• Clinical Research •

Causal role of oxidative stress in age-related macular degeneration: a bidirectional Mendelian randomization study

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Abstract

● **AIM:** To elucidate causal pathways between oxidative biomarkers and age-related macular degeneration (AMD) phenotypes.

● **METHODS:** A bidirectional Mendelian randomization (MR) analytical protocol was implemented, which utilized genome-wide association study (GWAS) summary statistics derived from the IEU OpenGWAS repositories. The investigation focused on 11 oxidative stress markers and AMD phenotypes, encompassing both wet and dry subtypes. The MR methodology incorporated inverse-variance weighted (IVW) calculations, MR-Egger statistical regression, weighted median approximation, and weighted mode assessments to estimate causative relationships. Sensitivity evaluations were conducted to verify result robustness and identify potential pleiotropy.

● **RESULTS:** Genetically predicted elevated catalase (CAT) concentrations demonstrated significant associations with heightened risks of overall AMD (IVW OR=1.084, 95%CI: 1.021-1.151, *P*=0.008) and wet AMD phenotype (IVW OR=1.113, 95%CI: 1.047-1.247, *P*=0.007). Higher genetically predicted albumin concentrations corresponded with reduced AMD risk (IVW OR=0.827, 95%CI: 0.715-0.957, *P*=0.013) but increased wet AMD risk (IVW OR=1.229, 95%CI: 1.036-1.458, *P*=0.018). Reverse MR analysis revealed that genetically predicted dry AMD exhibited significant association with reduced albumin levels (IVW OR=0.987, 95%CI: 0.979-0.996, *P*=0.004), while wet AMD corresponded with decreased total bilirubin (TBIL) and paraoxonase (PON) activity.

● **CONCLUSION:** The results offer strong support for a causal link between markers of oxidative stress and the development of AMD, indicating that oxidative processes play a role in driving the disease progression.

● **KEYWORDS:** age-related macular degeneration; oxidative stress; Mendelian randomization; antioxidant therapy; genetic epidemiology; retinal degeneration

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INTRODUCTION

Age-related macular degeneration (AMD) stands as a major contributor to vision loss worldwide, especially common in developed nations[1]. According to the World Health Organization, it is estimated that around 288 million people globally will face vision impairment due to AMD by 2040[2]. AMD constitutes a progressive chronic condition affecting the central retinal structures, with primary pathogenic mechanisms including age-related changes, lipofuscin accumulation in retinal pigment epithelial cells, choroidal ischemic complications, and oxidative damage[3].

Evidence strongly indicates interconnections between systemic oxidative processes and age-related macular deterioration[4]. Research demonstrates that individuals with wet AMD exhibit

enhanced oxidative stress markers such as high mobility group box 1 protein (HMGB-1), 3-nitrotyrosine, and aggregate oxidative status, indicating substantial tissue deterioration and inflammatory activation[5]. Alterations in antioxidant enzyme activity are evident in AMD patients, including decreased levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) compared to those in healthy individuals[6]. The Y402H polymorphic variant in complement factor H (CFH) intensifies oxidative damage by increasing inflammatory susceptibility. Previous research indicates that exogenous CFH can prevent cellular damage and death in adult retinal pigment epithelium cell line-19 (ARPE-19) ocular cells carrying the at-risk *CFH Y402H* variant under oxidative stress conditions[7]. Accumulation of oxidatively modified compounds in drusen deposits is a hallmark of AMD. Dietary antioxidants, including glutathione (GSH), may slow disease progression by scavenging free radicals. Notably, combining GSH with lutein significantly improves contrast sensitivity in AMD patients compared to lutein alone[8]. While these observations suggest connections between oxidative mechanisms and AMD development, the precise nature of this relationship remains incompletely characterized.

Recognizing the impact of oxidative stress in AMD, researchers have proposed various interventions. The age-related eye disease study (AREDS) revealed that dietary supplementation with antioxidants and zinc can effectively slow AMD progression, especially in the intermediate phase[9]. For instance, a 2023 Cochrane review indicated that although the AREDS formulation, consisting of vitamins C and E, beta-carotene, and zinc, may help delay the progression of intermediate AMD, there is insufficient evidence supporting its role in preventing disease onset[10]. Recent Meta-analyses and critiques have highlighted mixed outcomes regarding antioxidant treatments[11]. Furthermore, one study criticized the AREDS analysis, suggesting that it might have overstated benefits and pointing out limitations in its design and interpretation[12]. The applicability of AREDS results to diverse populations and the risks of adverse effects from excessive antioxidant intake also remain concerns[13-14].

Despite the growing body of evidence suggesting a link between oxidative stress and AMD, no studies have yet explored the causal relationship between these factors. Observational studies in this field are often limited by confounding factors and the potential for reverse causation, making it difficult to establish true causality.

To overcome these challenges and improve causal inference, we utilized Mendelian randomization (MR), which employs genetic markers as proxies to assess causal associations between risk factors and disease progression[15]. MR offers particular advantages for investigating oxidative stress-AMD relationships by leveraging natural genetic randomization to minimize biases inherent in traditional observational research. MR has been successfully implemented across various medical disciplines, including cardiovascular and neurological research, to identify potential therapeutic targets and elucidate disease mechanisms[16-17]. By applying this methodology to examine oxidative stress and AMD, we aim to address the limitations of observational studies and provide new perspectives on the potential causative relationship between these factors.

