• Mendelian Randomization •

Systematic genomic Mendelian randomization profiling of early molecular markers of optic atrophy

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Abstract

● **AIM:** To identify early molecular diagnostic biomarkers for optic atrophy (OPA) and explore potential mechanisms mediated by proteins.

● **METHODS:** Gene expression data was sourced from eQTLGen (31 684 samples; 19 960 genes). The OPA discovery cohort came from FinnGen (629 cases; 496 621 controls); the validation cohort came from the genome-wide association studies (GWAS) catalog (58 cases; 496 621 controls). Protein data for mediation analysis was obtained from the deCODE Genetics consortium (35 559 samples; 4907 proteins). Causal estimates were derived using two-sample Mendelian randomization (MR). Inverse-variance weighted (IVW) was the primary analysis method. Sensitivity analyses included MR-Egger intercept, Cochran's *Q* test for heterogeneity, and leave-one-out analysis. Colocalization analysis validated identified genes. Additionally, interaction analysis identified potential biomarkers. Finally, mediation analysis assessed potential mediating mechanisms.

● **RESULTS:** Multi-cohort validation revealed that increased expression levels of the SEC61A2 and THNSL2 were causally associated with an elevated risk of OPA. Furthermore, we identified 386 genes potentially associated with OPA. Hormone secretion and immune-related pathways were found to play significant roles in OPA pathogenesis. Mediation analysis indicated that Upper zone of growth plate and cartilage matrix associated (UCMA) potentially mediates the effect of SEC61A2 in increasing OPA risk.

● **CONCLUSION:** SEC61A2 and THNSL2 may serve as early diagnostic biomarkers for OPA. Additionally, SEC61A2 likely increases OPA risk through UCMA.

● **KEYWORDS:** optic atrophy; genome-wide association studies; biomarkers; Mendelian randomization

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INTRODUCTION

Optic atrophy (OPA), also known as optic neuropathy, is characterized by degeneration of optic nerve fibers, glial hyperplasia, and capillary occlusion, eventually leading to decreased or even lost visual function. The core pathological feature is pale or grayish-white optic disc color, accompanied by decreased vision, visual field defect, and acquired color vision disorder[1]. OPA can be classified according to etiology into primary OPA, secondary OPA, continuous OPA, and glaucomatous OPA[2], with primary OPA and secondary OPA being the main types. Among them, primary OPA is caused by lesions of the posterior ethmoid optic nerve, optic chiasm or lateral geniculate body[3], with a clear optic disc boundary and pale color. Commonly seen in traumatic and hereditary optic neuropathy (Leber hereditary optic neuropathy, LHON, OMIM#535000)[4], methanol poisoning[5] and inflammatory optic neuropathy[6]. Secondary OPA is often accompanied by retinal vascular abnormalities, with blurred optic disc boundaries and dull color. It is commonly seen in extensive retinal diseases such as retinitis pigmentosa and chorioretinitis[7]. In addition, OPA secondary to intracranial germ cell tumors also exists[8].

At present, there is a lack of globally unified epidemiological data, but OPA is regarded as one of the important causes of irreversible blindness[9]. Studies show that LHON primarily

causes blindness in young adult men, with an incidence rate of approximately 1/25 000 in the UK, and the proportion of male patients exceeds that of female patients[10]. Furthermore, among children with OPA, 53.20% of optic nerve patients have nystagmus, and 69.5% of OPA patients have systemic diseases[11].

In recent years, new methods such as gene therapy[12], stem cell therapy[13], and hyperbaric oxygen therapy[14] have shown potential in OPA clinical trials. However, since optic nerve cells are non-renewable, these treatments can only delay progression rather than reverse functional growth, so early diagnosis and etiological intervention remain the key to improving prognosis. Although some progress has been made in the genetic basis of OPA, the key bottleneck for early diagnosis is the lack of high-quality transcriptome data. On the one hand, clinical acquisition of optic nerve tissue is extremely difficult, which limits direct transcriptome sequencing of diseased tissues; on the other hand, most of the existing studies focus on case reports or small-scale cohorts, and there is a lack of systematic comparative analysis of peripheral blood transcriptome between large-scale healthy control groups and OPA patients. This makes it difficult to identify disease-specific gene expression characteristics at the molecular level, which in turn hinders early development of biomarkers based on transcriptional regulatory networks.

Genome-wide association studies (GWAS) have provided valuable insights into the complex molecular interactions between environmental and genetic factors in the pathogenesis of diseases[15], and many single nucleotide polymorphisms (SNPs) have shown strong associations with OPA[16]. Mendelian randomization (MR) is a method that uses genetic variations as instrumental variables (IVs) to explore the causal impact of exposure on the outcomes in observational epidemiological studies[17]. This study aims to use MR, from the perspective of genomic associations, combined with expression quantitative trait loci (eQTL), to explore the potential biomarkers of OPA at the genetic level and construct an interaction network to identify potential associated genes. Finally, the possible mechanisms therein are explored through mediation analysis. In accordance with the new requirements for research transparency, this study complies with the TITAN 2025 Guidelines on artificial intelligence (AI) statements and use[18].

