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Exploring the Causal Relationships between Lipid Biomarkers and Anti-VEGF Treatment Response in Patients with Neovascular Age-related Macular Degeneration

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Purpose: To identify the connections between lipid biomarkers and the anti-VEGF therapy response in patients with neovascular age-related macular degeneration (nAMD).

Design: A bidirectional and multivariable Mendelian randomization study.

Participants: The summary statistics for anti-VEGF nAMD treatment response included a total of 128 responders, 51 nonresponders, and 6 908 005 genetic variants available for analysis. The sample size of lipid biomarkers is 441 016 and 12 321 875 genetic variants available for analysis.

Methods: Two-sample Mendelian randomization (MR) method was conducted to exhaustively appraise the causalities among 13 lipid biomarkers and the risk of different anti-VEGF treatment responses (including visual acuity [VA] and central retinal thickness [CRT]) for nAMD subtypes.

Main Outcome Measures: Thirteen lipid biomarkers, VA, and CRT.

Results: A positive causal relationship was identified between triglycerides (TGs), apolipoproteins (Apos) E2, ApoE3, total cholesterol (TC), and VA response to anti-VEGF therapy in patients with nAMD, as confirmed by MR-Egger, weighted median, and weighted mode models. The MR-Egger model yielded statistically significant results for TC, ApoA-I, ApoB, and ApoA-V in relation to the CRT response to anti-VEGF treatment in patients with nAMD. In the reverse MR, the MR-Egger model identified significant causal relationships between ApoA-I, low-density lipoprotein cholesterol (LDL-c), ApoE3, and ApoF and the VA response. However, this was not the case in the weighted median and weighted mode models. In the MR-Egger model, ApoB, LDL-c, ApoE3, and ApoM were identified as significantly influencing the CRT response. In the multisample MR analysis, TC, high-density lipoprotein cholesterol, LDL-c, and TG were found to be causally related to VA response, and TC was also identified as being causally related to the CRT response to anti-VEGF therapy in patients with nAMD.

Conclusions: This MR study suggests unidirectional causality between TG and ApoE3 and the response to anti-VEGF treatment in patients with nAMD.

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Age-related macular degeneration (AMD) represents the primary cause of irreversible vision loss among adults >50 years of age.[1] The onset of AMD is influenced by a combination of age, genetic susceptibility, and environmental factors. Age-related macular degeneration is classified into 2 main categories: dry AMD and wet AMD, the latter of which is also referred to as neovascular age-related macular degeneration (nAMD). Despite nAMD representing only 10% to 15% of all AMD cases, it is responsible for vision loss in 90% of patients with AMD.[2] At present, the principal therapeutic intervention for nAMD is the repeated intraocular administration of anti-VEGF pharmaceutical agents. The advent of anti-VEGF biologics has markedly diminished the incidence of legal blindness resulting from nAMD.[3–5] However, the efficacy of anti-VEGF therapy varies considerably between individuals, and there is a paucity of data regarding the long-term prognosis of patients with nAMD after anti-VEGF treatment. The identification of key biomarkers that can predict the treatment effect and prognosis of nAMD is of significant clinical importance for the management of patients with nAMD.

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The retina is a lipid-rich structure, and lipid metabolism plays a pivotal role in the pathogenesis of nAMD.[6–8] The principal lipids are triglycerides (TGs), cholesterol (in the form of very-low-density lipoprotein, low-density lipoprotein [LDL], and high-density lipoprotein [HDL]), and a variety of Apolipoproteins (Apos). Nevertheless, a consensus on the relationship between lipid metabolism and the pathogenesis of AMD has yet to be reached.[9–13] Nevertheless, the study by Gehlbach et al[14] posits that the extant randomized controlled trials evidence is inadequate to substantiate the purported role of statins in the prevention or postponement of the onset or progression of AMD. The current understanding of the relationship between lipids and the onset of AMD is complex and requires further investigation. Research on tumor antiangiogenic drugs (AADs) revealed that resistance to AADs is closely related to lipid metabolic reprogramming. The strategy of targeting angiogenesis and lipid metabolism is anticipated to emerge as a novel paradigm in cancer therapy.[15,16] Anti-VEGF agents represent a significant advancement in the treatment of nAMD. Investigating the correlation between lipids and the prognosis of patients with nAMD receiving anti-VEGF treatment is highly clinically important.

In light of the limitations of existing randomized controlled trial evidence, the utilization of genetics can provide an additional line of evidence for prognostic studies of anti-VEGF treatment for nAMD, thereby enhancing the probability of successful discovery of prognostic markers. Mendelian randomization (MR) studies employ genetic variants that are randomly assigned at the time of conception, thereby rendering this study design less susceptible to confounding factors than other study designs.[17] Accordingly, to ascertain whether lipid levels can be utilized for prognostic evaluation of nAMD while circumventing the limitations of traditional methods (such as residual confounding), we constructed a multivariate complex MR analysis model, employing single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to elucidate the causal effects of 13 lipid biomarkers. The objective of this study was to elucidate the causal relationship between lipid levels and the prognosis of patients with nAMD receiving anti-VEGF treatment, and to identify reliable prognostic markers.

