Molecular Vision 2026; 32:308-323 <http://www.molvis.org/molvis/v32/308>
Received 9 September 2025 | Accepted 22 July 2026 | Published 31 July 2026

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Association and interaction of glucose transporter 3 and monocarboxylate transporter 2 gene polymorphisms in Japanese patients with normal-tension glaucoma

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Purpose: Animal models have shown that stress to retinal ganglion cells and the optic nerve compromises the astrocyte-to-neuron lactate shuttle (ANLS) and increases glucose transporter (GLUT) expression and intraretinal glucose concentration. The aim of this study is to investigate the risk of glaucoma development associated with genes related to ANLS and GLUT by conducting single-nucleotide polymorphism (SNP) association and gene–gene interaction analyses in Japanese patients with normal-tension glaucoma (NTG) and controls.

Methods: Comprehensive association analysis of 120 SNPs in the genes aquaporin 9 (*AQP9*), glucose transporter 1 (*GLUT1*), glucose transporter 2 (*GLUT2*), glucose transporter 3 (*GLUT3*), glucose transporter 4 (*GLUT4*), monocarboxylate transporter 1 (*MCT1*), monocarboxylate transporter 2 (*MCT2*), monocarboxylate transporter 4 (*MCT4*), lactate dehydrogenase A (*LDHA*), lactate dehydrogenase B (*LDHB*), lactate dehydrogenase C (*LDHC*), lactate dehydrogenase D (*LDHD*), lactate dehydrogenase A-like 6A (*LDHAL6A*), and lactate dehydrogenase A-like 6B (*LDHAL6B*), as well as gene–gene interaction analysis of 153 SNP pairs from 18 SNPs, was performed in two different populations, comprising a total of 888 patients with NTG and 3,178 normal controls, both within each population and by integrating the populations.

Results: Three SNPs, *GLUT3* rs7976243, *MCT2* rs2706299, and *LDHD* rs11648082, were significantly associated with NTG in the combined analysis of the two populations (*P*c = 0.00044–0.030). The minor allele of rs7976243 was protective (odds ratio [OR] = 0.86), while those of the other two SNPs increased NTG risk (OR = 1.16). SNP–SNP interaction analysis identified one significant pair, *GLUT3* rs11610602–*MCT2* rs11173115, with a protective effect on NTG (*P*c = 0.0060, OR = 0.43). A joint additive effect analysis of 12 SNPs selected using a $p < 0.05$ threshold in the meta-analysis demonstrated a significant combined effect ($p = 0.0017$), and the SNP sum score was associated with NTG risk (OR = 1.09 per allele, $p = 2.7 \times 10^{-7}$).

Conclusions: SNPs in genes of ANLS-related proteins and GLUT, as well as their interactions, may contribute to the genetic risk of NTG in the Japanese population.

Glaucomatous optic neuropathy (GON) is a multifactorial disease involving a complex interplay of genetic factors, epigenetic modifications, and environmental influences, resulting in irreversible degeneration of retinal ganglion cells (RGCs) and their axons [1]. In particular, in normal-tension glaucoma (NTG), where intraocular pressure (IOP) is within the normal range, it is hypothesized that IOP-independent factors are involved in the onset of GON [1].

It is known that brain neurons, including RGCs, utilize not only glucose taken directly from the bloodstream via specific glucose transporters (GLUT) but also lactate produced by astrocytes through aerobic glycolysis from the glucose they absorb. This lactate is transported to neurons via monocarboxylate transporters (MCTs) [2–6]. Lactate transferred from astrocytes to RGCs is converted to pyruvate by lactate dehydrogenase, which is then metabolized through the tricarboxylic acid cycle. During this process, adenosine triphosphate, the primary energy source for cells, is generated through the coupling of oxidative phosphorylation and the electron transport chain.

The transport of lactate as an energy substrate from astrocytes to neurons is referred to as the astrocyte-to-neuron lactate shuttle (ANLS). Although such glia–neuron compartmentalized glucose metabolic processes were discovered in *Caenorhabditis elegans* [7] and honeybees [8] over a quarter century ago, recent studies have increasingly revealed that they also play a critical role in maintaining physiologic functions in the mammalian brain and inner retina [2–6].

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GLUT and MCT belong to the solute carrier transporter (SLC) superfamily, which does not require energy for solute transport and passively transports substrates along their concentration gradients [9,10]. The GLUT family is classified as SLC2A, while MCT is classified as SLC16A. Both families are further subdivided into transporters specific to glial or vascular endothelial cells and those specific to neurons. In the case of GLUT, the former corresponds to GLUT1 and the latter to GLUT3; for MCT, the former includes MCT1 and MCT4, and the latter includes MCT2.

In RGCs, lactate transport is also mediated by aquaporin (AQP) 9, which belongs to the broader class of water channels. The AQP family includes 13 isoforms, which are generally classified into water-selective AQPs that permit only water molecules; aquaglyceroporins that allow the passage of uncharged solutes such as lactate, glycerol, and urea; and super-AQPs that can transport other substances [11,12]. AQP9 belongs to the aquaglyceroporin group, along with AQP3, AQP7, and AQP10.

Previously, we reported that in the retina, MCT1, MCT2, and MCT4 form complexes with AQP9; that MCT2 and AQP9 are expressed in RGCs; that expression of AQP9 is reduced, and these complexes are disrupted in models of optic nerve crush and experimental ocular hypertension; and that these changes are accompanied by a reduction in RGC number and function, along with increased expression of GLUT1 and GLUT3 [13,14]. Furthermore, metabolomic analysis of retinas from a mouse model of ocular hypertension revealed elevated retinal glucose levels, along with increased glutamate concentrations [15].

These observations suggest that MCT and AQP9 cooperatively participate in ANLS at the protein level in RGCs and that stress to RGCs or the optic nerve may disrupt the ANLS interaction between MCT and AQP9. As a result, this disruption may induce a state of excessive glucose dependence, at least in part, via changes in GLUT expression. These findings prompted us to consider the hypothesis that genetic factors regulating ANLS-related molecules or GLUT expression may constitute one of the determinants for the onset of GON in patients with NTG.

The aim of this study is to investigate the risk of GON development associated with genes related to the ANLS and GLUT by conducting a single-nucleotide polymorphism (SNP) association analysis and gene–gene interaction (epistasis) analysis [16] in Japanese patients with NTG and normal controls.

