Interactive Effects of *ATOH7* and *RFTN1* in Association with Adult-Onset Primary Open-Angle Glaucoma

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Purpose. Genome-wide association studies have shown association of the atonal homolog 7 (*ATOH7*) and raftlin lipid raft linker 1 (*RFTN1*) genes with glaucoma-related optic disc parameters. *ATOH7* and *RFTN1* sequence variations were investigated in patients with primary open-angle glaucoma (POAG) and their relationships with vertical cup-to-disc ratio (VCDR) and central corneal thickness (CCT) were determined.

Methods. In 289 unrelated controls and 142 patients with adult-onset POAG, including 117 with high-tension glaucoma (HTG) and 25 with normal-tension glaucoma (NTG), the single exon of *ATOH7* was sequenced by direct sequencing. Additional single-nucleotide polymorphisms (SNP) at upstream *ATOH7* (rs1900004 and rs3858145) and an *RFTN1* SNP (rs690037) were genotyped. Quantitative trait and disease associations were analyzed by linear and logistic regression respectively, controlling for sex and age.

Results. *ATOH7* rs61854782 was associated with VCDR (P = 0.004) in controls and *RFTN1* rs690037 was associated with CCT in combined POAG (HTG+NTG; P = 0.026). No coding mutation was detected in POAG, and no SNP was associated with POAG (P between 0.441 and 0.996). However, *ATOH7* rs3858145 showed significant interaction with *RFTN1* rs690037 in NTG and combined POAG (P = 0.026 and 0.013 respectively). *ATOH7* rs3858145 GG combined with *RFTN1* rs690037 TT conferred risk for glaucoma in HTG, NTG, and combined POAG (odds ratio = 2.11, 8.44, and 2.69, respectively).

Conclusions. Coding mutations of *ATOH7* were unlikely to be involved in POAG. But combination of *ATOH7* and *RFTN1* SNPs increased risk to POAG, indicating their diversified effects in the complex genetics of glaucoma. (*Invest Ophthalmol Vis Sci.* 2012;53:779–785) DOI:10.1167/iovs.11-8277

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Glaucoma is a heterogeneous group of degenerative optic neuropathies with multifactorial etiology.[1,2] It is one of the leading causes of visual loss and blindness worldwide.[3,4] Primary open angle glaucoma (POAG) is one of the most common forms of glaucoma.[5–7] A series of clinical features have been known as risk factors for POAG, such as a thinner retina nerve fiber layer, elevated intraocular pressure, lower central corneal thickness (CCT), and altered optic disc parameters, especially vertical cup-to-disc ratio (VCDR). There are genetic components in the etiology of POAG, as evident from family, twin, and linkage studies.[8–14] Likewise, glaucoma-related biometric parameters such as VCDR and CCT have also been found to be inheritable.[15–18]

Recent genome wide association studies (GWAS) on quantitative traits have identified genes associated with optic disc parameters including optic disc area, optic cup area, and VCDR.[19–21] Among them, atonal homolog 7 (*Drosophila*; *ATOH7*, OMIM 609875; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ National Center for Biotechnology Information [NCBI], Bethesda, MD) has been reported to be strongly associated with optic disc area in a GWAS involving Australian and U.K. cohorts.[19] The finding was replicated in a GWAS in the Netherlands and an Asian GWAS.[20,21] *ATOH7* spans 1.5 kb at chromosome 10, region q21.3. It is a member of the basic helix-loop-helix (bHLH) protein family with similarity to its *Drosophila* homolog, which controls photoreceptor development.[22,23] *ATOH7* participates in the ontogenesis of the vertebrate retina,[24] and deletion of its remote enhancer can cause nonsyndromic congenital retinal nonattachment.[25] Its links with a glaucoma parameter may be due to its role in retinal ganglion cell development.