Utilizing a two-sample bidirectional MR approach and comprehensive GWAS data, this study explores whether genetically inferred oxidative stress biomarker levels contribute to AMD risk and subtypes, while also assessing whether genetic predisposition to AMD impacts oxidative stress markers. This comprehensive bidirectional analysis seeks to establish causative relationships that could inform targeted therapeutic strategies and enhance our understanding of AMD pathophysiology.

PARTICIPANTS AND METHODS

Ethical Approval This investigation utilized exclusively published or publicly accessible summary-level statistics from the IEU OpenGWAS and FinnGen consortia. The original studies secured ethical approval and informed consent, and all data used in this study were de-identified. The study protocol for this secondary analysis was reviewed and approved by The Medical Ethics Committees of Tianjin Eye Hospital: KY-2025023.

Assumptions and Study Design of MR To explore causal relationships between oxidative stress biomarkers (OSIBs) and AMD, a bidirectional two-sample MR framework was utilized. This approach is based on three core assumptions: 1) single nucleotide polymorphism (SNPs) serving as instrumental variables (IVs) must exhibit a strong association with the exposure; 2) these IVs should remain unaffected by confounders influencing both exposure and outcome; 3) IVs must impact the outcome solely through the exposure pathway[18] (Figure 1). Causal effects were estimated using multiple MR techniques, with inverse-variance weighted (IVW) analysis under random effects as the primary method, assuming no horizontal pleiotropy. Additionally, MR-Egger was applied for pleiotropy detection and adjustment[19], while weighted median estimation provided robustness even when up to 50% of IVs were invalid[20]. MR-PRESSO methodology was utilized to identify and correct outlier SNPs that might distort analytical outcomes[21]. Sensitivity analyses, including MR-Egger, MR-PRESSO, Cochran's *Q*, Rucker's *Q*, and leave-one-out assessments, were conducted to verify result reliability[22]. In this context, horizontal pleiotropy describes situations where genetic variants influence outcome variables through mechanisms independent of their effect on the

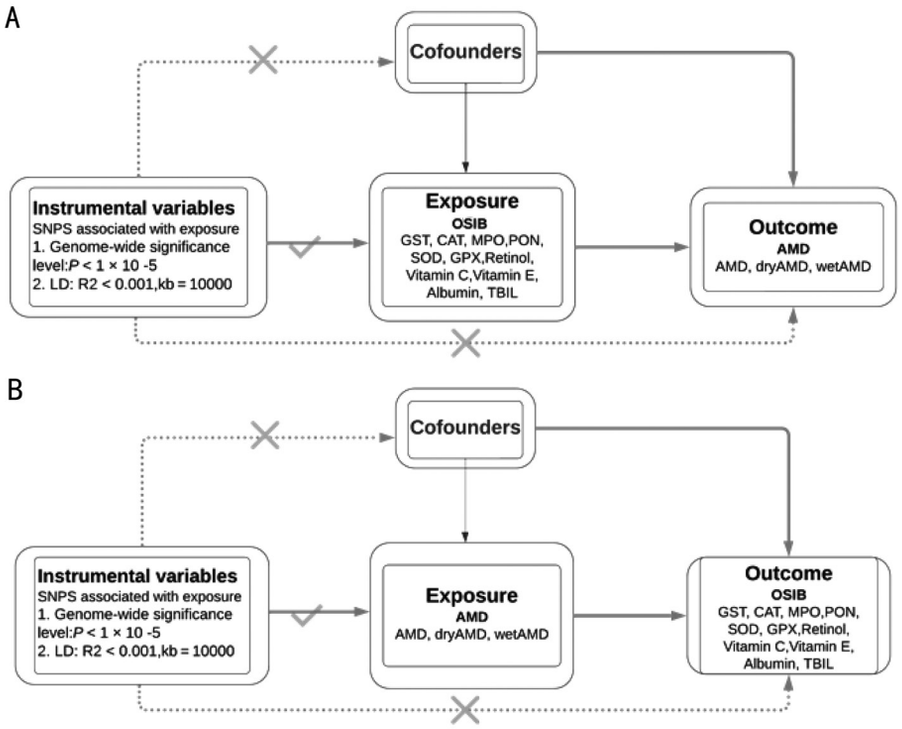


Figure 1 Bidirectional MR methodology and key assumptions MR: Mendelian randomization; OSIB: Oxidative stress biomarker; GST: Glutathione S-transferase; CAT: Catalase; MPO: Myeloperoxidase; PON: Paraoxonase; SOD: Superoxide dismutase; GPX: Glutathione peroxidase; TBIL: Total bilirubin; AMD: Age-related macular degeneration; LD: Linkage disequilibrium.

exposure variable. The F-statistic, calculated as the squared *t*-statistic from exposure regression, evaluates the association strength between the genetic variant and exposure.