METHODS

Ethics statement: This study was approved by the Medical Ethical Review Board of Kobe University Graduate School of Medicine (approval numbers: B212043 and B242041), Yokohama City University School of Medicine (approval numbers: A150122005 and B240100055), and Tohoku University Graduate School of Medicine (approval numbers: 2021–1-184 and 2024–1-150) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Participants: For the discovery study, 407 patients with NTG aged 40 years or older and 1,096 normal controls aged 50 years or older were recruited from patients attending Kobe University Hospital or Yokohama City University Hospital. For the replication study, 481 patients with NTG aged 40 years or older from Tohoku University Hospital and 2,082 individuals over 50 years of age from a general population cohort collected by the Tohoku Medical Megabank Organization [17] were enrolled.

The inclusion and exclusion criteria were based on previously published reports [18–20] as follows: NTG was diagnosed in the patients based on the following stringent inclusion criteria: the existence of GON accompanied by corresponding visual field loss, normal open angle with a Shaffer grade of 2 or higher, absence of IOP exceeding 21 mm Hg upon repeated measurement using Goldmann applanation tonometry without pharmacologic intervention, and the exclusion of any other retinal, optic nerve, or cerebral pathologies. Glaucomatous optic nerve abnormality was diagnosed when the vertical cup/disc ratio of the optic nerve head was 0.7 or greater, the rim width at the superior (11 to 1 o'clock) or inferior (5 to 7 o'clock) regions was less than or equal to 10% of the disc diameter, the interocular difference in the vertical cup/disc ratio was 0.2 or more, or a nerve fiber bundle defect was present. The Humphrey visual field test was conducted using the Swedish Interactive Standard Algorithm Program 30–2 or 24–2 (Carl Zeiss Meditec, Oberkochen, Germany). In accordance with Anderson and Patella's criteria [21], the hemifield was considered abnormal when the pattern deviation probability plot revealed a cluster of three or more nonedge contiguous points (when the 30–2 program was used) with a probability of less than 5% in the upper or lower hemifield and in one of these points with a probability of less than 1%.

Additionally, the patient groups were rigorously categorized using the following inclusion and exclusion criteria. Patients with a refractive error of less than −8.0 D (the spherical equivalent [SE]) and those with a diagnosis of NTG at age under 40 or over 60 were excluded. The following changes

were made to the selection criteria based on participant age to account for the detrimental effects of aging on the RGCs [18,19]: (1) no modification if the patient was diagnosed before the age of 50, (2) mean deviation (MD) of −10.00 dB or worse in at least one eye if the disease was diagnosed when the patient was between the ages of 50 and 55, and (3) MD of −15.00 dB or worse in at least one eye if the disease was diagnosed when the patient was older than 55. Patients diagnosed before 40 years of age were excluded to reduce potential genetic heterogeneity associated with early-onset disease. Patients whose refractions had altered during diagnosis—for instance, as a result of refractive surgery or cataract surgery—were not included. When only one eye had glaucomatous visual field loss, the affected eye's refraction was used, and the glaucomatous visual field loss was assessed. When both eyes had glaucomatous visual field loss, the more seriously affected eye's refraction and glaucomatous visual field loss were used.

Healthy volunteers from a geographic area comparable to that of the patients with NTG served as the control participants. They had no myopia or mild myopia with refractive errors of −3.00 D or less, nor any glaucoma or systemic or local illnesses that could cause glaucoma or alterations to the optic disc.

Candidate gene association analysis: A comprehensive SNP analysis was conducted on 14 gene regions: *AQP9*, *GLUT1*, *GLUT2*, *GLUT3*, *GLUT4*, *MCT1*, *MCT2*, *MCT4*, *LDHA*, *LDHB*, *LDHC*, *LDHD*, *LDHAL6A*, and *LDHAL6B*. We selected a total of 120 tagging SNPs that collectively covered the entire region of each gene, including 5 kb upstream and downstream. SNP selection was based on the HapMap Japanese dataset (minor allele frequency ≥5%, pairwise $r^2 \geq 0.8$), using the LD TAG SNP selection tool available on the SNPinfo web server.

SNP genotyping for the discovery cohort was performed using the TaqMan 5′ exonuclease assay, and primer–probe sets were supplied by Thermo Fisher Scientific (Foster City, CA). The polymerase chain reaction (PCR) for each SNP was performed with a 10-µl reaction mixture that contained 1× TaqMan GTXpress Master Mix (Thermo Fisher Scientific), 1× TaqMan SNP Genotyping Assay primer/probe mix, and 3 ng genomic DNA. The PCR conditions were as follows: 95 °C for 20 s, followed by 40 cycles of denaturation at 95 °C for 3 s and annealing/extension at 60 °C for 20 s. The fluorescent probe signal was detected using the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific), according to the manufacturer's instructions.

In the replication cohort, we used genome-wide association study data generated using the Japonica Array NEO by Tohoku Medical Megabank Organization [22]. These data were imputed following the method described in a previous study [23]. Specifically, a cross-imputed reference panel was first constructed from the 3.5KJPNv2 [24] and 1000 Genomes Project phase 3 [25] datasets using IMPUTE2 [26]. Genotype imputation was then performed using IMPUTE4 [27], with the resulting merged reference panel, after prephasing with SHAPEIT2 [28]. Of the 120 SNPs selected for analysis, one SNP (rs11024665) could not be imputed and was excluded. The remaining 119 SNPs, all with an INFO score >0.8, were used for subsequent analyses.

Association analyses of SNPs were performed under an additive genetic model using logistic regression for binary traits, as implemented in SNP & Variation Suite software version 8.8.3 (Golden Helix, Bozeman, MT) and PLINK software [29]. Age and sex were included in the model as covariates. The obtained *p* values were corrected for multiple testing with the Bonferroni method by multiplying the nominal *p* values by the number of tagging SNPs (n = 120). A corrected *P* (*Pc*) value <0.05 was considered significant. Linkage disequilibrium (LD) between SNPs was assessed using SNP & Variation Suite software.

SNP–SNP interaction analysis: We also performed SNP–SNP interaction analysis using the epistasis function of PLINK. During this process, SNPs in strong LD, defined as $r^2 > 0.70$, were subjected to LD pruning by selecting one representative SNP from each LD group, choosing the variant with the lowest meta-analysis *p* value within each group, resulting in the exclusion of 20 SNPs. In addition, SNPs with a minor allele frequency <5% or a Hardy–Weinberg equilibrium *p* value <0.05 were excluded to further ensure data quality. As a result, 19 SNPs were removed, leaving 81 after quality control and pruning. Of these 81 SNPs, 18 that showed nominal significance ($p < 0.10$) in the meta-analysis of SNP associations combining the discovery and replication cohorts were selected. A total of 153 SNP pairs derived from these 18 SNPs were then included in the SNP–SNP interaction analysis. Asymptotic *p* values for the interaction terms were computed based on logistic regression for binary traits. The obtained *p* values were corrected for multiple testing with the Bonferroni method by multiplying the nominal *p* values by the number of SNP pairs (n = 153), and a *Pc* value <0.05 was considered significant.