MATERIALS AND METHODS

Ethical Approval The study was conducted in accordance with the guidelines for Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR)[19] and received ethical approval in previous studies, without the need for additional supplementation. All analyses were based on publicly available genome-wide association summary statistics. No individual-level data or new human participants were involved, and therefore formal ethical review was not required. The study was conducted in accordance with the principles of the Declaration of Helsinki (2008).

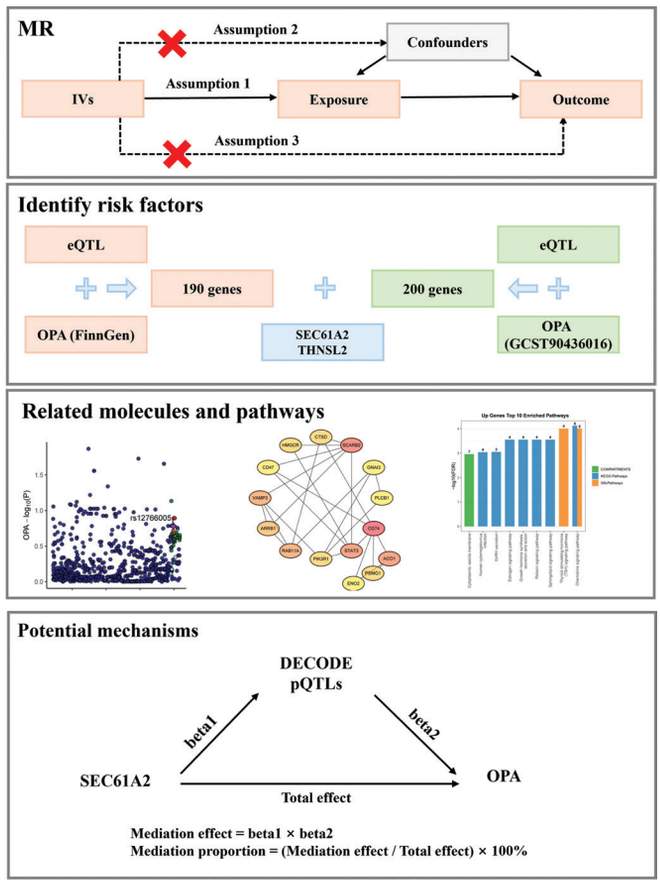

Figure 1 Flow chart of the overall work "MR" illustrates the three core assumptions of Mendelian randomization. "Identify risk factors" summarizes the biomarkers identified in the discovery cohort and replicated in the validation cohort. "Related molecules and pathways" lists the molecular markers and enriched pathways obtained from the transcriptomic datasets. "Potential mechanisms" presents the triangular framework used for mediation analysis. MR: Mendelian randomization; IVs: Instrumental variables; OPA: Optic atrophy; eQTL: Expression quantitative trait loci; pQTL: Protein quantitative trait loci.

Study Design The research process is shown as Figure 1. First, the causal relationship between eQTL and OPA was explored through the two-sample MR analysis. And the reliability of the results was verified using multiple cohorts to obtain genes with a stronger association with OPA. For the results obtained from multiple cohorts, we conducted co-localization analysis to obtain the key loci related to the gene and OPA, and performed functional analysis on them. Then, we conducted an interaction analysis on the genes with potential causal relationships that were initially screened and identified the hub gene. Furthermore, the biological pathways that may be related to OPA were explored through enrichment

analysis. Finally, we conducted a mediation analysis to assess whether there was a potential mechanism between plasma protein-mediated genes and OPA.

To ensure the validity of potential causal effects, we rely on the three core hypotheses of MR: 1) Genetic variations are closely related to exposure; 2) Genetic variations have no relation to confounding factors; 3) Genetic variations only affected the outcome through exposure.

Data Resource The eQTL data was from the eQTLGen[20] (https://eqtlgen.org/phase1.html), which contains 31 684 clinical samples of 19 960 genes. The cis-eQTL for each gene were selected as exposure data to explore that could be used as OPA biomarkers. OPA discovery cohort dataset was selected from the large sample cohort[21] (https://r12.finngen.fi/) of the FinnGen R12 and contains 629 cases and 496 621 controls. And the OPA validation cohort was derived from the GWAS catalog independent dataset (GCST90436016) and contained 58 British cases and 401 245 British controls. The protein quantitative trait locus (pQTL) data used for mediation analysis were obtained from the deCODE Genetics Consortium[22] and included 4907 proteins from 35 559 Icelander populations.