Exposure Sources The IEU OpenGWAS repository provided data on oxidative stress biomarkers (OSIBs), with 11 key biomarkers selected based on their roles in oxidative stress pathways and the availability of comprehensive GWAS data. These included glutathione S-transferase (GST), CAT, myeloperoxidase (MPO), paraoxonase (PON), SOD, GPX, retinol, vitamin C, vitamin E, albumin, and total bilirubin (TBIL). Sample sizes spanned from 1322 to 342 829 participants. To control for demographic variability, only European ancestry individuals were considered.

Outcome Sources The FinnGen consortium supplied AMD summary statistics, covering 298 486 to 4 191 981 participants of European ancestry. This dataset offered strong statistical power for identifying genetic associations. Additional GWAS details, such as sample size and population characteristics, appear in Table 1.

Statistical Analysis We implemented multiple rigorous statistical methodologies to investigate causative connections between OSIBs and AMD. These included IVW (random effects) calculations, MR-Egger regression analysis, weighted median approximation, weighted mode assessment, and MR-PRESSO statistical evaluation. In situations where horizontal pleiotropy is absent, IVW results are considered unbiased. MR-Egger regression, which accommodates pleiotropy across all analyzed SNPs, follows the Instrument Strength Independent of Direct Effect assumption, providing more reliable outcomes when pleiotropy exists[23]. The weighted median approach provides stable estimates even if up to 50% of IV weight is derived from invalid instruments, reducing Type I error compared to IVW and complementing MR-Egger analysis. MR-PRESSO corrects horizontal pleiotropy by identifying and removing outliers, reassessing causal estimates pre- and post-exclusion[21]. Sensitivity analyses were conducted to check MR bias, with pleiotropy tested using the MR-Egger intercept test and MR-PRESSO global assessment (P<0.05 indicating significance). Heterogeneity was analyzed *via* I^2 statistics, Cochran's Q for IVW, and Rucker's Q for MR-Egger, where I^2<50% or P>0.05 suggested no notable heterogeneity. Leave-one-out analysis determined SNP influence on IVW estimates. Only SNPs with F-statistics above 10 (F>10) were considered to ensure strong IV-exposure associations. The sample populations for OSIBs varied substantially, from 1322 to 342 829 participants, while AMD sample populations ranged from 298 486 to 4 191 981. This variability may affect estimation precision and MR analysis reliability, with larger sample populations providing more robust outcomes. To minimize weak instrument bias, we ensured all selected SNPs demonstrated F-statistics greater than 10; however, OSIBs with smaller sample populations may have limited statistical power, potentially increasing bias risk, and results should be interpreted cautiously pending replication

Table 1 Characteristics of GWAS Datasets for enzymatic and non-enzymatic oxidative stress markers

Biomarker	Source ID	Cohort size	Publication year	Citation
Enzymatic markers				
GST	prot-a-1283	3301	2018	[26]
CAT	prot-a-367	3301	2018	[26]
SOD	prot-a-2800	3301	2018	[26]
GPX	prot-a-1265	3301	2018	[26]
MPO	ebi-a-GCST90012031	21758	2020	[27]
PON	ebi-a-GCST90010252	1322	2020	[28]
Non-enzymatic markers				
Retinol	ukb-b-17406	62991	2018	UKB
Vitamin C	ukb-b-19390	64979	2018	UKB
Vitamin E	ukb-b-6888	64979	2018	UKB
Albumin	met-d-Albumin	115060	2018	UKB
TBIL	ukb-d-30840_raw	342829	2018	UKB

GST: Glutathione S-transferase; CAT: Catalase; SOD: Superoxide dismutase; GPX: Glutathione peroxidase; MPO: Myeloperoxidase; PON: Paraoxonase; TBIL: Total bilirubin; UKB: UK Biobank.

Table 2 Summary of AMD phenotype cohorts from FinnGen Database

Phenotype	Dataset ID	Cases/controls	Year
Degeneration (whether dry or wet)	finngen_R10_H7_AMD	11023/4191981	2019
Wet age-related macular degeneration	finngen_R10_WET_AMD	5890/300152	2019
Dry age-related macular degeneration (includes geographic atrophy)	finngen_R10_DRY_AMD	7589/298486	2019

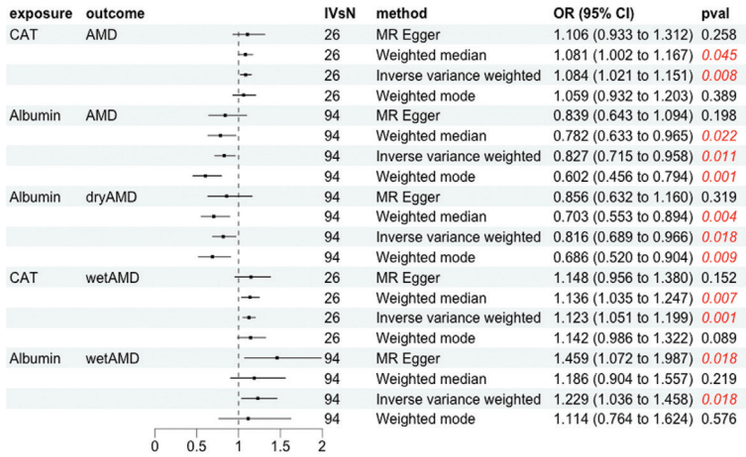
AMD: Age-related macular degeneration.

in larger datasets. For genetic variants demonstrating robust exposure associations (significance threshold: $P<1\times10^{-5}$), we implemented linkage equilibrium assurance through clumping methodology (parameters: R^2 threshold 0.001, genomic distance 10 Mb). Statistical procedures were executed *via* R computational environment (v4.2.2) employing specialized genetic analysis extensions—TwoSampleMR package (v0.5.6) and MR-PRESSO suite (v1.0)[24-25]. The investigation defined statistical significance as $P<0.05$ when evaluating potential causal connections between redox biomarkers and macular pathology (Table 2).