Meta-analysis: Meta-analyses combining the discovery and replication cohorts were conducted for both the SNP association results and the SNP–SNP interaction results, using the inverse-variance method under a random-effects model with the META software, which accounts for heterogeneity in effect sizes (odds ratios [ORs]) between cohorts. The

obtained *p* values were corrected for multiple testing with the Bonferroni method by multiplying the nominal *p* values by the number of tagging SNPs (n = 120) or SNP pairs (n = 153). A *P*c value <0.05 was considered significant.

Joint additive effect analysis: A joint additive effect analysis was performed to evaluate the combined contribution of multiple candidate SNPs. Twenty SNPs that met the selection threshold of $p < 0.05$ in the meta-analysis of single-variant association tests were first selected as candidate variants. These SNPs were further filtered by LD pruning ($r^2 > 0.70$), minor allele frequency (<5%), and deviation from Hardy–Weinberg equilibrium ($p < 0.05$), resulting in 12 SNPs for analysis.

For the joint additive effect analysis, genotype and phenotype data from the discovery and replication cohorts were merged into a single dataset, and a cohort indicator variable was included as a covariate to account for between-cohort differences. Missing genotype data were imputed using the cohort-specific mean allele dosage for each SNP. Joint additive effects were assessed using a multivariable logistic regression model in which all 12 SNPs were simultaneously included under an additive genetic model, with adjustment for age, sex, and cohort. For each SNP, mutually adjusted ORs, 95% confidence intervals (CIs), and *p* values were estimated. The overall joint effect of the 12 SNPs was evaluated using a likelihood ratio test comparing the full model (covariates + all SNPs) with the covariate-only model. In addition, a SNP sum score, defined as the total number of risk alleles across the 12 SNPs, was calculated for each individual, and its association with NTG was examined using a separate logistic regression model.

All statistical analyses were conducted in R (version 4.3.1) using the glm function with a binomial family. Regression summaries and effect estimates were obtained with the broom and dplyr packages.

Functional annotation: We investigated the functional roles of the identified SNPs using HaploReg v4.2 [30] and RegulomeDB [31]. Expression quantitative trait locus data were extracted from the GTEx Portal online database, version 10, which provides expression quantitative trait locus results across 47 different human tissues for assessment of associations between an identified SNP and the target gene [32].

RESULTS

Demographic and clinical characteristics of the study populations: The demographic and clinical characteristics of patients with NTG and controls are summarized in Table 1. In the discovery cohort, the mean ages of patients and controls were 49.3 ± 5.4 years and 63.1 ± 8.5 years, respectively, while in the replication cohort, they were 61.5 ± 12.1 years and 64.0 ± 6.8 years, respectively. A significant difference in age was observed between patients and controls in both cohorts. The NTG cases in the discovery cohort were significantly younger than those in the replication cohort. In contrast, there was no difference in age among the controls between the two cohorts. In terms of sex distribution, 43.0% of patients and 44.5% of controls in the discovery cohort were male, and 49.9% of patients and 50.1% of controls in the replication cohort were male. No significant difference in sex distribution was observed in either cohort. The proportion of females among NTG cases in the discovery cohort was significantly higher than that in the replication cohort, whereas there was no difference between the two cohorts among the controls. Age and sex were included as covariates in the association analyses (see Methods). The mean SE in the discovery cohort was −3.82 ± 2.95 D for patients and 0.28 ± 1.88 D for controls, showing a significant difference between the groups. In the replication cohort, the mean SE for patients was −4.51 ± 4.12 D. The NTG cases in the replication cohort had significantly greater myopic refractive error than those in the discovery cohort. SE data were not available for controls in the replication cohort; therefore, SE was not included as a covariate in the association analyses to ensure consistent covariate adjustment across both cohorts. The mean MDs of patients in the discovery cohort and replication cohort were −9.57 ± 7.81 dB and −14.12 ± 8.14 dB, respectively. The latter had significantly worse MD compared with the former. The mean IOPs of patients in the discovery cohort and replication cohort were 15.73 ± 2.78 mmHg and 13.13 ± 2.60 mmHg, respectively. The former was significantly higher than the latter.

To assess the potential confounding effect of SE, we performed a quantitative linear regression analysis within the discovery cohort, treating SE as a continuous outcome variable and each SNP as a predictor. None of the SNPs tested in this study showed a significant association with SE, suggesting that SE is unlikely to be a confounding factor in our genetic association analyses.

Association analysis: In this study, we conducted association analyses on 120 tagging SNPs covering 14 gene regions (*AQP9*, *GLUT1*, *GLUT2*, *GLUT3*, *GLUT4*, *MCT1*, *MCT2*, *MCT4*, *LDHA*, *LDHB*, *LDHC*, *LDHD*, *LDHAL6A*, and *LDHAL6B*). First, these 120 SNPs were analyzed in the discovery cohort, and subsequently, the same set of SNPs was examined in an independent replication cohort to validate the findings. Of these SNPs, 12 showed an association with NTG with $p < 0.05$ in the discovery cohort, with the strongest association observed for rs3820549 located in *GLUT1* (Appendix

Table 1. Demographic and clinical characteristics of the study populations.

Characteristic	Discovery cohort			Replication cohort			Inter-cohort difference	
	NTG cases	Controls	*P*	NTG cases	Controls	*P*	NTG cases	Controls
Number of subjects	407	1096		481	2082		*P*	
Mean age, year	49.3±5.4	63.1±8.5	<0.00001	61.5±12.1	64.0±6.8	<0.00001	<0.0001	0.0012
Male/female, %	43.0/57.0	44.5/55.5	0.60	49.9/50.1	49.7/50.3	0.98	0.040	0.12
Mean spherical equivalent, diopter	−3.82±2.95	0.28±1.88	<0.00001	−4.51±4.12	NA	NA	0.0049	NA
Mean deviation, dB	−9.57±7.81	NA	NA	−14.12±8.14	NA	NA	<0.0001	NA
Intraocular pressure, mmHg	15.73±2.78	NA	NA	13.13±2.60	NA	NA	<0.0001	NA

NA, not applicable. Differences in age and spherical equivalent were tested using the Mann–Whitney U test; sex distribution was compared using the chi-square test.

1). However, none of these associations remained significant after Bonferroni correction for multiple testing (*Pc* > 0.05). In the replication cohort, six SNPs showed an association with NTG with $p < 0.05$, with the strongest signal observed for rs12313154 located in *GLUT3* (Appendix 1); however, none remained significant after Bonferroni correction (*Pc* > 0.05). Among these six SNPs, only rs7976243 in *GLUT3* showed $p < 0.05$ in the discovery cohort, exhibited a consistent direction of effect (OR) between the two cohorts, and achieved statistical significance in the subsequent meta-analysis (*Pc* = 0.00044; Table 2, Appendix 1).