Selection of IVs For all eQTL summary statistics, genes significantly associated with peripheral blood ($P<5\times10^{-8}$) were selected as IVs, and linkage disequilibrium (LD; $r^2<0.001$, window size=10 000 kb) was removed to ensure SNP independence. For the pQTL summary statistics used in the mediation analysis, $r^2<0.01$ was used to guarantee the number of IVs when LD was removed. Moreover, we all used the *F*-statistic to evaluate the strength of the selected IVs, where SNPs with an *F*-statistics below 10 were excluded to avoid weak instrument bias [$F=R^2\times(N-2)/(1-R^2)$; $R^2=2\times EAF\times(1-EAF)\times beta^2$][23].

MR Analysis and Sensitivity Analysis The MR was used for causal relationships between genes and OPAs. When only one IV was available, Wald ratio was used to infer causality. For exposures with multiple IVs, causality was inferred using the inverse-variance weighted method (IVW), MR Egger, Weighted median, Simple Mode, and Weighted Mode. IVW generally provided the highest statistical power[24], so we used IVW as the main method. When there was heterogeneity, the random-effects IVW test provided more conservative and robust estimates. Otherwise, a fixed effects model was used. MR-Egger was used to test the existence of multilevel effects. It can provide valid causal estimates when pleiotropy is present[25]. Sensitivity analyses were performed to assess the robustness of causality. Horizontal pleiotropy was assessed using MR-Egger regression and MR-PRESSO. The non-zero intercept of the MR-Egger regression indicated directional pleiotropy. Heterogeneity between IVs was assessed using Cochran's *Q* test. In addition, leave-one-out was used to assess whether individual SNPs drive causal estimates.

Colocalization Analysis Bayesian co-localization analysis was used to assess the probability that two traits share the same causal variant using the "coloc" package[26] with default parameters (v. 5.2.3). Bayesian co-localization provides posterior probabilities of five hypotheses about whether a single variant is shared between two traits. The analysis assessed support for five exclusivity hypotheses: 1) no association with either phenotype; 2) only associated with phenotype 1; 3) only associated with phenotype 2; 4) both phenotypes were associated with different causal variants; 5) both phenotypes are associated with the same causal variant. Strong colocalization support was considered when the posterior probability (PPH4) of the shared causal variable exceeded 0.8.

Construction of Interaction Networks and Screening of Hub Genes Interaction analysis was performed for potential marker genes of OPA. First, a gene interaction network was constructed from the STRING database (https://cn.string-db.org/) with a minimum interaction threshold of 0.4 (medium confidence). Then, based on the construction and analysis of gene interaction network, the obtained network was further analyzed by CytoHubba, and the key nodes in the network were identified by Maximal Clique Centrality (MCC). Finally, Cytoscape software (version: 3.10.2) was used to visualize and analyze the network.

Functional Enrichment Analysis of Genes Cytoscape functional enrichment module was used to perform functional enrichment analysis on the selected top 15 hub nodes. The biological processes, cellular components, molecular functions, metabolic pathways and signaling pathways involved in these nodes were revealed to verify their correlation with OPA by using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), REACTOME, WikiPathways, and Uniport databases.

Mediation Analysis Mediation analysis aims to assess the pathway from exposure to outcome through mediation, which helps to explore the potential mechanisms by which exposure affects outcome[27]. pQTL from deCODE consortium were used to explore the underlying mechanism of "gene-disease". First, the causal relationship between genes and proteins was evaluated using a two-sample MR to obtain $\beta1$. Second, the causal relationship between protein and OPA was evaluated using the same method to obtain $\beta2$. Mediation effect=$\beta1\times\beta2$. The total effect of genes on OPA was obtained in the previous two-sample MR, direct effect=(total effect−mediation effect). The following formula was used for mediating proportion: mediating proportion=(mediation effect/total effect)×100%. The delta method[27] was used to estimate the 95% confidence interval (CI) of the mediation effect and proportion.

Table 1 IVs for *SEC61A2* and *THNSL2* used for MR analysis

Exposure	SNP	effect_allele.exposure	other_allele.exposure	pval.exposure	se.exposure	beta.exposure	*F*
SEC61A2	rs11257354	A	G	1.13E-09	0.0322458	-0.196383	37.08776681
	rs12766360	T	A	1.00E-200	0.0117018	0.370922	1004.689202
	rs150536510	C	A	5.33E-14	0.050354	0.378849	56.60038296
	rs7897943	G	A	7.52E-72	0.0139134	-0.249397	321.2834223
THNSL2	rs62160181	T	C	5.29E-26	0.0176736	-0.186385	111.2059427
	rs2970924	C	T	1.00E-200	0.0159581	0.754203	2233.503027
	rs72845822	A	C	1.00E-200	0.0172389	0.890794	2669.979114
	rs112584171	A	C	1.00E-200	0.0371261	1.19716	1039.721673

IVs: Instrumental variables; MR: Mendelian randomization; SNP: Single nucleotide polymorphism.