RESULTS

Causal Effect of Genetically Predicted OSIB on AMD The bidirectional genetic instrumental examination demonstrated causal associations between genetically-determined oxidative indicators and macular degeneration phenotypes, as depicted in Figure 2. The findings demonstrated that genetically predicted CAT concentrations exhibited significant associations with increased risk of AMD [IVW random effects, odds ratio (OR)=1.084, 95% confidence interval (CI): 1.021-1.151, P=0.008] and significantly corresponded with elevated risk of wet AMD (IVW random effects, OR=1.113, 95%CI: 1.047-1.247, P=0.007). Additionally, genetically predicted albumin levels were significantly associated with reduced AMD risk (IVW random effects, OR=0.827, 95%CI: 0.715-0.957, P=0.013) but significantly associated with increased wet AMD risk (IVW random effects, OR=1.229, 95%CI: 1.036-1.458, P=0.018). Finally, genetically predicted albumin levels were significantly associated with decreased dry AMD risk (IVW random effects, OR=0.816, 95%CI: 0.689-0.966, P=0.018). Comprehensive analysis results indicate a robust causative relationship between the examined OSIBs and AMD with its subtypes. The MR-Egger intercept did not significantly deviate from zero ($P>0.05$), suggesting absence of pleiotropy or heterogeneity. Notably, the MR-PRESSO test identified only one outlier SNP (rs34284056, *ZCCCHC2*) in the analysis of albumin and dry AMD among the various OSIB and AMD groupings. However, outlier correction demonstrated that removing this SNP did not significantly alter the estimates or significance values, further validating the robustness of the results. Additionally, leave-one-out analysis of individual SNPs demonstrated that the MR results remained stable, confirming the reliability and stability of the findings (Figure 3). Overall, despite minor heterogeneity in some instances, these analytical results strongly support a robust causative relationship between the studied OSIBs and AMD and its subtypes, without compromising the overall reliability of the conclusions.

Causal Effect of Genetically Predicted AMD on OSIB Examining bidirectional relationships through reverse genetic instrumental analysis revealed several significant associations. Atrophic AMD genetic determinants demonstrated substantial influence on albumin metabolism (IVW random effects, OR=0.987, 95%CI: 0.979-0.996, P=0.004), establishing reduced serum levels. The analysis of neovascular AMD genetic architecture showed multiple downstream effects on oxidative pathways: mild suppression of albumin synthesis



exposure	outcome	IVsN	method	OR (95% CI)	pval
CAT	AMD	26	MR Egger	1.106 (0.933 to 1.312)	0.258
		26	Weighted median	1.081 (1.002 to 1.167)	*0.045*
		26	Inverse variance weighted	1.084 (1.021 to 1.151)	*0.008*
		26	Weighted mode	1.059 (0.932 to 1.203)	0.389
Albumin	AMD	94	MR Egger	0.839 (0.643 to 1.094)	0.198
		94	Weighted median	0.782 (0.633 to 0.965)	*0.022*
		94	Inverse variance weighted	0.827 (0.715 to 0.958)	*0.011*
		94	Weighted mode	0.602 (0.456 to 0.794)	*0.001*
Albumin	dryAMD	94	MR Egger	0.856 (0.632 to 1.160)	0.319
		94	Weighted median	0.703 (0.553 to 0.894)	*0.004*
		94	Inverse variance weighted	0.816 (0.689 to 0.966)	*0.018*
		94	Weighted mode	0.686 (0.520 to 0.904)	*0.009*
CAT	wetAMD	26	MR Egger	1.148 (0.956 to 1.380)	0.152
		26	Weighted median	1.136 (1.035 to 1.247)	*0.007*
		26	Inverse variance weighted	1.123 (1.051 to 1.199)	*0.001*
		26	Weighted mode	1.142 (0.986 to 1.322)	0.089
Albumin	wetAMD	94	MR Egger	1.459 (1.072 to 1.987)	*0.018*
		94	Weighted median	1.186 (0.904 to 1.557)	0.219
		94	Inverse variance weighted	1.229 (1.036 to 1.458)	*0.018*
		94	Weighted mode	1.114 (0.764 to 1.624)	0.576

Figure 2 Associations between genetically predicted OSIB and AMD IVW: Inverse-variance weighted; OSIBs: Oxidative stress biomarkers; AMD: Age-related macular degeneration; CAT: Catalase.

(IVW random effects, OR=0.991, 95%CI: 0.982-1.001, *P*=0.048), marked reduction in TBIL concentration (IVW random effects, OR=0.976, 95%CI: 0.955-0.998, *P*=0.036), and pronounced inhibition of PON enzymatic activity (IVW random effects, OR=0.902, 95%CI: 0.841-0.968, *P*=0.004). These relationships are visualized in Figure 4, illustrating how macular pathology genetically influences systemic redox parameters.