Methods

Overview of the Study Design

Figure 1 shows a systemic overview of a bidirectional MR study design. In summary, the objective of our study was to investigate the causal impact of anti-VEGF nAMD treatment response on the lipid biomarkers. Furthermore, an investigation was conducted to ascertain the causal relationship between lipid biomarkers and the anti-VEGF nAMD treatment response. Genetic variants are typically regarded as IVs, which must satisfy 3 rigorous assumptions. The genetic variations exhibit a significant correlation with the exposure and are not associated with any known confounders. Furthermore, it is important to note that genetic variants do not exert a direct influence on the outcome; instead, they are hypothesized to affect the outcome indirectly through the exposure pathway.

Genetic Data Acquisition

The 15 Biobank Japan release of disease traits of genome-wide association studies (GWAS) data of clinical lipid biomarkers were downloaded from the Integrative Epidemiology Unit (IEU) OPEN GWAS PROJECT official website (https://gwas.mrcieu.ac.uk/). The sample size and characteristics of each lipid biomarker are presented in Table S1 (available at www.ophthalmologyscience.org). The summary statistics for anti-VEGF nAMD treatment response, including visual acuity (VA) and central retinal thickness (CRT) responses, in our study were obtained from comprehensive aggregated data in the Zenodo database (https://zenodo.org/) provided by Strunz et al.[18] The data set included a total of 128 responders, 51 nonresponders, and 6 908 005 genetic variants available for analysis. Subsequently, the readr package (version 4.40) and the bbjMRP program script (https://github.com/YLCHEN1992/bbjmrp) were employed to facilitate the reading and analysis of the aforementioned GWAS data. During the reading process, the tgtable function of the readr package was employed to read the compressed vcf.gz file while the read.table function was employed to read and parse the SNP site information, β values, standard error (SE) variances, and trait correlation *P* values related to lipid biomarkers and nAMD treatment response.

Ethics Statement

Participant consent and ethical approval were obtained from the original studies. This study was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki. The local ethics committee determined that an ethical review was not necessary for this study.

Single-Nucleotide Polymorphism Tool Variable Filtering

By screening SNPs for exposure factors or traits using correlation *P* values, we identified the top 1000 SNP sites with significant *P* values <0.05 and arranged them in ascending order of *P* values as candidate sites for further filtering. After removing the linkage SNP sites with a distance of <10 000 bp, the first quarter of the SNP sites in ascending order of *P* values were selected as IVs. The SNP sites were further filtered, and a MR analysis model was constructed via Mendelian Randomization PRESSO (MR-PRESSO). Specifically, the inverse variance weighted (IVW) model was initially constructed on the basis of the extracted information regarding the SNP sites associated with the outcome events, as determined by IVs. Subsequently, the discrepancy between each SNP site and the IVW model was calculated, and SNPs with deviations smaller than the median were retained as the final IV.

Mendelian Randomization Model Construction

The exposure and outcome of β values of SNP IVs associated with exposure factors were transformed into quadrants 1 and 3. Subsequently, IVW and MR-Egger models were constructed before and after quadrant transformation via the lm function. The weighted median and weighted mode models were constructed using the mr_weighted_median and mr_weighted_mode functions, respectively, within the 2-sample MR package, and the F statistic was calculated using the following formula: F = (N-k-1)/k*R^2/(1-R^2), which was employed to determine any potential weak IV bias. Finally, a loop function was set to perform bidirectional MR analysis on the lipid biomarkers and nAMD treatment response of the GWAS data to obtain causal relationships. Four indicators with statistically significant *P* values <0.05 were identified as the results.

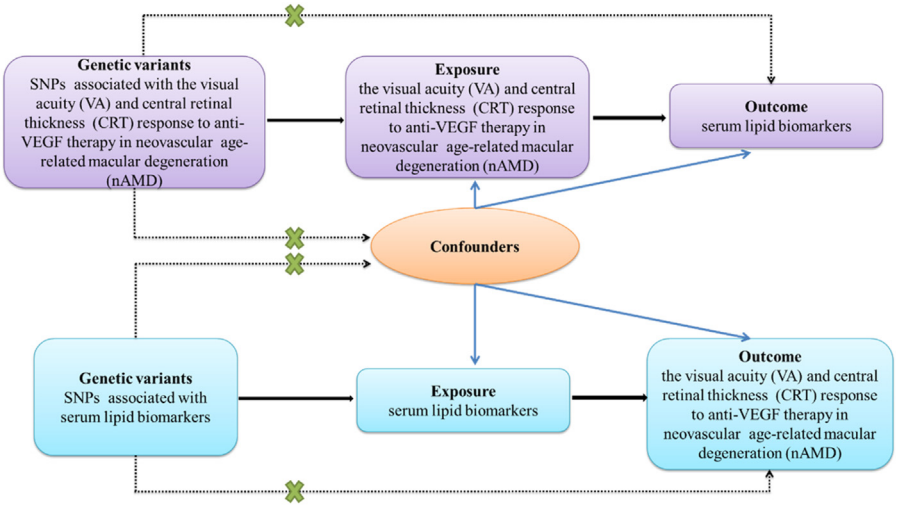

Fig 1. The methodology of applying a bidirectional Mendelian randomization analysis. The sign "×" indicates that genomic alterations are disassociated from confounders or do not directly influence the outcome. On the contrary, genomic alterations can exert their effects via the exposure elements. The solid line represents the existence of substantial connections, while the dashed line indicates the absence of interrelationships between the variables. SNP = single-nucleotide polymorphism.