Raftlin lipid linker 1 (*RFTN1*) showed association with optic cup area in a meta-analysis of GWAS.[19] *RFTN1* is located on 3p24.3 and is necessary for the integrity of lipid raft and B-cell response signal transduction.[26] It modulates T-cell receptor signaling and enhances th17-mediated autoimmune responses.[27] *RFTN1* has been reported to be associated with Alzheimer's disease,[28] with a likely role in neuronal degeneration. Whether *RFTN1* affects the development of POAG remains to be elucidated.

In this study, we investigated the association of *ATOH7* and *RFTN1* with POAG in a cohort of Southern Chinese.

Materials and Methods

Patient Recruitment and Clinical Examination

The study subjects were unrelated and included 142 adult-onset POAG patients and 289 controls recruited at the Joint Shantou International Eye Center in Shantou, a city in South China (Table 1). Both POAG patients and controls received a complete ophthalmic examination. Their highest intraocular pressure (IOP), maximum VCDR, and mean CCT were documented. POAG was defined based on the following criteria: (1) exclusion of secondary causes (e.g., trauma, uveitis, and

Table 1. Demographic and Clinical Features of the Study Subjects

			Age (y)*		IOP (mmHg)†	VCDR†	CCT (μm)†
	n	Female	Range	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	289	147	50–96	71.2 ± 8.6	13.3 ± 2.9	0.3 ± 0.1	542.2 ± 35.8
HTG	117	25	36–85	59.1 ± 12.6	34.1 ± 10.2	0.8 ± 0.2	537.6 ± 37.4
NTG	25	13	40–85	64.3 ± 12.5	16.8 ± 2.7	0.7 ± 0.2	540.3 ± 27.9

* The age at recruitment for controls or the age of disease onset for POAG patients was shown.

† The intraocular pressure (IOP) value is the recorded highest IOP, the vertical cup/disc ratio (VCDR) is the recorded maximal VCDR, and the central corneal thickness (CCT) is the mean of the measurement at the latest follow-up visit before study enrollment.

steroid-induced or exfoliation glaucoma); (2) gonioscopically open anterior chamber angle of Shaffer grade III or IV; (3) characteristic optic disc damage and typical visual field loss by automated perimetry (Humphrey; Carl Zeiss Meditec, Dublin, CA) using the Glaucoma Hemifield test. All the patients with adult-onset POAG had an age at disease onset of 35 years or older; 117 had high-tension glaucoma (HTG; highest IOP, ≥21 mm Hg), and 25 had normal-tension glaucoma (NTG; highest IOP, <21 mm Hg). All controls were ≥50 years of age without a family history or any sign of glaucoma. Their highest IOP was lower than 21 mm Hg, and VCDR was <0.5. Peripheral blood was collected from all participants, and genomic DNA was extracted (Qiamp Blood Kit; Qiagen, Hilden, Germany).

This study was approved by the Ethics Committee of Joint Shantou International Eye Center and was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from each participating subject after an explanation of the nature of the study.

Sequencing of *ATOH7*

The whole *ATOH7* gene (1.5 kb; Fig. 1) plus the 1.0-kb upstream and 0.5-kb downstream sequences were amplified by polymerase chain reactions (PCR) and sequenced in all the 142 POAG patients and 289 controls by direct sequencing, as previously described.[30] The primers listed in Table 2 were designed using PerlPrimer version 1.1.19, referring to the published gene sequence of *ATOH7* in the NCBI Reference Sequence database (http://www.ncbi.nlm.nih.gov).[31]

SNP Genotyping

Five SNPs, including rs61854782, rs7916697, rs1900004, and rs3858145 in *ATOH7*, and rs690037 in *RFTN1* were genotyped. SNPs rs1900004, rs3858145, and rs690037 were genotyped (TaqMan SNP Genotyping Assay; Applied Biosystems, Inc. [ABI], Foster City, CA) according to the protocol suggested by the manufacturer. SNPs rs61854782 and rs7916697 in the 5′-untranslated region (UTR) of *ATOH7* were genotyped by direct sequencing of PCR-amplified DNA.