In addition to *GLUT3* rs7976243, the meta-analysis combining the discovery and replication cohorts identified two more SNPs that showed statistically significant associations with NTG: rs2706299 in *MCT2* and rs11648082 in *LDHD* (*Pc* = 0.030 and 0.0084, respectively; Table 2, Appendix 1). Among these three SNPs, the minor allele of *GLUT3* rs7976243 was associated with a decreased risk of NTG (OR = 0.86), whereas the minor alleles of the other two SNPs were associated with an increased risk of NTG (*MCT2* rs2706299: OR = 1.16; *LDHD* rs11648082: OR = 1.16).

SNP–SNP interaction analysis: In the discovery cohort, seven SNP pairs showed associations with NTG at $p < 0.05$, and 12 SNP pairs did so in the replication cohort; however, none of these associations remained significant after Bonferroni correction (Appendix 2). Among them, two SNP pairs, *GLUT3* rs11610602-*MCT2* rs11173115 and *AQP9* rs2414535-*LDHD* rs755099, showed $p < 0.05$ in both cohorts and exhibited consistent directions of effect (ORs). Of these, the *GLUT3* rs11610602–*MCT2* rs11173115 pair reached statistical significance in the meta-analysis after multiple testing correction (*Pc* = 0.0060), with an odds ratio of 0.43, indicating a protective interaction between their minor alleles in NTG development (Table 3). In contrast, the *AQP9* rs2414535–*LDHD* rs755099 pair did not reach significance in the meta-analysis (*Pc* = 0.18; Table 3). In total, 16 SNP pairs showed $p < 0.05$ in the meta-analysis, but only the *GLUT3* rs11610602–*MCT2* rs11173115 pair remained significant after correction (Appendix 2).

Joint additive effect analysis: To further evaluate the combined effect of the identified variants, we performed a joint additive effect analysis of the 12 SNPs selected based on the meta-analysis of association tests with $p < 0.05$ (Table 4). In the SNPwise analysis, none of the SNPs showed a significant independent association after correction for multiple testing, although rs7165001 showed a nominal association ($p = 0.034$). These findings suggest that the individual effects of each SNP are modest and may not be independently detectable when accounting for other variants. In contrast, the likelihood ratio test demonstrated that inclusion of all 12 SNPs significantly improved the model fit compared with the base model ($p = 0.0017$), indicating a significant joint effect of these variants. Furthermore, the SNP sum score, defined as the total number of risk alleles across the 12 SNPs, was significantly associated with the risk of NTG ($p = 2.7 \times 10^{-7}$, OR = 1.09), corresponding to an approximately 9% increase in NTG odds per additional risk allele. In other words, if all 12 alleles are risk alleles, the OR would be $1.09^{12} = 2.81$. Thus, having 12 risk alleles results in an OR 2.81 times higher compared with having zero risk alleles (OR = 1).

Functional analysis: The five SNPs identified in the association analyses and SNP–SNP interaction analyses are located within the noncoding region, including intronic and untranslated regions, which can significantly affect gene expression. Among the three SNPs identified in the association analyses, the HaploReg v4.2 database predicted that all

Table 2. Association results of 20 SNPs with p<0.05 in the meta-analysis for normal tension glaucoma.

SNP (Gene)	Chr:position (GRCh37)	Alleles (1>2)	Population	Minor allele frequency (cases/controls)	*P*	*Pc*	OR (95% CI)
rs7976243	12:8,086,083	A>C	Discovery	0.448/0.493	0.028	1.00	0.83 (0.71–0.98)
(*GLUT3*)			Replication	0.456/0.498	0.037	1.00	0.86 (0.80–0.92)
			Meta-analysis		3.7E-06	0.00044	0.86 (0.80–0.91)
rs11648082	16:75,145,538	C>T	Discovery	0.322/0.300	0.24		1.11 (0.93–1.31)
(*LDHD*)			Replication	0.298/0.268	0.049	1.00	1.18 (1.08–1.28)
			Meta-analysis		7.0E-05	0.0084	1.16 (1.08–1.25)
rs2706299	12:60,093,215	A>T	Discovery	0.275/0.249	0.14		1.15 (0.96–1.38)
(*MCT2*)			Replication	0.223/0.200	0.087		1.16 (1.06–1.27)
			Meta-analysis		2.5E-04	0.030	1.16 (1.07–1.26)
rs755099	16:75,148,604	G>A	Discovery	0.176/0.162	0.38		1.10 (0.89–1.36)
(*LDHD*)			Replication	0.146/0.129	0.084		1.20 (1.08–1.33)
			Meta-analysis		6.3E-04	0.076	1.18 (1.07–1.30)
rs7165001	15:59,505,191	C>T	Discovery	0.125/0.109	0.20		1.18 (0.92–1.52)
(*LDHAL6B*)			Replication	0.123/0.106	0.14		1.18 (1.05–1.32)
			Meta-analysis		0.0017	0.203	1.18 (1.06–1.31)
rs11173115	12:60,093,536	T>G	Discovery	0.081/0.097	0.18		0.82 (0.62–1.10)
(*MCT2*)			Replication	0.098/0.117	0.16		0.85 (0.75–0.95)
			Meta-analysis		0.0024	0.283	0.84 (0.75–0.94)
rs730168	16:75,150,275	T>C	Discovery	0.362/0.352	0.59		1.05 (0.89–1.23)
(*LDHD*)			Replication	0.348/0.323	0.12		1.13 (1.04–1.22)
			Meta-analysis		0.0030	0.364	1.11 (1.04–1.20)
rs1043503	16:75,144,297	G>A	Discovery	0.179/0.169	0.52		1.07 (0.87–1.32)
(*LDHD*)			Replication	0.178/0.157	0.14		1.16 (1.05–1.28)
			Meta-analysis		0.0036	0.431	1.14 (1.04–1.25)
rs7966327	12:8,089,824	A>G	Discovery	0.447/0.394	0.0070	0.84	1.26 (1.06–1.48)
(*GLUT3*)			Replication	0.366/0.338	0.16		1.12 (1.03–1.22)
			Meta-analysis		0.0036	0.433	1.16 (1.05–1.28)
rs2541279	12:8,085,547	T>C	Discovery	0.450/0.412	0.063		1.17 (0.99–1.37)
(*GLUT3*)			Replication	0.391/0.368	0.23		1.09 (1.01–1.18)
			Meta-analysis		0.0037	0.449	1.11 (1.03–1.18)
rs2178672	12:60,130,146	A>C	Discovery	0.065/0.062	0.76		1.05 (0.75–1.47)
(*MCT2*)			Replication	0.066/0.052	0.16		1.24 (1.06–1.44)