In our bulk MR analysis process, we elected to utilize the MR-PRESSO conceptual approach in lieu of directly employing its method. The optimal conditions for filtering candidate SNPs were not set, such as selecting the best choice according to the IVW model or the condition of minimum cost function. Instead, the filtering was conducted based on the median of the cost function values generated by the algorithm. Subsequently, the MR-Egger model was constructed as the primary reference result after the aforementioned conditional filtering. This approach ensures comparability among different factors under the same conditions during bulk analysis and helps reduce the impact of multiple testing effects. Furthermore, this approach helps mitigate the issue of high false positive rates associated with the MR-PRESSO method. The specific script source code [ftp://systempackage.cn/R/CMR.r] employed for analysis will facilitate a more profound understanding of the underlying methodologies.

Multivariable Mendelian Randomization

The multivariable Mendelian randomization (MVMR) analyzes may be applied to multiple genetic instruments, regardless of their association with exposure. In this MVMR analysis, the IVs can be connected with >1 exposure element, provided that they fulfill the equivalent IVs assumption. Therefore, we implemented this method with the objective of identifying the independent effects of all the IVs for ApoA-I, ApoB, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and TG to pinpoint their independent effects on the response to anti-VEGF treatment in patientswith nAMD.

Results

A 2-Sample MR Analysis for Potential Causality between the Lipid Biomarkers on the VA and CRT Response to Anti-VEGF Treatment in nAMD

In our study, we applied a 2-sample MR analysis in which genetic variants connected with nAMD prognosis with lipid biomarkers (including TGs, total cholesterol [TC], LDL-c, HDL-c, ApoM, ApoL1, ApoF, Apo E3, Apo E2, ApoD, ApoB, ApoA-V, and ApoA-I) originating from a contemporary GWAS of European ancestry are shown in Table S1. The results of independent variable MR analysis revealed a significant causal relationship between TG, ApoE2, ApoE3, and TC and the VA response to anti-VEGF treatment in nAMD in the MR-Egger model, and the causal relationship was still significant in the weighted median or weighted mode model TG (β: −13.651, SE: 1.655, *P*: 1.73E-05, IVW: 4.33E-12, weighted median: 5.08E-15, weighted mode: 0.0001); ApoE2 (β: 10.891, SE: 3.771, *P*: 0.009, IVW: 0.0006, weighted median: 2.83E-13, weighted mode: 1.08E-06); ApoE3 (β: 8.985, SE: 3.101, *P*: 0.009, IVW: 0.268, weighted median: 7.30E-09, weighted mode: 7.69E-07); TC (ID: ieu-a-301, beta: −27.857, SE: 8.929, *P*: 0.005, IVW: 0.803, weighted median: 1.07E-16, weighted mode: 1.67E-07); TC (ID: ieu-a-782, β: −29.997, SE: 4.469, *P*: 6.09E-07, IVW: 0.803, weighted median: 1.07E-16, weighted mode: 1.67E-07) (Table 2 and Fig 2A). In the CRT response to anti-VEGF treatment in nAMD, TC was significant among the MR-Egger, weighted median and weighted mode model TC (ID: ieu-a-782, β: −38.570, SE: 12.550, *P*: 0.005; IVW: 3.63E-20, weighted median: 3.7E-10, weighted mode: 0.031). In addition, ApoA-I, ApoB and ApoA-V were also significant in the MR-Egger model (ApoA-I [β: −147.200, SE: 48.328, *P*: 0.016]; ApoB [ID: ieu-b-108, β: 197.309, SE: 43.005, *P*: 0.004]; ApoA-V [β: −81.164, SE: 21.480,*P*: 0.001]), but the corresponding weighted median or weighted mode model results were not significant (Table 3 and Fig 2B).

Causal Effects of VA and CRT Response to Anti-VEGF Treatment in Patients with nAMD on Lipid Biomarkers

Next, we implemented a 2-sample MR to analyze cause-and-effect association between VA and CRT response to anti-VEGF treatment in patients with nAMD with respect to

Table 2. Independent Variables of MR Results for the Relationship between Lipid Biomarkers on the VA Response to Anti-VEGF Treatment in Patients with nAMD

ID	β	SE	*P*	Int	IVW	Weighted Median	Weighted Mode	N	Biomarkers
ieu-b-107	−1.968	1.104	0.113	1.069	**1.98E-06**	**6.69E-09**	**0.001**	393 193	Apolipoprotein A-I
ieu-b-108	−8.339	6.070	0.219	1.560	**0.001**	**3.23E-06**	**0.003**	439 214	Apolipoprotein B
ieu-b-109	−0.625	1.898	0.749	1.039	**1.19E-06**	**6.40E-06**	**0.001**	403 943	HDL cholesterol
ieu-b-110	4.555	6.545	0.537	−1.481	**0.017**	**0.004**	**0.012**	440 546	LDL cholesterol
ieu-b-111	−13.651	1.655	**1.73E-05**	0.364	**4.33E-12**	**5.08E-15**	**0.000**	441 016	Triglycerides
prot-a-126	−4.148	4.156	0.330	0.465	0.170	**1.36E-10**	**6.03E-06**	3301	Apolipoprotein A-V
prot-a-127	3.589	3.922	0.369	0.128	**4.14E-05**	**9.89E-18**	**1.51E-08**	3301	Apolipoprotein B
prot-a-130	0.375	3.768	0.922	−0.505	**0.045**	**1.12E-12**	**6.89E-08**	3301	Apolipoprotein D
prot-a-131	8.985	3.105	**0.009**	−1.610	0.268	**7.30E-09**	**7.69E-07**	3301	Apolipoprotein E (isoform E3)
prot-a-132	10.891	3.771	**0.009**	−1.019	**0.001**	**2.83E-13**	**1.08E-06**	3301	Apolipoprotein E (isoform E2)
prot-a-133	−2.624	1.499	0.090	0.246	**0.031**	**4.81E-11**	**0.000**	3301	Apolipoprotein F
prot-a-135	0.070	0.535	0.900	−0.538	**0.017**	0.918	**0.023**	3301	Apolipoprotein L1
prot-a-137	3.926	3.841	0.320	−1.113	**0.002**	**3.46E-08**	**1.82E-06**	3301	Apolipoprotein M
ieu-a-301	−27.857	8.929	**0.005**	2.553	0.633	**1.49E-17**	**1.73E-08**	187 365	Total cholesterol
ieu-a-782	−29.997	4.469	**6.09E-07**	2.766	0.803	**1.07E-16**	**1.67E-07**	92 260	Total cholesterol