The robustness of our findings was confirmed through comprehensive sensitivity analyses. Pleiotropy testing revealed no significant evidence (*P*>0.05), and advanced MR techniques such as MR-PRESSO were employed to detect horizontal pleiotropy and evaluate result sensitivity to potential outlier SNPs. We identified several potential outliers: rs28645199 and rs72765510 (*PXDN*) for wet AMD and albumin, along with rs241425, rs3775221 (*AFF1*), and rs429358 (*APOE*) for wet AMD and TBIL. Importantly, MR-PRESSO outlier correction demonstrated that excluding these SNPs did not substantially alter estimates or significance values. Additionally, leave-one-out analysis verified that individual SNPs had minimal influence on MR results, supporting the causal relationship between AMD and OSIB (Figure 5).

DISCUSSION

Using bidirectional two-sample MR, this study examines the causal link between OSIBs and AMD. Our findings demonstrate significant associations between genetically predicted OSIBs and AMD, offering insights into underlying disease mechanisms and potential therapeutic targets.

Our analysis demonstrated that elevated genetically predicted CAT levels significantly increase AMD risk. Higher CAT levels were associated with 8.4% increased odds of general AMD and 11.3% increased odds of wet AMD specifically. This finding appears paradoxical, as previous studies have shown that AMD patients exhibit significantly lower actual CAT activity, suggesting insufficient antioxidant defense as a contributor to AMD development[29-30]. This discrepancy may reflect a compensatory mechanism where genetically elevated CAT levels attempt to mitigate heightened oxidative stress, yet fail to prevent disease progression under persistent insult, as evidenced by reduced enzymatic activity in AMD patients. This highlights the complex interplay between genetic predisposition, functional enzyme capacity, and environmental influences.

Higher genetically predicted albumin levels were associated with decreased dry AMD risk, highlighting the complex relationship between oxidative stress and AMD. Albumin, known for its antioxidant properties[4], demonstrates the multifaceted nature of oxidative mechanisms in AMD pathogenesis. This protective effect aligns with findings linking higher C-reactive protein levels, a marker of inflammation, to increased AMD risk[31-32]. However, contradicting this trend, higher albumin levels were unexpectedly associated with an increased risk of wet AMD. This dichotomy likely stems from the distinct oxidative stress dynamics and inflammatory responses characterizing different AMD subtypes. For instance, research has identified differentially expressed proteins in neovascular AMD, including those involved in oxidative stress and inflammation, which could explain the increased risk observed with higher albumin[33]. The inverse correlation between diabetic retinopathy (DR) severity and AMD presence, marked by elevated vitreous retinol-binding protein 3 to vascular endothelial growth factor (VEGF) ratios in AMD patients with milder DR, suggests distinct molecular mechanisms[34]. While albumin may protect against oxidative stress in dry AMD, it might simultaneously aggravate pathological neovascularization in wet AMD through VEGF and inflammatory mediator pathways.

Our reverse MR analysis, investigating the genetically predicted effects of AMD on OSIBs, further emphasized the critical roles of oxidative stress and chronic inflammation in AMD