SNP (Gene)	Chr:position (GRCh37)	Alleles (1>2)	Population	Minor allele frequency (cases/controls)	*P*	*Pc*	OR (95% CI)
			Meta-analysis		0.0083	0.994	1.20 (1.05–1.38)
rs2706289	12:60,138,111	T>C	Discovery	0.065/0.062	0.76		1.05 (0.75–1.47)
(*MCT2*)			Replication	0.066/0.052	0.16		1.24 (1.06–1.44)
			Meta-analysis		0.0083	0.994	1.20 (1.05–1.38)
rs16939881	15:58,471,979	G>C	Discovery	0.079/0.073	0.57		1.09 (0.81–1.48)
(*AQP9*)			Replication	0.096/0.073	0.022	1.00	1.34 (1.18–1.53)
			Meta-analysis		0.0098	1.00	1.27 (1.06–1.52)
rs3820547	1:43,398,147	A>G	Discovery	0.037/0.041	0.65		0.91 (0.59–1.38)
(*GLUT1*)			Replication	0.043/0.055	0.20		0.80 (0.67–0.95)
			Meta-analysis		0.012	1.00	0.82 (0.69–0.96)
rs6581273	12:60,178,940	C>T	Discovery	0.074/0.069	0.65		1.08 (0.78–1.48)
(*MCT2*)			Replication	0.071/0.059	0.20		1.20 (1.04–1.39)
			Meta-analysis		0.013	1.00	1.18 (1.04–1.35)
rs8035807	15:58,440,194	T>C	Discovery	0.136/0.162	0.079		0.82 (0.65–1.03)
(*AQP9*)			Replication	0.112/0.124	0.41		0.90 (0.80–1.02)
			Meta-analysis		0.025	1.00	0.88 (0.79–0.98)
rs4405356	12:60,135,670	C>T	Discovery	0.393/0.354	0.044	1.00	1.19 (1.00–1.40)
(*MCT2*)			Replication	0.324/0.310	0.36		1.07 (0.99–1.16)
			Meta-analysis		0.031	1.00	1.10 (1.01–1.19)
rs10877334	12:60,156,120	G>A	Discovery	0.077/0.078	0.99		1.00 (0.74–1.35)
(*MCT2*)			Replication	0.080/0.095	0.23		0.85 (0.75–0.97)
			Meta-analysis		0.031	1.00	0.88 (0.78–0.99)
rs1579333	16:75,152,974	C>T	Discovery	0.208/0.204	0.83		1.02 (0.84–1.25)
(*LDHD*)			Replication	0.210/0.184	0.097		1.17 (1.06–1.28)
			Meta-analysis		0.043	1.00	1.13 (1.00–1.26)
rs11173120	12:60,109,110	G>A	Discovery	0.167/0.172	0.73		0.96 (0.77–1.20)
(*MCT2*)			Replication	0.190/0.203	0.31		0.91 (0.83–1.00)
			Meta-analysis		0.0499	1.00	0.92 (0.84–1.00)

1, major allele; 2, minor allele; OR, odds ratio; CI, confidence interval.

Table 3. Interaction results of SNP pairs with p<0.05 in the meta-analysis for normal-tension glaucoma

SNP–SNP pairs		Population	*P*	*Pc*	OR (95% CI)
SNP 1 (Gene/Chr:position)	SNP 2 (Gene/Chr:position)				
rs11610602	rs11173115	Discovery	0.0013	0.20	0.33 (0.17–0.65)
(*GLUT3*/Chr12:8,070,815)	(*MCT2*/Chr12:60,093,536)	Replication	0.0065	0.99	0.49 (0.29–0.82)
		Meta-analysis	3.9E-05	6.0E-03	0.43 (0.28–0.64)
rs2414535	rs755099	Discovery	0.019	1.00	1.44 (1.06–1.94)
(*AQP9*/Chr15:58,433,290)	(*LDHD*/Chr16:75,148,604)	Replication	0.025	1.00	1.41 (1.04–1.89)
		Meta-analysis	0.0012	0.18	1.42 (1.15–1.76)
rs7976243	rs7165001	Discovery	0.013	1.00	0.64 (0.45–0.91)
(*GLUT3*/Chr12:8,086,083)	(*LDHAL6B*/Chr15:59,505,191)	Replication	0.16		0.79 (0.57–1.10)
		Meta-analysis	0.0068	1.00	0.72 (0.57–0.91)
rs2300141	rs11173115	Discovery	0.21		1.30 (0.86–1.96)
(*GLUT3*/Chr12:8,081,949)	(*MCT2*/Chr12:60,093,536)	Replication	0.034	1.00	1.45 (1.03–2.05)
		Meta-analysis	0.015	1.00	1.39 (1.07–1.81)
rs2706299	rs9901910	Discovery	0.19		0.82 (0.61–1.10)
(*MCT2*/Chr12:60,093,215)	(*MCT4*/Chr17:80,198,007)	Replication	0.035	1.00	0.71 (0.52–0.98)
		Meta-analysis	0.017	1.00	0.77 (0.62–0.95)
rs7966327	rs2414535	Discovery	0.051		1.27 (1.00–1.61)
(*GLUT3*/Chr12:8,089,824)	(*AQP9*/Chr15:58,433,290)	Replication	0.15		1.18 (0.94–1.47)
		Meta-analysis	0.017	1.00	1.22 (1.04–1.44)
rs1554203	rs16939881	Discovery	0.026	1.00	0.51 (0.28–0.92)
(*AQP9*/Chr15:58,429,547)	(*AQP9*/Chr15:58,471,979)	Replication	0.21		0.73 (0.45–1.19)
		Meta-analysis	0.017	1.00	0.63 (0.44–0.92)
rs7976243	rs11173115	Discovery	0.23		0.78 (0.51–1.18)
(*GLUT3*/Chr12:8,086,083)	(*MCT2*/Chr12:60,093,536)	Replication	0.037	1.00	0.70 (0.50–0.98)
		Meta-analysis	0.018	1.00	0.73 (0.56–0.95)
rs7966327	rs1554203	Discovery	0.12		1.32 (0.93–1.86)
(*GLUT3*/Chr12:8,089,824)	(*AQP9*/Chr15:58,429,547)	Replication	0.077		1.35 (0.97–1.87)
		Meta-analysis	0.018	1.00	1.33 (1.05–1.69)
rs1554203	rs8035807	Discovery	0.32		0.78 (0.48–1.27)
(*AQP9*/Chr15:58,429,547)	(*AQP9*/Chr15:58,440,194)	Replication	0.031	1.00	0.57 (0.35–0.95)
		Meta-analysis	0.027	1.00	0.67 (0.47–0.96)
rs7976243	rs2414535	Discovery	0.036	1.00	0.78 (0.61–0.98)
(*GLUT3*/Chr12:8,086,083)	(*AQP9*/Chr15:58,433,290)	Replication	0.28		0.90 (0.73–1.09)