β = β coefficients of MR-Egger model; HDL = high-density lipoprotein; ID = identity document; Int = intercept of MR-Egger model; IVW = *P* values of inverse variance weight model; LDL = low-density lipoprotein; MR = Mendelian randomization; N = sample size of GWAS; nAMD = neovascular age-related macular degeneration; *P* = *P* values of MR-Egger model; SE = standard error; VA = visual acuity; weighted median = *P* values of weighted median model; weighted mode = *P* values of weighted mode model.
Causal effect of the lipid biomarkers on the VA response to anti-VEGF therapy in patients with nAMD. Bold values indicate that their *P* values are less than 0.05, demonstrating statistical significance.

lipid biomarkers. The results of reverse MR analysis revealed a causal relationship between the VA response to anti-VEGF treatment in nAMD and 4 lipid biomarkers (ApoA-I, LDL-c, ApoE3, and ApoF) in the MR-Egger model, but in the weighted median and weighted mode models, the relationships were not significant (ApoA-I [β: 0.0003, SE: 0.0001, *P*: 0.0125]; LDL-c [β: 0.0002, SE: 8.44E-05, *P*: 0.044]; ApoE3 [β: 0.003, SE: 0.001, *P*: 0.006]; ApoF [β: 0.003, SE: 0.0009, *P*: 0.008]) (Table 4 and Fig 3A). Similarly, in the CRT response to anti-VEGF treatment in nAMD, 3 lipid biomarkers (ApoB [ID: ieu-b-108], LDL-c and ApoE3) were significant in the MR-Egger model, but the weighted median and weighted mode models were not significant (ApoB [ID: ieu-b-108, β: −6.49E-05, SE: 1.93E-05, *P*: 0.002]; LDL-c [beta: −6.25E-05, SE: 2.05E-05, *P*: 0.005]; ApoE3 [beta: 0.003, SE: 0.001, *P*: 0.006]); 2 lipid biomarkers (ApoB [ID: prot-a-127] and ApoM) were significant in the MR-Egger model and weighted median model, but in the weighted mode model was not significant (ApoB [ID: prot-a-127, β: −0.0002, SE: 7.60E-05, *P*: 0.004, IVW: 2.51E-10, weighted median: 0.0007]; ApoM [β: 0.0004, SE: 0.0002,

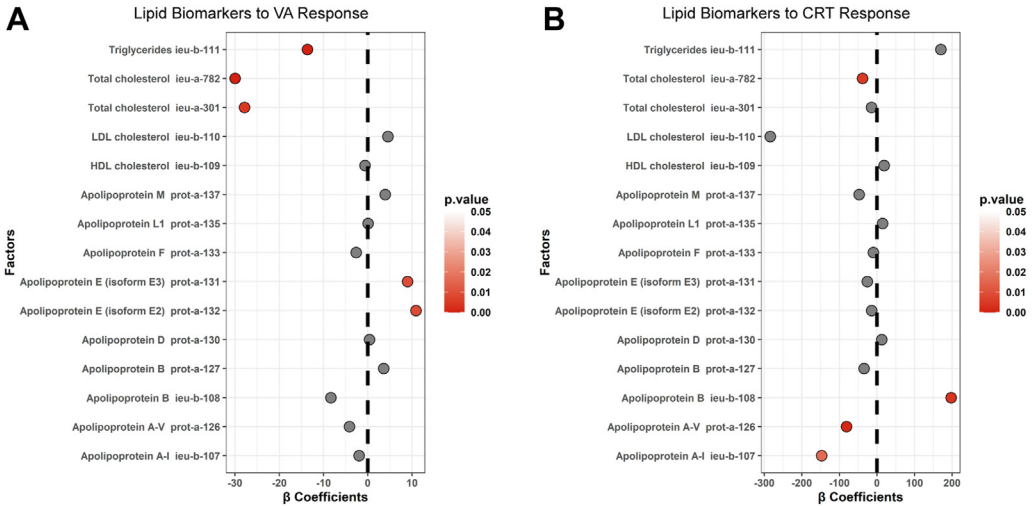

Figure 2. Forest plot showing the β coefficients of bidirectional independent MR results of the serum lipid biomarkers response to anti-VEGF therapy in nAMD. (**A**) Lipid biomarkers to VA response; (**B**) Lipid biomarkers to CRT response. CRT = central retinal thickness; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MR = Mendelian randomization; nAMD = neovascular age-related macular degeneration; VA = visual acuity.