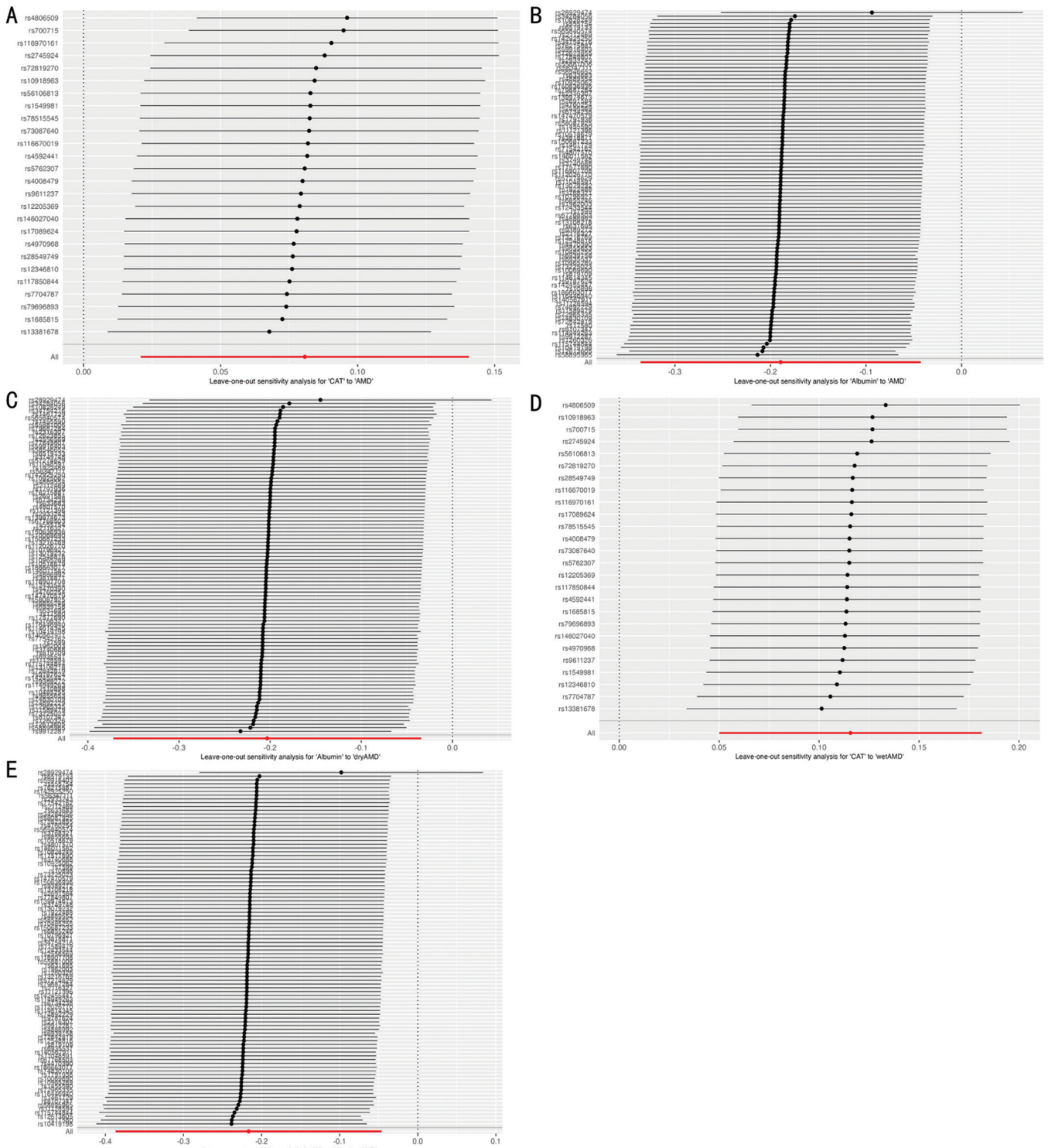


Figure 3 Sensitivity assessment using leave-one-out analysis for OSIBs-AMD causal relationships OSIBs: Oxidative stress biomarkers; AMD: Age-related macular degeneration; wetAMD: Wet age-related macular degeneration; dryAMD: Dry age-related macular degeneration; CAT: Catalase.

pathogenesis. This supports the multifactorial nature of AMD. However, these associations may reflect pleiotropic effects where SNPs influence both AMD and OSIBs independently, rather than direct causation. Cautious interpretation is therefore necessary, and longitudinal studies are needed for confirmation. Genetically predicted dry AMD was significantly associated with decreased albumin levels, while genetically predicted wet AMD was linked to lower albumin, TBIL levels, and reduced PON activity. These associations highlight the importance of these OSIBs in AMD development. Albumin and bilirubin are known for their antioxidant properties, while PON1, an enzyme involved in high-density lipoprotein metabolism, protects against oxidative modification of low-density lipoprotein (LDL) cholesterol[35-36]. The reduced PON activity observed in our study aligns with findings of altered PON1 phenotypes and elevated oxidative stress markers like

Exposure	Outcome	IVsN	Method	OR (95% CI)	Pvalue
dryAMD	Albumin	61	MR Egger	0.980 (0.967 to 0.993)	*0.004*
		61	Weighted median	0.987 (0.976 to 0.998)	*0.017*
		61	IVW(random effects)	0.987 (0.979 to 0.996)	*0.004*
		61	Weighted mode	0.984 (0.960 to 1.008)	0.187
wetAMD	Albumin	63	MR Egger	0.984 (0.970 to 0.999)	*0.041*
		63	Weighted median	0.987 (0.977 to 0.997)	*0.015*
		63	IVW(random effects)	0.991 (0.982 to 1.000)	*0.048*
		63	Weighted mode	0.995 (0.974 to 1.015)	0.607
wetAMD	TBIL	63	MR Egger	0.993 (0.957 to 1.030)	0.703
		63	Weighted median	0.974 (0.947 to 1.001)	0.060
		63	IVW(random effects)	0.976 (0.955 to 0.998)	*0.036*
		63	Weighted mode	1.006 (0.952 to 1.063)	0.845
wetAMD	PON	61	MR Egger	0.921 (0.821 to 1.032)	0.162
		61	Weighted median	0.928 (0.838 to 1.028)	0.153
		61	IVW(random effects)	0.902 (0.841 to 0.968)	*0.004*
		61	Weighted mode	0.917 (0.837 to 1.005)	0.070

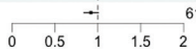


Figure 4 Reverse MR analysis: AMD genetic variants' effects on OSIBs IVW: Inverse-variance weighted; OSIBs: Oxidative stress biomarkers; AMD: Age-related macular degeneration; TBIL: Total bilirubin; PON: Paraoxonase; MR: Mendelian randomization.