SNP–SNP pairs		Population	*P*	*Pc*	OR (95% CI)
SNP 1 (Gene/Chr:position)	**SNP 2 (Gene/Chr:position)**				
		Meta-analysis	0.029	1.00	0.84 (0.72–0.98)
rs7976243	rs1554203	Discovery	0.14		0.77 (0.55–1.09)
(*GLUT3*/Chr12:8,086,083)	(*AQP9*/Chr15:58,429,547)	Replication	0.11		0.79 (0.59–1.05)
		Meta-analysis	0.030	1.00	0.78 (0.62–0.98)
rs16939881	rs755099	Discovery	0.14		1.53 (0.87–2.71)
(*AQP9*/Chr15:58,471,979)	(*LDHD*/Chr16:75,148,604)	Replication	0.12		1.48 (0.90–2.43)
		Meta-analysis	0.032	1.00	1.50 (1.03–2.18)
rs7966327	rs7165001	Discovery	0.20		1.28 (0.88–1.84)
(*GLUT3*/Chr12:8,089,824)	(*LDHAL6B*/Chr15:59,505,191)	Replication	0.087		1.36 (0.96–1.93)
		Meta-analysis	0.033	1.00	1.32 (1.02–1.70)
rs11610602	rs2414535	Discovery	0.33		0.86 (0.64–1.16)
(*GLUT3*/Chr12:8,070,815)	(*AQP9*/Chr15:58,433,290)	Replication	0.055		0.77 (0.58–1.01)
		Meta-analysis	0.038	1.00	0.81 (0.66–0.99)
rs11173115	rs11173120	Discovery	0.038	1.00	0.51 (0.27–0.96)
(*MCT2*/Chr12:60,093,536)	(*MCT2*/Chr12:60,109,110)	Replication	0.24		0.77 (0.50–1.19)
		Meta-analysis	0.039	1.00	0.67 (0.46–0.98)

OR, odds ratio; CI, confidence interval. SNP–SNP interactions with p<0.05 in the meta-analysis are shown. Chromosomal positions are based on the GRCh37 genome build.

Molecular Vision 2026; 32:308-323 <http://www.molvis.org/molvis/v32/308>

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would be located in promoter histone marks and enhancer histone marks, three in DNase hypersensitivity regions, and two in protein-binding regions (Appendix 3). The database also predicted that all of these three SNPs would alter the regulatory motif of transcription factors, suggesting a potential role for them in regulating gene expression. In addition, the RegulomeDB scores of these three SNPs were 1f, indicating that these SNPs are likely to affect transcription factor binding and the expression of a target gene (Appendix 3).

Among the two SNPs identified in the SNP–SNP interaction analyses, HaploReg predicted that *GLUT3* rs11610602 would be located in a DNase hypersensitivity region and a protein-binding region and would alter the regulatory motif of transcription factors. In addition, its RegulomeDB score was 1f, suggesting its potential role in regulating gene expression (Appendix 3). *MCT2* rs11173115 was predicted to be located within promoter histone marks, enhancer histone marks, DNase hypersensitivity, and protein-binding regions and alter the regulatory motif of transcription factors in HaploReg. Its RegulomeDB score was also 1f (Appendix 3).

The GTEx Portal database indicated that two of the three SNPs identified in the association analyses, *GLUT3* rs7976243 and *LDHD* rs11648082, affected expression levels of their target genes. The C allele of *GLUT3* rs7976243 was significantly associated with increased *GLUT3* expression in multitissue meta-analysis ($p = 8.0 \times 10^{-15}$; Appendix 3). The T allele of rs11648082 in *LDHD* was significantly associated with increased expression of *LDHD* in many tissues/organs ($p = 3.0 \times 10^{-9}$; Appendix 3). On the other hand, although *MCT2* rs2706299 showed a significant increase or decrease in *MCT2* expression in a limited number of tissues, the multitissue meta-analysis did not demonstrate a significant association between rs2706299 and *MCT2* expression (p = 0.28; Appendix 3). Regarding heterogeneity across tissues, rs7976243 ($I^2 = 0.58$, $p = 4.0 \times 10^{-7}$) and rs2706299 ($I^2 = 0.55$, $p = 5.0 \times 10^{-6}$) showed moderate to high levels, indicating substantial variation in expression effects. rs11648082 showed a lower degree of heterogeneity ($I^2 = 0.38$, p = 0.0050), suggesting relatively consistent effects across tissues.

Among the two SNPs identified in the SNP–SNP interaction analyses, *GLUT3* rs11610602 exhibited tissue-specific expression differences. The T allele of rs11610602 was significantly associated with decreased *GLUT3* expression specifically in brain tissues ($p = 4.0 \times 10^{-7}$ in brain tissue meta-analysis). However, this association was not observed in the meta-analysis across all GTEx tissues (p = 0.73; Appendix 3). For *MCT2* rs11173115, the direction of effect on *MCT2* expression varied across tissues. In some tissues, the SNP was associated with a significant increase in expression, while in others, a significant decrease was observed. Nonetheless, the overall multitissue meta-analysis did not show a significant association between rs11173115 and *MCT2* expression (p = 0.84; Appendix 3). Across tissues, rs11173115 showed high heterogeneity ($I^2 = 0.73$, $p = 2.0 \times 10^{-16}$), whereas rs11610602 showed minimal heterogeneity ($I^2 = 0.14$, p = 0.21).

Table 4. Results of joint additive effect analysis.

Metric	Gene	Chr:position	*P*	*Pc*	OR (95% CI)
Independent SNP effects in the multivariable model					
rs7976243	*GLUT3*	12:8,086,083	0.27		1.09 (0.94–1.26)
rs7966327	*GLUT3*	12:8,089,824	0.27		1.09 (0.93–1.29)
rs2706299	*MCT2*	12:60,093,215	0.14		1.18 (0.95–1.46)
rs11173115	*MCT2*	12:60,093,536	0.30		1.11 (0.91–1.36)
rs11173120	*MCT2*	12:60,109,110	0.51		1.05 (0.90–1.23)
rs4405356	*MCT2*	12:60,135,670	0.85		0.98 (0.82–1.18)
rs8035807	*AQP9*	15:58,440,194	0.22		1.12 (0.93–1.34)
rs16939881	*AQP9*	15:58,471,979	0.09		1.20 (0.97–1.47)
rs7165001	*LDHAL6B*	15:59,505,191	0.034	0.41	1.21 (1.01–1.45)
rs1043503	*LDHD*	16:75,144,297	0.27		1.21 (0.87–1.68)
rs11648082	*LDHD*	16:75,145,538	0.75		0.95 (0.70–1.29)
rs755099	*LDHD*	16:75,148,604	0.10		1.26 (0.96–1.67)
Overall joint effect (LRT)			0.0017	ΔDeviance=31.4, df=12	
SNP sum score (per 1 additional risk allele)			2.7E-07		1.09 (1.05–1.13)

LRT; likelihood ratio test, OR, odds ratio; CI, confidence interval. Chromosomal positions are based on the GRCh37 genome build.