RESULTS

Evaluation of Causal Effects in the OPA Discovery Cohort The current OPA discovery dataset was derived from the largest cohort in Finland. According to our quality control criteria, leaving 26 152 IVs for 5430 genes. In the discovery cohort, the IVW revealed 190 genes with potentially causal relationship between expression levels and OPA. Among them, an increase in the expression level of 98 genes increased the risk of OPA, and an increase in the expression level of 92 genes decreased the risk of OPA. *ENO2* [odd ratio (OR): 3.48, 95%CI: 1.65–7.34, *P*=0.001] and *PIK3CB* (OR: 0.29, 95%CI: 0.09–0.89, *P*=0.03) showed the greatest effect with OPA, respectively. *ENO2* is closely related to neurodegenerative diseases[28] and has been used as a diagnostic biomarker for glaucoma in previous studies[29]. *PIK3CB* has also been identified as a candidate gene for diabetic retinopathy in previous studies[30], and diabetic retinopathy also causes OPA[31].

After Bonferroni correction ($P<9.20\times10^{-6}=0.05/5430$), there were no genes that met the correction significance, so we considered these genes as suggestive genes for OPA. In addition, sensitivity analysis showed that there were no SNPs with horizontal pleiotropy in our results ($P>0.05$), and there was no heterogeneity among the results ($P>0.05$). The leave-one-out results also revealed no causal association between any single SNP driver in each pair of linked traits. Therefore, our results are robust.

Evaluation of Causal Effects in the OPA Validation Cohort Similarly, independent OPA data derived from the GWAS Catalog were used for causal association estimation. All thresholds were the same as for the discovery cohort, and the IVW results indicated that 200 genes were potentially associated with OPA. Among them, 96 genes had a positive association with OPA, and 104 genes had a negative association with OPA. *RASSF1* (OR: 62.45, 95%CI: 2.32–1675.23, *P*=0.01) and *C2orf68* (OR: 0.03, 95%CI: 0.003–0.24, *P*=0.001) were the genes with the largest effect size, which might play an important role in the disease. As with the above methods, our results were reliable and stable after sensitivity analysis verification, and there was no bias caused by other factors.

Combining the results of the discovery cohort and the validation cohort, we finally identified two genes with a consistent causal association with OPA after rigorous screening for intersection and consistent effect directions: *SEC61A2* and *THNSL2* (Figure 2A), that is, increased gene expression levels showed a consistent trend with increased OPA risk (Figure 2B–2E), and IVs are shown in Table 1. This result suggests that *SEC61A2* and *THNSL2* may play an important role in the pathogenesis of OPA and provides important clues for subsequent biological studies and potential therapeutic target development.

Colocalization Analysis and Functional Annotation of Variants To further explore the possible causal variation between genes and OPA, we performed a colocalization analysis. The results showed that rs12766005 (PPH4=0.94) might drive the causal relationship between *SEC61A2* and OPA. However, rs149620706 (PPH4=0.99) may drive the causal relationship between *THNSL2* and OPA (Figure 3).

The priority scores of the two SNPs were 3a and 4 by RegulomeDB[32]. rs12766005 showed more evidence of regulation, that is, mapping to transcription factor binding sites (GATA1 and IKZF1) and altering motifs (ARNTL, BHLHE41, MAX, TFE3, TFEB, USF1, *etc*.), as well as DNase sites. In contrast, rs149620706 only mapped to transcription factor binding sites (SP3, DNMT3B, IKZF5, CREB1, RELB, and CEBPG; Table 2).

Interactions among Potential Biomarkers To further expand the potential biomarkers of OPA, we combined the potential genes obtained from the discovery cohort and the validation cohort to obtain 192 up-regulated genes and 194 down-regulated genes. Protein-protein interaction (PPI) analysis was performed on the above-mentioned genes using STRING database to observe the interaction between the downstream proteins encoded by the genes. The top 15 hub genes were identified by Cytoscape. Among the up-regulated