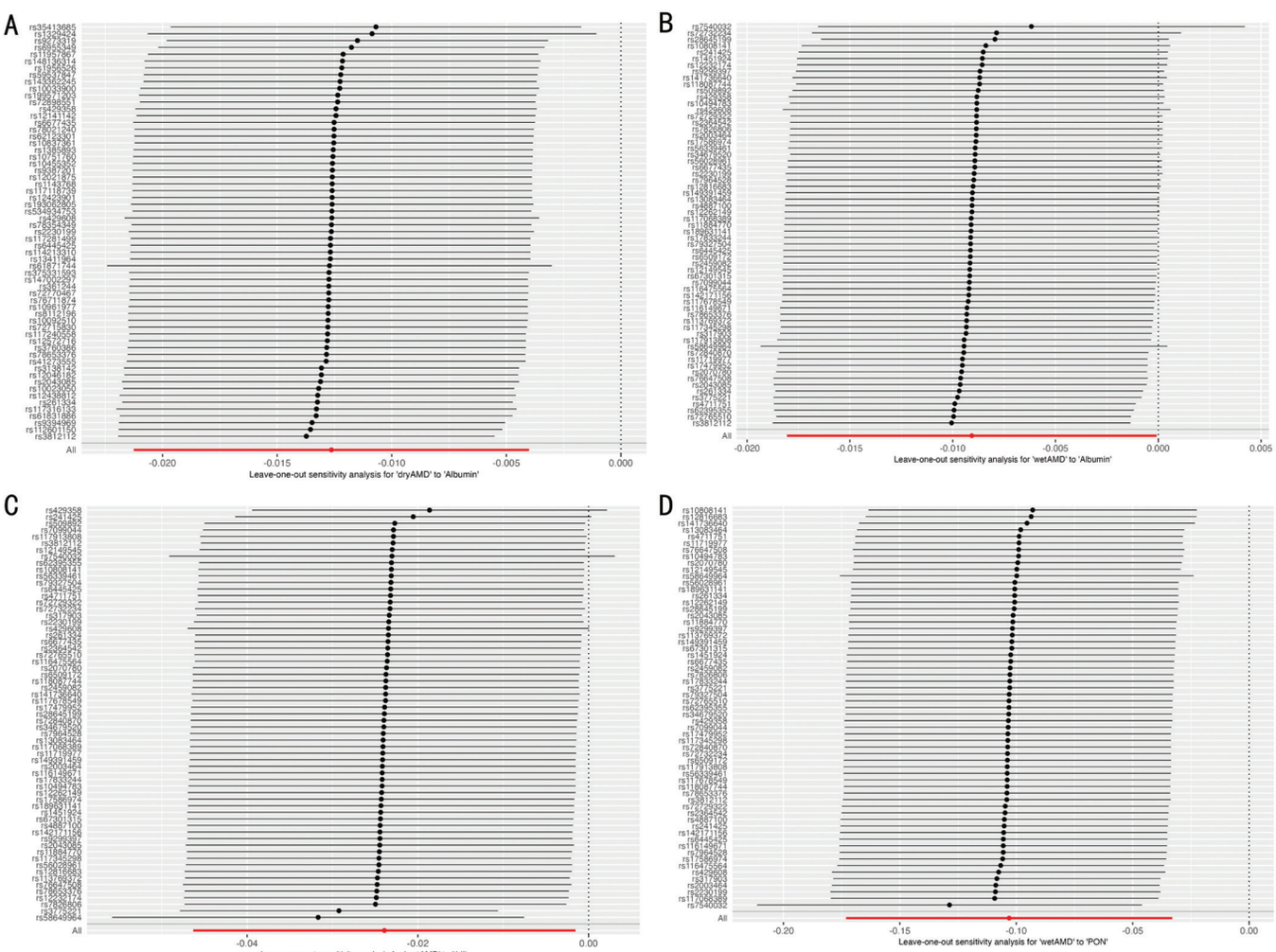


Figure 5 MR leave-one-out sensitivity analyses for AMD and OSIBs interactions OSIBs: Oxidative stress biomarkers; AMD: Age-related macular degeneration; wetAMD: Wet Age-related macular degeneration; dryAMD: Dry Age-related macular degeneration; TBIL: Total bilirubin; PON: Paraoxonase; MR: Mendelian randomization.

oxidized LDL and asymmetric dimethylarginine in AMD patients[37]. Elevated plasma homocysteine in AMD patients contributes to oxidative stress and vascular endothelial injury[38], while abnormal lipid profiles with increased LDL cholesterol underscore the involvement of dyslipidemia in AMD development[39]. Genetic and environmental factors including smoking and alcohol consumption affect AMD-related protein expression[40-41], highlighting the multifaceted nature of AMD pathogenesis. Early molecular alterations due to oxidative damage offer promising targets for preventive intervention.

Existing literature supports our results, with multiple studies documenting elevated oxidative stress markers and altered antioxidant enzyme activities in AMD patients[42]. The MR

methodology we employed represents a significant strength, minimizing confounding through genetic randomization. However, we acknowledge that lifestyle factors such as smoking, poor diet, or physical inactivity—known AMD risk factors—could potentially influence our results if SNPs were indirectly associated with these traits. To address this concern, we carefully selected SNPs strongly linked to OSIBs and verified the absence of associations with known confounders using tools like PhenoScanner. Environmental factors can intensify oxidative stress, compounded by retinal pigment epithelium (RPE) cell mitochondrial dysfunction, which features reduced oxidative phosphorylation, heightened reactive oxygen species production, and mitochondrial DNA mutations[43]. The age-related decline of autophagy, mitophagy, and heterophagy pathways further exacerbates oxidative stress by impairing the removal of damaged cellular components[44]. Polymorphisms in *CFH* and dysregulation of non-coding RNAs affecting mitochondrial function and antioxidant responses contribute to oxidative stress, leading to cellular damage and disease progression[7].