DISCUSSION

Recent large-scale genome-wide association studies have been reported to explain approximately 14% of the genetic predisposition to glaucoma [33]. Nevertheless, many genetic factors remain unaccounted for as part of the so-called missing heritability [34].

In the present study, we conducted a discovery and replication study in two distinct cohorts using a comprehensive set of SNPs from 14 candidate genes involved in ANLS and GLUT, followed by a meta-analysis. Among the 120 SNPs analyzed in the association study, SNP rs7976243 in *GLUT3* showed nominal significance in the additive model in both the discovery and replication cohorts. In the meta-analysis, it reached statistical significance after Bonferroni correction, with the minor C allele being associated with a reduced risk of NTG (meta-analysis: OR = 0.86; 95% CI, 0.80–0.91; *P*c = 0.00044; Table 2 and Appendix 1).

Previously, we reported increased expression of GLUT1 and GLUT3, along with elevated retinal glucose levels in a mouse model of optic nerve crush [13]. Similarly, in a metabolomic analysis using a mouse model of ocular hypertension, we also observed increased glucose concentrations in the retina [15]. According to prior metabolomic studies, retinal glucose levels were approximately threefold higher 2 weeks after optic nerve crush compared to controls [35] and nearly 50-fold higher during the IOP elevation phase in DBA/2J mice, a model of spontaneous glaucoma [36].

Under physiologic conditions, glucose levels in brain parenchyma are tightly regulated to remain below half of blood glucose levels. When blood glucose rises, cerebral glucose uptake significantly decreases to maintain normal brain metabolic activity [37]. Artificially elevating glucose levels in the brain also leads to a doubling of glutamate concentrations [38]. It is well known that excess glutamate induces neuronal cell death via excitotoxicity [39]. In emergency medicine, elevated blood glucose or increased brain glucose levels following traumatic brain injury are associated with increased patient mortality [40,41]. Proposed mechanisms include tissue acidification in injured brain regions, increased glucose–sodium exchange, mitochondrial fragmentation, enhanced inflammatory responses, promotion of advanced glycation end-product formation, excessive production of reactive oxygen species, and activation of endothelial protease C.

Recently, it has been reported that patients with glaucoma showed higher levels of serum glucose compared to patients without glaucoma and that glaucoma incidence was much greater in patients with hyperglycemia than in those with a high polygenic risk score (PRS) but normoglycemia [42]. Moreover, another recent study showed that while glucose levels were positively associated with glaucoma risk, glycolytic metabolites showed a significant negative correlation [43].

Taken together, the observed risk-reducing effect of the neuron-specific *GLUT3* SNP rs7976243 on NTG may suggest that Japanese individuals with a genetic predisposition for excessive glucose uptake in RGCs are more susceptible to developing NTG.

However, our functional annotation analysis in this study suggests that rs7976243 has a regulatory effect on the expression of a target gene, with the minor C allele being significantly associated with increased *GLUT3* expression across multiple tissues. Nevertheless, the analysis also found moderate heterogeneity of GLUT3 expression (I^2 = 0.58), particularly among brain tissues, with both upregulation and downregulation depending on the brain region. However, since the GTEx database does not include retinal tissue, the effect of rs7976243 on *GLUT3* expression in the retina remains unclear. Therefore, further studies using retina-specific datasets are needed to clarify its role in NTG pathogenesis.

In the association analysis, although rs2706299 in *MCT2* and rs11648082 in *LDHD* did not reach statistical significance in each individual cohort, all three showed a trend toward increased odds ratios for NTG onset under the additive model. In the meta-analysis, they reached statistical significance after Bonferroni correction (Table 2).

MCT2 is a neuron-specific monocarboxylate transporter known to play a critical role in the survival and functional maintenance of RGCs. In our previous study, mice receiving intravitreal injections of an MCT2 inhibitor exhibited more severe RGC damage after optic nerve crush compared to untreated controls. Harun-Or-Rashid et al. [44] reported that knockout of *Mct2* in DBA/2J mice—a model of spontaneous glaucoma—resulted in significantly reduced visual evoked potentials and retinal adenosine triphosphate production, whereas overexpression of *Mct2* preserved RGC density and axon count at levels comparable to controls. These meta-analysis findings may suggest that Japanese individuals genetically predisposed to lower MCT2 expression have a higher risk of developing NTG.

LDHD is a mitochondrial enzyme that converts lactate into pyruvate. Although the precise function of LDHD remains unclear, genetic variants in LDHD have been reported to cause central nervous system disorders accompanied by elevated blood D-lactate levels [45]. On

the other hand, a recent study reported that high levels of lactate, pyruvate, and citrate can reduce glaucoma risk by 29%, even among individuals in the top PRS quartile, and that administration of pyruvate in drinking water suppressed optic nerve degeneration in a spontaneous glaucoma mouse model [46]. It is known that the physiologic isomer of lactate is the L-form, whereas the D-form is inactive or even exerts toxic effects on retinal neurons [47]. Taken together, it has been suggested that in a Japanese population carrying certain *LDHD* gene polymorphisms, the serum or local concentration of the toxic D-lactate isomer increases, resulting in enhanced oxidative stress in the electron transport chain and a potentially elevated risk of developing GON.

Next, we examined pairwise interactions among SNPs from the 14 candidate genes. As a result, individuals carrying both *GLUT3* rs11610602 and *MCT2* rs11173115 showed nominal significance in both the discovery and replication cohorts and reached statistical significance after Bonferroni correction in the meta-analysis, indicating a reduced risk of NTG (meta-analysis: OR = 0.43; 95% CI, 0.28–0.64; *P*c = 0.0060; Table 3). Although these SNPs are located within the same genes as those identified in the single-SNP association analyses (*GLUT3* rs7976243 and *MCT2* rs2706299), they are in low LD at each locus (r^2 = 0.20 for *GLUT3* and r^2 = 0.02 for *MCT2*), suggesting that the observed interaction effect likely involves genetically distinct signals relative to the single-SNP findings.