Table 3. Independent Variables of MR Results for the Relationship between Lipid Biomarkers on the CRT Response to Anti-VEGF Treatment in Patients with nAMD

ID	β	SE	P	Int	IVW	Weighted Median	Weighted Mode	N	Biomarkers
ieu-b-107	−147.200	48.328	**0.016**	18.263	0.263	0.818	0.295	393 193	Apolipoprotein A-I
ieu-b-108	197.309	43.005	**0.004**	−15.863	**0.002**	0.313	0.394	439 214	Apolipoprotein B
ieu-b-109	19.151	27.199	0.499	−5.258	**0.026**	0.774	0.317	403 943	HDL cholesterol
ieu-b-1010	284.336	196.460	0.244	50.621	0.156	0.963	**0.039**	440 546	LDL cholesterol
ieu-b-111	170.031	93.517	0.102	−9.741	**0.001**	0.075	0.083	441 016	Triglycerides
prot-a-126	−81.165	21.480	**0.001**	11.538	**0.011**	0.057	0.053	3301	Apolipoprotein A-V
prot-a-127	−34.403	30.846	0.275	3.401	0.161	0.421	0.960	3301	Apolipoprotein B
prot-a-130	12.746	25.639	0.625	1.270	**0.000**	0.178	0.986	3301	Apolipoprotein D
prot-a-131	−25.888	29.912	0.398	4.124	0.876	0.839	0.927	3301	Apolipoprotein E (isoform E3)
prot-a-132	14.168	26.532	0.599	1.224	0.409	0.071	0.420	3301	Apolipoprotein E (isoform E2)
prot-a-133	−9.764	9.112	0.292	1.213	0.305	**0.006**	0.829	3301	Apolipoprotein F
prot-a-135	14.686	8.294	0.120	−10.137	0.504	0.983	0.598	3301	Apolipoprotein L1
prot-a-137	−47.405	28.168	0.109	4.595	**0.027**	**0.029**	0.204	3301	Apolipoprotein M
ieu-a-301	−14.574	53.281	0.787	9.123	**3.34E-11**	**4.01E-06**	**0.017**	187 365	Total cholesterol
ieu-a-782	−38.570	12.550	**0.005**	12.678	**0.000**	**3.70E-10**	**0.031**	92 260	Total cholesterol

β = β coefficients of MR-Egger model; CRT = central retinal thickness; HDL = high-density lipoprotein; ID = identity document; Int = intercept of MR-Egger model; IVW = *P* values of inverse variance weight model; LDL = low-density lipoprotein; MR = Mendelian randomization; N = sample size of GWAS; nAMD = neovascular age-related macular degeneration; *P* = *P* values of MR-Egger model; SE = standard error; weighted median = *P* values of weighted median model; weighted mode = *P* values of weighted mode model.
Causal effect of the lipid biomarkers on the CRT response to anti-VEGF therapy in patients with nAMD. Bold values indicate that their *P* values are less than 0.05, demonstrating statistical significance.

P: 0.022, IVW: 0.0005, weighted median: 0.001]) in the Table 5 and Figure 3B.

Multivariable MR Analysis

Six lipid biomarkers (including TC, HDL-c, LDL-c, TG, ApoA-I, and ApoB) from the IEU cohort were selected for MVMR analysis. The MVMR analysis demonstrated that TC (β: −37.052, SE: 17.039, *P*: 0.036) and LDL-c (β: 58.283, SE: 23.874, *P*: 0.019) were significantly associated with the VA response to anti-VEGF treatment in nAMD in the IVW model (Table S6, available at www.ophthalmologyscience.org). In contrast, the MR-Egger model (Table S7, available at www.ophthalmologyscience.org) yielded

Table 4. Reverse MR Results for the Relationship between the VA Response to Anti-VEGF Treatment in Patients with nAMD on the Lipid Biomarkers

ID	β	SE	P	Int	IVW	Weighted Median	Weighted Mode	N	Biomarkers
ieu-b-107	0.000286	0.000	**0.012**	0.000	**9.49E-05**	0.539	0.984	179	Apolipoprotein A-I
ieu-b-108	0.000124	0.000	0.243	0.000	0.411	0.081	0.962	179	Apolipoprotein B
ieu-b-109	0.000211	0.000	0.077	0.000	**0.001**	0.507	0.996	179	HDL cholesterol
ieu-b-1010	0.000177	8.44E-05	**0.044**	−0.001	0.741	0.045	0.708	179	LDL cholesterol
ieu-b-111	4.48E-08	0.000	1.000	0.000	0.577	0.132	0.852	179	Triglycerides
prot-a-126	0.00138	0.002	0.367	0.001	0.139	0.152	0.984	179	Apolipoprotein A-V
prot-a-127	0.001785	0.001	0.072	0.000	**0.028**	0.191	0.976	179	Apolipoprotein B
prot-a-130	−0.00177	0.001	0.139	0.002	0.208	0.086	0.974	179	Apolipoprotein D
prot-a-131	0.002931	0.001	**0.006**	0.002	**1.29E-05**	0.101	0.994	179	Apolipoprotein E (isoform E3)
prot-a-132	0.00104	0.001	0.184	0.005	**0.001**	0.001	0.901	179	Apolipoprotein E (isoform E2)
prot-a-133	0.002526	0.001	**0.008**	−0.004	**0.026**	0.046	0.792	179	Apolipoprotein F
prot-a-135	0.001521	0.001	0.244	−0.001	0.228	0.106	0.722	179	Apolipoprotein L1
prot-a-137	−0.00161	0.001	0.123	0.002	0.202	0.056	0.764	179	Apolipoprotein M