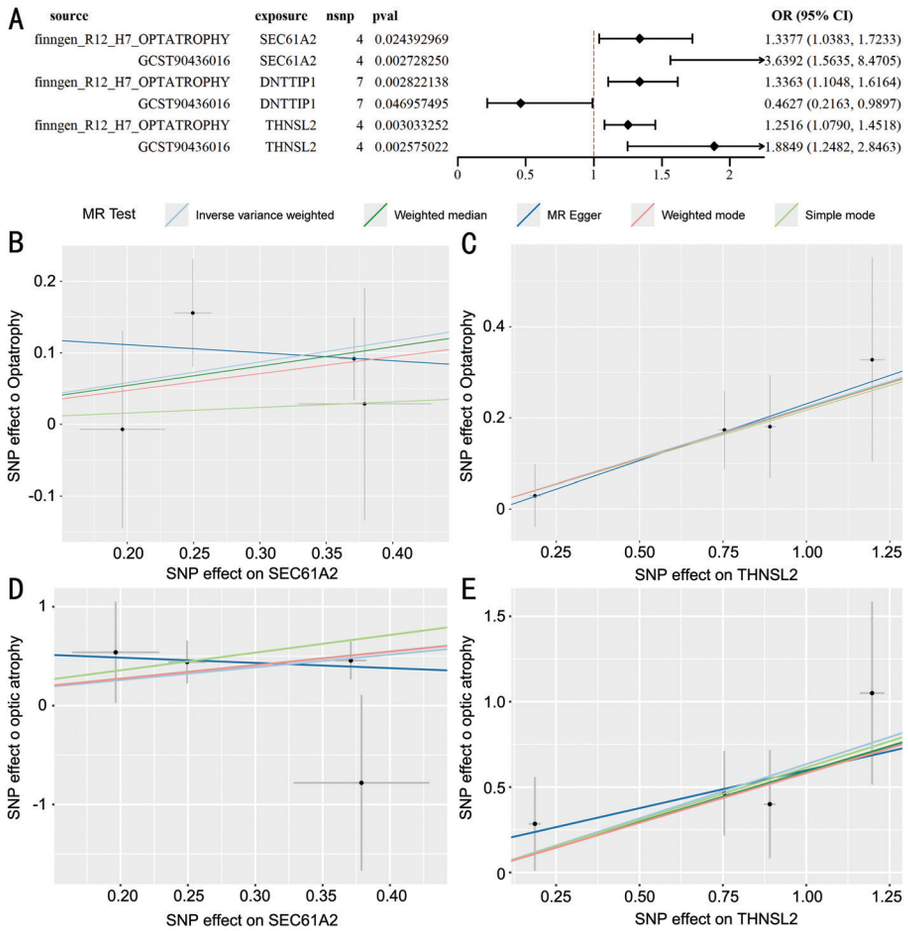

Figure 2 Causality estimates based on OPA discovery and verification cohorts A: Forest plot of causal effects for the discovery and validation cohorts; B: Scatter plot of SEC61A2 in the discovery cohort; C: Scatter plot of THNSL2 in the discovery cohort; D: Scatter plot of SEC61A2 in the validation cohort; E: Scatter plot of THNSL2 in the validation cohort. OR: Odds ratio; CI: Confidence interval; SNP: Single nucleotide polymorphism; MR: Mendelian randomization; OPA: Optic atrophy.

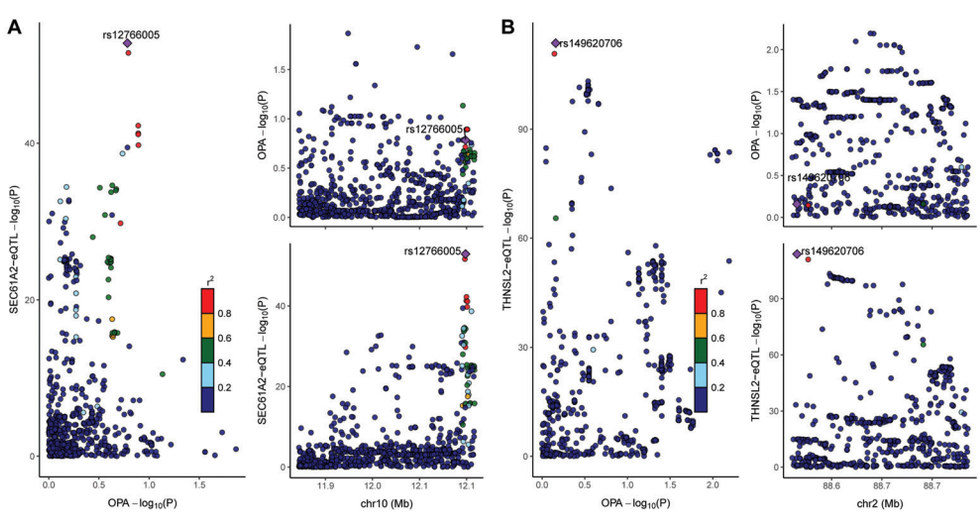

Figure 3 LocusZoom plot for colocalization analysis A: LocusZoom plot for SEC61A2; B: LocusZoom plot for THNSL2. OPA: Optic atrophy; eQTL: Expression quantitative trait loci.

genes, they were obtained and ranked according to the degree of interaction (Figure 4A), namely: *CD74* (14), *SCARB2* (10), *STAT3* (9), *ACO1* (9), *VAMP3* (8), *RAB11A* (8), *ARRB1* (7), *CTSD* (6), *PSMG1* (6), *PIK3R1* (6), *HMGCR* (6), *ENO2* (5), *CD47* (5), *GNAI3* (5), and *PLCB1* (5). In addition, among the down-regulated genes, we obtained another 15 hub genes ranked by the degree of interaction (Figure 4B), namely: *KRAS* (18), *GSK3B* (14), *TLR1* (8), *SIRT1* (7), *RELB* (7), *PPP1CB* (6), *CTSS* (6), *TANK* (6), *LY96* (6), *H2AJ* (5), *TNFRSF13C* (5), *SNAP23* (5), *GNB5* (5), *JARID2* (4), and *PRKCQ* (4).