Notably, neither rs11610602 nor rs11173115 reached statistical significance in the single-SNP association analyses in either cohort when considered individually. Epistasis refers to interactions between multiple, often distant, genes that influence phenotypic expression [16,48–50]. In some cases, each gene may have an additive or multiplicative effect on the trait individually, while in others, no single gene shows an effect on its own, yet a significant phenotypic effect emerges when specific combinations occur. The present findings appear to correspond to the latter case, highlighting the importance of SNP–SNP interactions that cannot be detected through single-marker effects.

Both *GLUT3* rs7976243 and *MCT2* rs2706299 are located in regions where histone modifications characteristic of promoters or enhancers have been identified, suggesting a high likelihood that they function as promoter/enhancer elements. This may explain why each SNP independently contributes significantly to disease risk without requiring interaction with other SNPs. In contrast, although *MCT2* rs11173115 also exhibits regulatory features, including promoter and enhancer histone marks, it did not show a significant independent effect. Instead, its association with NTG emerged through interaction with *GLUT3* rs11610602. These findings suggest that regulatory potential alone may not be sufficient for independent disease association and that certain variants may exert their effects in combination with other genetic factors.

In recent years, PRS, which incorporates a large number of SNPs, has been explored as a means to improve prediction of various phenotypes or diseases, including glaucoma [51,52]. However, SNPs included in the PRS are typically those that show statistically significant associations on their own, and current PRS models remain insufficient to explain the missing heritability. One possible reason is that interactions between SNPs that do not individually affect phenotypic expression may not have been taken into account [16,47,48]. Since no consensus exists regarding significance thresholds in epistasis analysis, the current study limited its discussion to the SNP pair described above. However, when we conducted interaction analysis on 153 SNP pairs derived from 18 selected SNPs, 23 SNP pairs reached nominal significance in either or both cohorts and/or in the meta-analysis (Appendix 2). These interactions showed both increased and decreased odds ratios for NTG onset, depending on the SNP pair. How to best analyze and interpret such data remains an open question.

In this study, we also performed a joint additive effect analysis to evaluate the combined contribution of the 12 SNPs selected based on the meta-analysis of association tests with $p < 0.05$ with PRS in mind (Table 4). Although none of the individual SNPs showed a significant independent association after mutual adjustment, the likelihood ratio test indicated a significant joint effect, and the SNP sum score was significantly associated with NTG, with risk increasing according to the number of risk alleles, with an approximately 9% increase in NTG odds per additional risk allele. These findings suggest that multiple variants with modest individual effects may collectively contribute to NTG susceptibility, consistent with a modest cumulative polygenic effect. As an example, the ORs for *GLUT3* rs11610602 and *MCT2* rs11173115 were 0.86 and 0.82 in the discovery cohort, 0.94 and 0.85 in the replication cohort, and 0.93 and 0.84 in the meta-analysis, respectively. Therefore, if a joint additive effect were present, the expected OR in the meta-analysis would be 0.93 × 0.84 = 0.78. In contrast, the interaction OR obtained from the epistasis analysis in the meta-analysis was 0.43 (*P*c = 0.0060). Thus, the interaction effect of this specific pair greatly exceeded the expected additive effect. Taken together, our data suggest the involvement of both additive and interaction effects in the genetic architecture of NTG with a different power of effect depending on the combination of SNP pairs.

The limitations of this study include a relatively small sample size and the restriction to a Japanese population, which limits the generalizability of our findings. A more stringent threshold (e.g., $p < 0.001$) might help confirm associations in future studies with larger samples or independent cohorts. Diurnal IOP variation over 24 hours was not assessed, and central corneal thickness was not measured. For these reasons, the possibility that some patients with high-tension glaucoma were inadvertently included cannot be entirely excluded. Differences in control subject backgrounds between the discovery and replication cohorts may also explain the limited consistency between association and interaction results across the two groups. Furthermore, we did not conduct functional validation of the SNPs examined, so the interpretations discussed above remain speculative. Nevertheless, our findings are consistent with previous reports showing altered expression and interaction of ANLS-related transporters and GLUTs, as well as altered retinal glucose metabolism, in glaucoma model animals [13–15].

In conclusion, SNPs in genes of ANLS-related proteins and GLUT, as well as their interactions, may contribute to the genetic risk of NTG in the Japanese population.

APPENDIX 1. ASSOCIATION RESULTS OF ALL 120 ANALYZED SNPS WITH NORMAL TENSION GLAUCOMA.

To access the data, click or select the words "Appendix 1." Association analysis results under the additive genetic model for all 120 tagging SNPs covering 14 gene regions (*AQP9, GLUT1, GLUT2, GLUT3, GLUT4, MCT1, MCT2, MCT4, LDHA, LDHB, LDHC, LDHD, LDHAL6A, LDHAL6B*). Minor allele frequencies, p values (nominal and Bonferroni-corrected), and odds ratios with 95% confidence intervals are shown for each cohort and the meta-analysis. SNPs with p<0.05 in the meta-analysis are shown in Table 2 of the main text.

APPENDIX 2. RESULTS OF SNP–SNP INTERACTION ANALYSIS FOR 153 PAIRS DERIVED FROM 18 NOMINALLY ASSOCIATED SNPS.

To access the data, click or select the words "Appendix 2." Pairwise SNP-SNP interaction analysis results for all 153 SNP pairs derived from 18 SNPs selected based on nominal significance (p<0.10) in the meta-analysis. Asymptotic p values for interaction terms, Bonferroni-corrected p values (Pc), and odds ratios with 95% confidence intervals are shown for each cohort and the meta-analysis. The significant pair (*GLUT3* rs11610602 and *MCT2* rs11173115) and the pair that met nominal significance (*AQP9* rs2414535-*LDHD* rs755099) are shown in Table 3 of the main text.

APPENDIX 3. FUNCTIONAL ANNOTATION OF SIGNIFICANT SNPS ASSOCIATED WITH NORMAL TENSION GLAUCOMA.

To access the data, click or select the words "Appendix 3." Functional annotation data for the three SNPs identified in the single-SNP association analyses (*GLUT3* rs7976243, *MCT2* rs2706299, and *LDHD* rs11648082) and the two SNPs identified in the SNP-SNP interaction analysis (*GLUT3* rs11610602 and *MCT2* rs11173115). Data include HaploReg v4.2 predictions (chromatin state, DNase hypersensitivity, protein binding, motif alterations), RegulomeDB scores, and GTEx v10 eQTL results across tissues.

ACKNOWLEDGMENTS

This study was supported in part by JSPS KAKENHI Grant Number 24K02603 (MN, AM). We thank Mses. Sachiyo Sugimoto and Chinatsu Gocho in Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan for their technical assistance. None of the authors have any conflict of interest.

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Articles are provided courtesy of Emory University and The Abraham J. & Phyllis Katz Foundation. The print version of this article was created on 31 July 2026. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.