C2/CFB* and *C3* confirm complement dysregulation. Additional signals in *INHBB, GLI2* and *TYR* implicate immune modulation, retinal development and melanin biosynthesis. SNPs on Chromosomes 1 and 10 show strong associations ($p<0.05$, effect sizes 1.39–2.38). Despite clear clustering, regression shows low explanatory power ($R^2 = 0.11$), supporting AMD as a polygenic disorder requiring multi-SNP models. However, there are few limitations of the study. Bioinformatics analysis was performed on publicly available datasets, and population-specific variations or ethnic differences were not considered. The findings presented are preliminary and require further experimental validation to confirm their biological relevance. Additionally, enrichment and pathway analyses should be conducted in future studies to provide a more comprehensive understanding of the underlying molecular mechanisms.

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