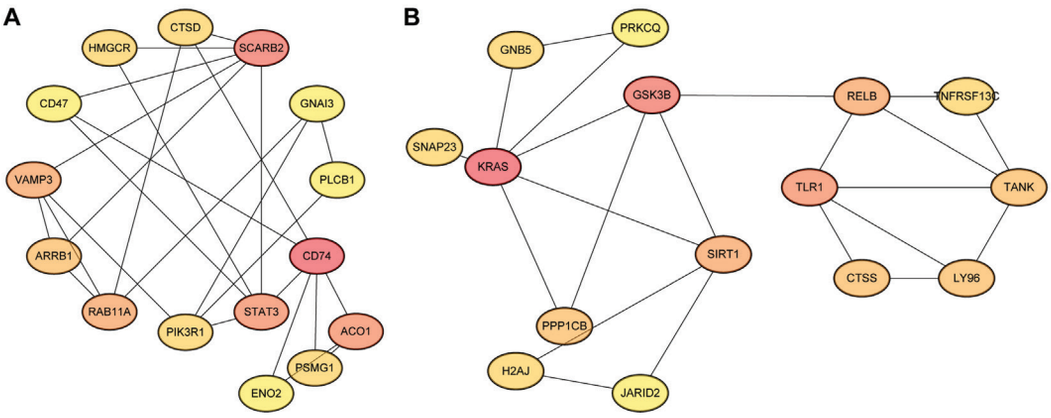

Figure 4 Interaction network plot of potential markers A: Network interaction plot of upregulated hub genes; B: Network interaction plot of downregulated hub genes.

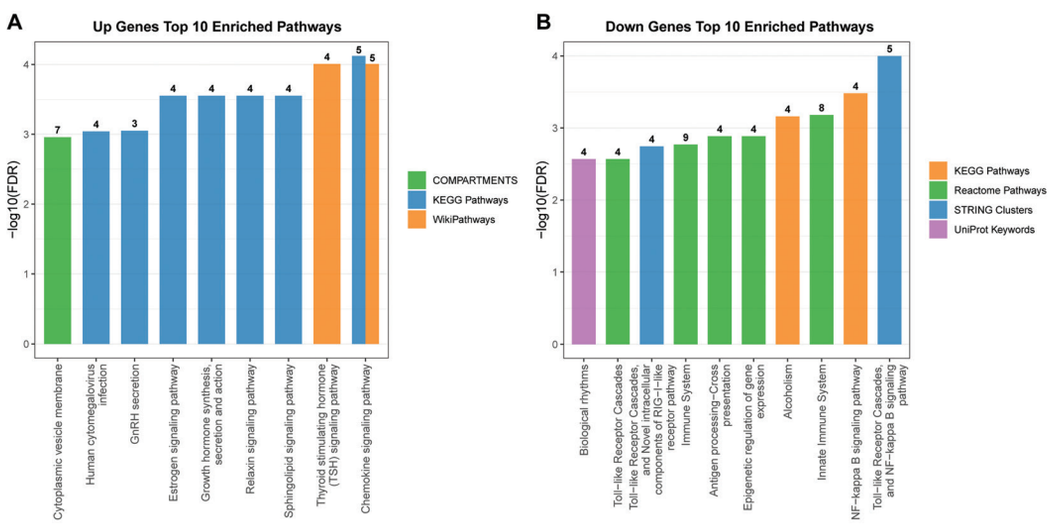

Figure 5 Functional enrichment analysis of hub gene A: Functional enrichment analysis of upregulated genes; B: Functional enrichment analysis of downregulated genes. FDR: False discovery rate; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Enrichment Analysis of Biomarker-Related Pathways Functional enrichment analysis was performed on the Hub genes to explore the links between genes involved in biological regulation. Among the top ten up-regulated hub genes, chemokine signaling pathway was enriched in both KEGG pathway and WikiPathways databases, and there were many pathways related to hormones. For examples, it includes thyroid-stimulating hormone signaling, estrogen signaling, growth hormone synthesis, and gonadotropin-releasing hormone signaling (Figure 5A). For down-regulated genes, the top ten pathways were mainly enriched in immune-related aspects, with two pathways directly related to the immune system, and others such as Toll-like receptor and nuclear factor kappa-B (NF-κB) were also highly related to immunity. In addition, immune-related chemokine signaling was also suggested in the upregulated pathways (Figure 5B). In summary, we speculate that hormonal dysregulation may contribute to the outcome of OPA, and it can also lead to OPA through the imbalance of immune homeostasis.

Mediation Analysis To further explore the potential mechanisms that may be in the "gene-OPA" relationship to explore the proteins that may potentially play a role in the intermediate process, we performed a two-stage MR and mediation analysis. In this part, we mainly explored the genes that were satisfied in both the discovery cohort and the validation cohort.