β = β coefficients of MR-Egger model; HDL = high-density lipoprotein; ID = identity document; Int = intercept of MR-Egger model; IVW = *P* values of inverse variance weight model; LDL = low-density lipoprotein; MR = Mendelian randomization; N = sample size of GWAS; nAMD = neovascular age-related macular degeneration; *P* = *P* values of MR-Egger model; SE = standard error; weighted median = *P* values of weighted median model; weighted mode = *P* values of weighted mode model.
Causal effect of the VA response to anti-VEGF therapy in patients with nAMD on the lipid biomarkers. Bold values indicate that their *P* values are less than 0.05, demonstrating statistical significance. The filtering of single-nucleotide polymorphisms for total cholesterol (ieu-a-301, ieu-a-782) does not meet the conditions for MR model construction, leading to a lack of MR data analysis results for the reverse total cholesterol factor.

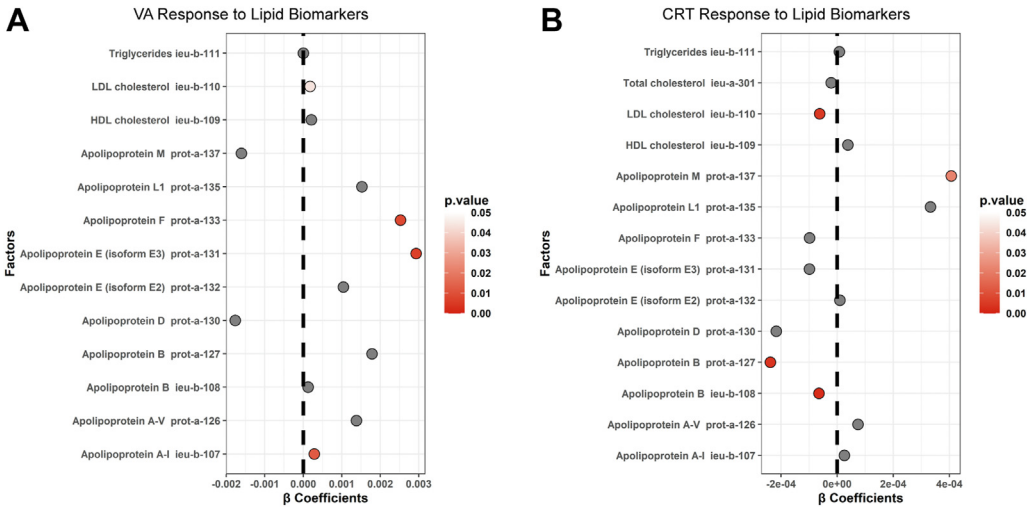

Figure 3. Forest plot showing β coefficients of bivariate independent MR results for serum lipid biomarkers in response to anti-VEGF therapy in nAMD patients. (**A**) VA response to lipid biomarkers; (**B**) CRT response to lipid biomarkers. CRT = central retinal thickness; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MR = Mendelian randomization; nAMD = neovascular age-related macular degeneration; VA = visual acuity.

significant results for TC (β: 7.673, SE: 3.308, *P*: 0.026), HDL-c (β: −6.017, SE: 2.213, *P*: 0.010), LDL-c (β: −10.765, SE: 4.713, *P*: 0.028), and TG (β: −4.434, SE: 1.136, *P*: 0.000). Furthermore, in the CRT response to anti-VEGF treatment in patients with nAMD, only TC (β: 214.758, SE: 70.838, *P*: 0.004) demonstrated a statistically significant result in the IVW model (Table S8, available at www.ophthalmologyscience.org). The MR-Egger model yielded 6 lipid biomarkers that were not statistically significant (Table S9, available at www.ophthalmologyscience.org).

Discussion

This MR study demonstrated that the TG and ApoE3 levels had a significant direct causal relationship with the prognosis of anti-VEGF treatment for nAMD. Conversely, no obvious causal relationship was identified between the other 11 lipid factors, including LDL and HDL levels, and the prognosis of anti-VEGF treatment for nAMD.

Research has revealed a correlation between lipids and the development of nAMD. Lipids constitute the primary component of AMD drusen, comprising >40% of the

Table 5. Reverse MR Results for the Relationship between the CRT Response to Anti-VEGF Treatment in Patients with nAMD on the Lipid Biomarkers