Based on oxidative stress's significant role in AMD, various antioxidant approaches have been investigated. AREDS and AREDS2 showed that supplementing with vitamins C and E, beta-carotene, and zinc slows AMD progression, especially in intermediate stages[4]. Our results indicate that targeting specific oxidative stress pathways may offer effective AMD treatment. For example, patients with genetically predicted elevated CAT levels might benefit from strengthened antioxidant defenses using vitamin E or zinc, as demonstrated in AREDS[44-45], while those with lower albumin levels linked to dry AMD could potentially benefit from albumin-boosting therapies—though such interventions should be avoided in wet AMD patients due to the increased risk of neovascularization. Using genetic testing to identify high-risk individuals for specific OSIBs could enable tailored antioxidant supplementation, aligning interventions with individual genetic and biochemical profiles, given the complexity of AMD subtypes and individual variability. Beyond supplementation, leveraging endogenous antioxidant systems like thioredoxin, glutaredoxin, glutathione, and coenzyme Q10 could complement these strategies. Dietary intake of antioxidants, including plant-derived polyphenols, carotenoids, and omega-3 polyunsaturated fatty acids, has also been suggested as a preventive strategy[46]. Despite the theoretical benefits, the efficacy of antioxidant supplementation has yielded mixed results in clinical studies. For instance, while some studies have demonstrated that supplements like glutathione and resveratrol precursors can significantly improve redox status and reduce inflammation, others have found limited or inconsistent health benefits[47-48]. This inconsistency may be due to factors such as bioavailability, dosage, and individual variations in oxidative stress levels and antioxidant needs[49]. High doses of supplements may even interfere with normal physiological processes. The potential for antioxidants to act as both beneficial and harmful agents underscores the need for a holistic approach to understanding their role in health.

Our study results contribute to this understanding by highlighting the complex interactions between genetically predicted OSIBs and AMD. For instance, our results showing paradoxically increased risk of overall and wet AMD with higher genetically predicted CAT levels suggest that compensatory increases in CAT may be insufficient to counteract oxidative damage. Similarly, the multifaceted relationship between albumin levels and AMD subtypes suggests that while albumin may be protective against dry AMD, it may exacerbate wet AMD through mechanisms involving VEGF and other inflammatory mediators. Furthermore, our reverse MR analysis emphasizes the importance of antioxidants such as albumin, bilirubin, and PON in AMD pathogenesis, again highlighting the critical roles of oxidative stress and chronic inflammation. This study pioneers the use of bidirectional two-sample Mendelian randomization to examine causal links between comprehensive oxidative stress biomarkers and AMD. While previous MR investigations have explored various AMD risk factors including smoking, alcohol intake, blood pressure, BMI, and glycemic traits, none have specifically targeted oxidative stress biomarkers[50]. These insights suggest that while antioxidant supplementation may be beneficial, its effectiveness may vary depending on specific oxidative stress biomarkers and AMD subtypes. Personalized antioxidant therapies, tailored to individual genetic and biochemical profiles, may offer more effective strategies for AMD prevention and treatment. Integrating genetic, clinical, and biochemical data will be key to developing these targeted interventions, ultimately leading to better outcomes for AMD patients.

Although informative, this study has limitations. The MR analyses were based on European ancestry data, which may limit generalizability due to population stratification risks. Genetic variants associated with OSIBs may differ in frequency or effect size across populations, potentially altering the observed associations in non-European cohorts such as East Asians or Africans, necessitating validation in diverse populations to ensure broader applicability. Second, while MR analysis helps infer causality, it does not elucidate the exact biological mechanisms behind the observed associations. Additional experiments, including ARPE-19 cell assays assessing CAT's role in oxidative stress-induced damage, are needed to clarify AMD-related molecular mechanisms and uncover therapeutic targets.

In conclusion, this study provides strong evidence for a causal relationship between oxidative stress and AMD, highlighting the potential of antioxidant therapies in AMD prevention and treatment. Our findings underscore the need for personalized approaches that consider the differential impacts of oxidative stress biomarkers on AMD subtypes.

Based on our results, we propose several specific clinical strategies. First, genetic screening for CAT variants to identify individuals who might benefit from targeted vitamin E or zinc supplementation, particularly those at risk for wet AMD. Second, monitoring albumin levels in AMD patients, with potential albumin-enhancing interventions for dry AMD patients while carefully avoiding such therapies in wet AMD patients. Third, developing subtype-specific antioxidant formulations that address the distinct oxidative stress mechanisms in dry versus wet AMD. Finally, incorporating PON and bilirubin assessments into AMD risk profiling, especially for identifying patients at risk for wet AMD progression.

These personalized approaches require further mechanistic validation but represent promising directions for improving AMD management.

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Data Availability: All analyses were conducted using summary statistics from IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/) and FinnGen consortium(https://www.finngen.fi/en) which provide publicly available data on genetic associations with various traits and diseases.

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