In the first stage, genes were used as exposures and 4907 plasma proteins were considered as outcomes. The results showed that *SEC61A2* had potential causal relationships with 68 proteins and *THNSL2* had potential causal relationships with 67 proteins. In the second phase, 4907 plasma proteins were used as exposure and OPA as outcome. The results showed that 74 proteins had potential causal relationships with OPA. Sensitivity analyses showed that these results were robust. Finally, the results that met the total effect, mediation effect, and indirect effect direction were screened. Only upper zone of growth plate and cartilage matrix associated (UCMA; mediation proportion=10.20%; beta=0.03; 95%CI: 0.01–0.19; *P*=0.02) played a regulatory role in "*SEC61A2*-OPA", that is, *SEC61A2* may increase the risk of OPA through UCMA (Table 3).

Table 2 Function SNP annotation results

SNP	Method	Peak location	Biosample	Targets	Value
rs12766005	CHIP	chr10:12098866..12099084	CD36-positive erythrocyte precursor cell	GATA1	12
	CHIP	chr10:12098559..12099043	K562	IKZF1	59.07143
rs149620706	CHIP	chr2:88565108..88565658	HEK293	SP3	39.876
	CHIP	chr2:88565247..88565727	HepG2	DNMT3B	8.55349
	CHIP	chr2:88565210..88565754	HepG2	IKZF5	15.66225
	CHIP	chr2:88565138..88565698	HepG2	CREB1	21.43031
	CHIP	chr2:88565086..88565686	GM12878	RELB	23.57308
	CHIP	chr2:88565120..88565680	K562	CEBPG	39.07665

SNP: Single nucleotide polymorphism.

Table 3 The results of mediation analysis of potential regulation

Exposure	Mediate	Outcome	Beta	Se	p_mediation	med_lci	med_uci	med_por
SEC61A2	10977_55_UCMA_UCMA	OPA	0.029685	0.013204	0.024564	0.013081	0.190989	0.102035

OPA: Optic atrophy; med_lci: Mediation proportion lower confidence interval; med_uci: Mediation proportion upper confidence interval; med_por: Mediation proportion.

DISCUSSION

This study employed a two-sample MR to systematically reveal, for the first time from a genetic association perspective, potential causal links between genes and OPA, and identified candidate biomarkers potentially characterizing OPA. By integrating multiple large-scale datasets, we identified a total of 386 genes with potential causal associations with OPA. Among these, *SEC61A2* and *THNSL2* showed consistent effect directions across two independent cohorts, suggesting their potential value as key genes. Colocalization results indicated that rs12766005 and rs149620706 may play important roles. Furthermore, based on gene interaction network and pathway enrichment analyses, we found that hormone secretion and immune-related pathways may play a central role in the pathogenesis of OPA. Finally, our mediation analysis suggests that UCMA potentially mediates the effect of *SEC61A2* in increasing OPA risk.

This study found that *SEC61A2* and *THNSL2* were both positively causally associated with OPA risk in both the discovery and validation cohorts. *SEC61A2* encodes the Sec61α subunit, a transmembrane protein of the endoplasmic reticulum (ER) and a core component of the ER protein translocation channel. It participates in the cotranslational translocation of nascent polypeptide chains and the degradation of misfolded proteins. Studies indicate that ER stress is closely associated with various neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, *etc*.)[33]. In the optic nervous system, ER stress can induce apoptosis of retinal ganglion cells (RGCs), leading to optic nerve fiber degeneration[34]. This study found that high expression of *SEC61A2* increases OPA risk, suggesting it may accelerate RGC axonopathy by exacerbating imbalances in ER protein transport or stress responses. This mechanism is highly consistent with the core pathological features of OPA, namely the degeneration of optic nerve fibers and gliosis.

THNSL2 encodes an enzyme related to threonine metabolism, but its function in the nervous system remains unclear and it represents a novel gene. Mitochondrial dysfunction is a core mechanism in LHON[35]; therefore, we speculate that *THNSL2* may represent a new target for energy metabolism imbalance in OPA. Additionally, the risk gene with the strongest effect size in the discovery cohort, *ENO2*, is a key enzyme in neuronal glycolysis. As a crucial enzyme for RGC energy metabolism, its high expression significantly increased OPA risk (OR=3.48, *P*=0.001). This result is consistent with previous studies: *ENO2* overexpression under hypoxic or metabolic stress conditions may trigger lactate accumulation and cellular acidosis, leading to neuronal damage[36]. Conversely, the protective gene *PIK3CB* inhibits apoptosis by regulating the AKT serine/threonine kinase (AKT)/mechanistic target of rapamycin (mTOR) signaling pathway[37], suggesting that activating the phosphoinositide 3-kinase (PI3K)-AKT pathway could be a potential therapeutic target for OPA.

Furthermore, SNPs identified by traditional GWAS are often located in non-coding regions, making their functional mechanisms difficult to interpret directly. By linking SNPs to cis-eQTLs, this study successfully mapped genetic signals to gene expression levels, providing functional clues for elucidating OPA's molecular mechanisms. For example, rs11573156, a cis-eQTL for *PLA2G2A*, upregulates *PLA2G2A* transcript levels influenced by tissue SP protein levels[38]. Through colocalization analysis, we found that rs12766005 and rs149620706 may drive the associations between *SEC61A2*/*THNSL2* and OPA, respectively. Querying GTEx Portal V10 revealed that rs12766005 is an eQTL for *SEC61A2* in the cerebellum, tibial artery, and tibial nerve; rs149620706

acts as an eQTL for *THNSL2* in multiple tissues, such as the cerebellum, pituitary, tibial nerve, esophagus, and colon. This further strengthens the association between these two genes and OPA.