ID	β	SE	P	Int	IVW	Weighted Median	Weighted Mode	N	Biomarkers
ieu-b-111	7.69E-06	2.30E-05	0.741	−0.003	**4.75E-06**	**7.82E-05**	**0.027**	179	Triglycerides
ieu-b-109	3.82E-05	2.52E-05	0.140	−0.001	**0.000**	**0.006**	0.556	179	HDL cholesterol
ieu-b-110	−6.25E-05	2.05E-05	**0.005**	0.003	0.891	0.079	0.555	179	LDL cholesterol
ieu-a-301	−2.12E-05	7.14E-05	0.773	0.004	**0.002**	0.488	0.101	179	Total cholesterol
ieu-b-107	2.58E-05	2.17E-05	0.245	0.000	**3.15E-05**	**0.005**	0.477	179	Apolipoprotein A-I
ieu-b-108	−6.49E-05	1.93E-05	**0.002**	0.003	0.351	0.925	0.974	179	Apolipoprotein B
prot-a-126	7.43E-05	0.000	0.552	−0.009	**0.001**	**0.013**	0.965	179	Apolipoprotein A-V
prot-a-127	0.000	7.60E-05	**0.004**	0.004	**2.51E-10**	**0.001**	0.946	179	Apolipoprotein B
prot-a-130	0.000	0.000	0.063	0.012	0.905	0.062	0.888	179	Apolipoprotein D
prot-a-131	−9.88E-05	0.000	0.583	−0.010	**9.45E-09**	**0.001**	0.237	179	Apolipoprotein E (isoform E3)
prot-a-132	9.44E-06	0.000	0.936	−0.014	**5.15E-08**	**0.001**	0.493	179	Apolipoprotein E (isoform E2)
prot-a-133	−9.82E-05	8.72E-05	0.268	−0.006	**5.18E-09**	**0.023**	0.176	179	Apolipoprotein F
prot-a-135	0.000	0.000	0.138	−0.007	**0.000**	**0.043**	0.982	179	Apolipoprotein L1
prot-a-137	0.000	0.000	**0.022**	−0.034	**0.000**	**0.001**	0.973	179	Apolipoprotein M

β = β coefficients of MR-Egger model; CRT = central retinal thickness; HDL = high-density lipoprotein; ID = identity document; Int = intercept of MR-Egger model; IVW = *P* values of inverse variance weight model; LDL = low-density lipoprotein; MR = Mendelian randomization; N = sample size of GWAS; nAMD = neovascular age-related macular degeneration; *P* = *P* values of MR-Egger model; SE = standard error; weighted median = *P* values of weighted median model; weighted mode = *P* values of weighted mode model.
Causal effect of the CRT response to anti-VEGF treatment in patients with nAMD on the lipid biomarkers. Bold values indicate that their *P* values are less than 0.05, demonstrating statistical significance. The filtering of single-nucleotide polymorphisms for total cholesterol (ieu-a-301, ieu-a-782) does not meet the conditions for MR model construction, leading to a lack of MR data analysis results for the reverse total cholesterol factor.

drusen volume.[7] The primary ultrastructural component of drusen is cholesterol-rich lipoproteins secreted by retinal pigment epithelium (RPE) cells, which contain a substantial amount of ApoB and ApoE.[19] Abnormalities in cholesterol resulting from dysfunction of RPE cells are pivotal factors in the deposition of lipids and the formation of drusen.[20] A cross-sectional study by Colijn et al[13] revealed a positive correlation between HDL and an elevated risk of AMD, whereas TG was negatively correlated. Furthermore, both HDL and TG are strongly associated with early AMD and drusen. Recent studies have also revealed that lipid droplets accumulate to an excessive degree in the retina of ApoE-deficient mice that have been fed a high-fat diet. This accumulation results in the development of AMD pathologies, including RPE degeneration, Bruch's membrane thickening, drusen deposits, and photoreceptor dysfunction. The elimination of lipid droplets has been demonstrated to rescue both RPE degeneration and photoreceptor dysfunction, thereby underscoring the pivotal role of lipid droplets and ApoE in the pathogenesis of AMD.[21] ApoE3 is one of 3 subtypes of ApoE (ApoE2, ApoE3, ApoE4), which is the primary component of LDL and very-low-density lipoprotein and primarily mediates the uptake of lipoproteins by cells. In the study of nAMD, it was demonstrated that ApoE2 and ApoE3 alleles elevated ApoE levels and the release of inflammatory factors in tissues.[22] Nevertheless, our findings indicated a causal relationship between ApoE3, but not ApoE2, and the response to anti-VEGF treatment in nAMD. Further investigation is required to determine whether the absence of ApoE2 is associated with anti-VEGF treatment outcomes.

Anti-VEGF therapy represents a pivotal treatment modality for nAMD. Treatment improves a patient's central vision to a certain extent by reducing choroidal neovascularization and macular edema. However, anti-VEGF therapy is not an effective treatment for all patients, and its efficacy is influenced by a number of factors.[23] The impact of lipids on the efficacy of anti-VEGF therapy remains understudied. A study investigating the efficacy of AADs for the treatment of neoplastic lesions revealed that tumors exhibiting a propensity to grow in proximity to adipose tissue—including breast cancer, prostate cancer, pancreatic cancer, and hepatocellular carcinoma—demonstrated considerable resistance to AAD treatment.[24] Research has demonstrated that in nonglycolytic tumor tissues, the utilization of exogenous free fatty acids and the fatty acid oxidation metabolic pathway represent crucial mechanisms through which such cancer cells obtain energy, as evidenced in prostate cancer and B-cell lymphoma.[25,26] Furthermore, adipose tissue and free fatty acids markedly increase the survival, proliferation, and migration of cancer cells.[27] Research has demonstrated that anti-VEGF therapy and tissue hypoxia enhance lipid transport and storage in cancer cells via hypoxia inducible factor-1α-dependent mechanisms.[28] Antiangiogenic drugs induce a metabolic switch from glucose-dependent to lipid-dependent metabolism by inhibiting the oxygen and nutrient depletion triggered by angiogenesis. In the presence of a minimal number of microvessels, the latter pathway plays a crucial role in tumor growth.