PPI network analysis showed that OPA-associated genes do not act independently but form complex regulatory networks. By constructing PPI networks for 192 risk genes and 194 protective genes, this study identified several hub genes. In the risk gene network, *CD74* and *STAT3* had the highest degree centrality, highlighting the central role of immune regulation in OPA. *CD74* is associated with optic nerve microglial populations[39]. *STAT3* participates in glial cell activation and cytokine signal amplification; its sustained activation can lead to neurogliosis and axonal damage[40]. Additionally, interactions among metabolism-related genes (*HMGCR*, *ENO2*) suggest a link between energy metabolism imbalance and OPA. *HMGCR*, the rate-limiting enzyme in cholesterol synthesis, may protect the optic nerve through its inhibition by reducing oxidized low-density lipoprotein deposition[41]. In the protective gene network, *KRAS* and *GSK3B* were the core hubs. *KRAS* regulates cell proliferation and survival *via* the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway; its low expression might inhibit RGC apoptosis[42]. *GSK3B* is a key negative regulator of the Wnt/β-catenin pathway; its inhibition promotes β-catenin nuclear translocation, enhancing neuronal anti-apoptotic capacity[43]. These interaction networks not only reveal individual gene functions but also suggest potential mechanisms of multi-gene synergy, providing a theoretical basis for multi-target therapeutic strategies.

Enrichment analysis indicated that upregulated genes were significantly enriched in endocrine signaling pathways such as thyroid hormone and estrogen, while downregulated genes were concentrated in Toll-like receptor and NF-κB pathways. This finding aligns with previous research: haploinsufficiency of the thyroid hormone-related gene *NR2F1* causes Bosch-Boonstra-Schaaf OPA Syndrome[44], and estrogen has a well-defined neuroprotective effect on RGCs[45]. Combining this with the functional annotation of *SEC61A2*, we speculate it might contribute to OPA by interfering with the ER transport of thyroid hormone receptors. Immune-related pathways were enriched among protective genes. *TLR9* expression is elevated in OPA1[46]. The NF-κB pathway plays a central role in regulating pro-inflammatory cytokine expression, and its inhibition can alleviate inflammatory optic nerve damage[47]. Additionally, the chemokine CXCL12 within the chemokine signaling pathway was found to be elevated in a mouse model of growth retardation, alopecia, pseudoanodontia, and OPA syndrome caused by loss-of-function mutations in ANTXR1[48]. Finally, we found that *SEC61A2* may increase the risk of OPA through UCMA. UCMA, also known as Gla-rich protein (GRP), is a vitamin K-dependent protein whose primary function is as a potent inhibitor of pathological calcification[49]. UCMA dysfunction leads to abnormal calcification in the vascular system[49-50]. Similarly, it might cause calcification of the vasculature surrounding the optic nerve. Such pathological calcification could lead to blood flow obstruction, insufficient nutrient supply, or direct mechanical damage to nerve fibers, potentially resulting in neurodegeneration and ultimately developing into OPA.

While MR has been increasingly applied to ophthalmic diseases such as diabetic retinopathy, myopia, glaucoma, age-related macular degeneration, and uveitis, OPA remains genetically unexplored, representing a critical gap in ocular genetics. Our study is the first to apply two-sample MR to dissect the genetic mechanisms of OPA, avoiding confounding biases inherent in traditional observational studies. Second, we characterized potential molecular biomarkers for OPA. Furthermore, we integrated multiple cohorts to validate causal associations, enhancing the robustness of the results. Finally, integrative multi-dimensional analyses enabled construction of a comprehensive molecular regulatory framework governing OPA pathobiology, revealing a novel causal axis wherein SEC61A2 potentiates OPA risk *via* UCMA-mediated mechanisms. Collectively, our investigation establishes a foundational genomic blueprint for OPA research and furnishes clinically actionable biomarker candidates for early diagnostic stratification.

However, the study still has some limitations: Firstly, the statistical power of GWAS is constrained by sample size. The discovery cohort (629 patients) and validation cohort (58 patients) in this study were relatively small, potentially leading to false negatives or overestimation of effect sizes[51]. Secondly, GWAS typically reflects population-level average effects and struggles to capture rare variants or gene-environment interactions. For example, LHON-related mitochondrial mutations (*e.g.*, m.11778G>A) might not be detected by GWAS due to their complex inheritance patterns[52]. Additionally, eQTL data primarily originate from blood tissues, while OPA's pathological processes are concentrated in the retina and optic nerve. The lack of tissue-specific eQTL data may reduce the biological relevance of the results[53].

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