During the progression of nAMD, the purpose of neovascularization is to alleviate damage to the retina caused by a series of pathological changes, including retinal inflammation, complement activation, RPE cell necrosis, ischemia, and hypoxia.[3] A theoretical model has been proposed that suggests a link between oxidative changes in lipoproteins and the development of proangiogenic factors, which in turn may contribute to the growth of new blood vessels in the context of AMD, particularly in the form of neovascular AMD.[29] The accumulation of oxidized lipoprotein fragments in the Bruch membrane of nAMD has been demonstrated to induce angiogenesis and alleviate retinal ischemia and hypoxia. However, as the disease progresses, the equilibrium of angiogenic factors (such as VEGF and PDGF) is disrupted, or the regulation of the complement cascade results in the transformation of nonexudative macular neovascularization (MNV) into exudative MNV, ultimately leading to the formation of nAMD.[29,30] Anti-VEGF treatment has been demonstrated to reduce the generation of exudative MNVs, improve macular exudation to a certain extent, and improve vision. However, it is likely to exacerbate retinal hypoxia and cell death.[31,32] Nevertheless, further investigation is needed to ascertain whether the hypoxic retina, following anti-VEGF treatment, is capable of ensuring retinal nutrition by converting glucose-dependent metabolism to lipid-dependent metabolism, as observed in tumor tissue. If a similar conversion of sugar to lipid metabolism occurs in the retina, it may explain, at least in part, the observed association between TG levels and a reduced risk of AMD. Nevertheless, the current research data are insufficient, and it is imperative to elucidate the relationship between lipid metabolism and nAMD by investigating alterations in lipid metabolism levels in patients with nAMD following anti-VEGF treatment. Our study revealed a notable causal relationship between TG and the efficacy of anti-VEGF treatment in patients with nAMD, indicating that anti-VEGF treatment exerts an influence on lipid metabolism in retinal tissue. While further demonstration is required to substantiate this result, it does provide preliminary support for the rationality of the aforementioned perspective.

This MR study is subject to several limitations. First, it should be noted that risk factors for disease prognosis are not always the same as risk factors for disease severity or onset. In other words, the direct inference of our results pertains to the treatment effect of nAMD rather than its onset. Second, it is not possible to make direct comparisons between MR estimates and drug treatments. Direct comparisons between the effects of anti-VEGF treatment and subtle differences in lipid levels caused by genetic variation are not possible. Lifelong exposure to MR is distinct from short-term pharmacological intervention. Genetic variations in systemic lipid-related genes may differ from those in the target tissues of intervention. Third, the exposure factors and outcomes are derived from disparate cohorts, and it is unclear whether the original data have been standardized, which renders the β value less referenceable. Fourth, the MVMR models IVW and MR-Egger are equally explanatory, and the corresponding index P value is <0.05, indicating that the index has a significant causal relationship with the outcome event. As the MR-Egger

model cutoff approaches 0, the interference of the multivariate effect of the tool diminishes. However, the MVMR model is not applicable to the weighted median and weighted mode models. Additionally, the study population was exclusively composed of individuals of European ancestry. Further studies in other ethnic populations are needed to assess the generalizability of the current findings.

This article presents the development of the bbjMRP program, which has been created independently using the R language. The R language is a statistical computing language that is primarily utilized for the automated execution of MR analysis on large batches of IEU public GWAS data. The program employs the MR-PRESSO analysis approach to filter the data set of SNPs, utilizing functions from the TwoSampleMR package. The weighted median and weighted mode models are calculated using this method. The magnitude of the intercept in the MR-Egger model is employed to ascertain the extent of relevant pleiotropy in the model. The significance of the weighted median and weighted mode models is employed to ascertain the irrelevance of the model. The exclusion of SNPs that deviate significantly from the IVM model through filtering ensures that the remaining SNPs align more closely with the principles of MR analysis. Concurrently, the MR-Egger model outcomes serve as the primary results for MR analysis, with F values, intercepts, weighted models, and mode models utilized as references.

The results of this MR study indicate a causal relationship between TG and ApoE3 levels and the prognosis of treatment with anti-VEGF agents for nAMD. TG and ApoE3 levels have the potential to serve as prognostic markers for anti-VEGF treatment of nAMD; nevertheless, further clinical studies are needed.

Footnotes and Disclosures

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Analysis and interpretation: He, Q. Chen, Y. Chen

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Abbreviations and Acronyms:
AAD = antiangiogenic drug; **AMD** = age-related macular degeneration; **Apo** = Apolipoprotein; **CRT** = central retinal thickness; **GWAS** = genome-wide association studies; **HDL** = high-density lipoprotein; **HDL-c** = high-density lipoprotein cholesterol; **IEU** = Integrative Epidemiology Unit; **IV** = instrumental variable; **IVW** = inverse variance weighted; **LDL** = low-density lipoprotein; **LDL-c** = low-density lipoprotein cholesterol; **MNV** = macular neovascularization; **MR** = Mendelian randomization; **MR-PRESSO** = Mendelian Randomization PRESSO; **MVMR** = multivariable Mendelian randomization; **nAMD** = neovascular age-related macular degeneration; **RPE** = retinal pigment epithelium; **SE** = standard error; **SNP** = single-nucleotide polymorphism; **TC** = total cholesterol; **TG** = triglyceride; **VA** = visual acuity.

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