Statistical Analysis

The Hardy-Weinberg test of each SNP and linkage disequilibrium (LD) analysis was conducted by using Haploview version 4.2 (http://www.broadinstitute.org/haploview, Broad Institute, Massachusetts Institute of Technology, Cambridge, MA).[32] Linear regression was used to analyze association with quantitative traits (VCDR and CCT). The χ^2 test and logistic regression are used for analysis of disease association.[30,33] Regression analysis was implemented by the R statistical language version 2.12.12. Regression *P* values were further adjusted for sex and age. Permutations (×10,000) were used to correct multiple comparisons. $P < 0.05$ after correction was considered significant. The effect size ± SE ($\beta \pm$ SE) in linear regression or odds ratio (OR) in logistic regression was calculated for different genetic models (additive, dominant, and recessive models). In regression analysis the homozygous major, heterozygous, and homozygous minor genotypes were code as 0, 1, and 2 for additive models, 0, 1, and 1 or dominant models, and 0, 0, and 1 for recessive models, respectively. Haplotype association was analyzed using UPHASED version 3.1.4.[33]

To assess the relationship between the *ATOH7* and *RFTN1* in association with POAG, two-locus analysis was performed. In logistic regression of disease association, a full model with the interaction term of SNPs, and a reduced model without the interaction term were compared, to assess the effects of interaction. χ^2 tests with $df = 1$ were used to test whether adding the interaction term would significantly reduce the model deviance.

Results

Association with VCDR and CCT

All the five SNPs genotyped in the present study showed no deviation from Hardy-Weinberg equilibrium in the control sub-

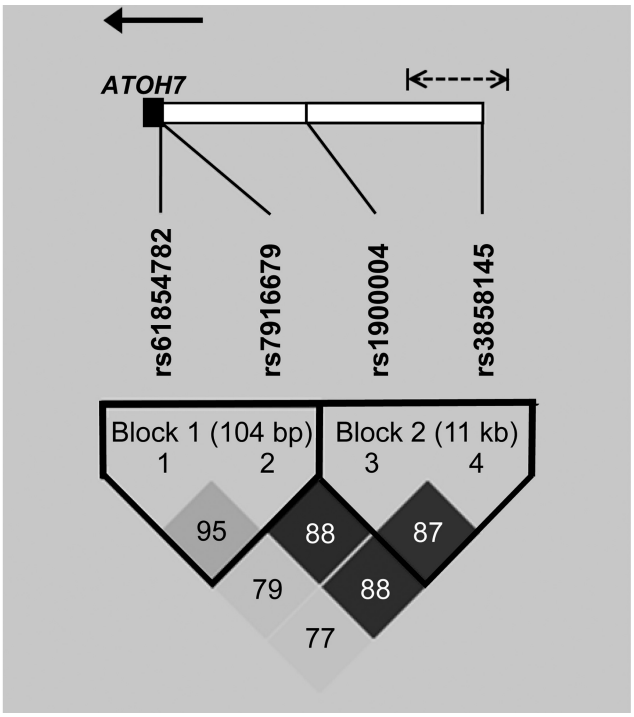

Figure 1. Linkage disequilibrium of the *ATOH7* SNPs in the present study. The single exon of *ATOH7* and 20-kb upstream genomic region is shown. The two SNPs in the promoter of *ATOH7* form a 104-bp haplotype block, and the other two form another haplotype block of 11 kb, according to the criteria of the confidence intervals, an algorithm proposed by Gabriel et al.[29] *Solid line with arrow*: transcription direction; *dashed line with double arrows*: the deletion reported by Ghiasvand et al.[25] causing nonsyndromic congenital retinal nonattachment.

TABLE 2. Primers and PCR Conditions for Sequencing of *ATOH7*

Amplicon Target	Primer Sequence		$MgCl_2$ (mM)	Annealing Temperature (°C)	Size (bp)
	Forward primer (5′→3′)	Reverse Primer (5′→3′)			
Promoter	AAGGAGTCTCAGGCTTTCCC	ATCAACCCATTCACAAGATCC	2	62	1212
Promoter-exon	AAAGCTGTCCAAGTACGAGAC	CTGATATCTCTTCACTTGCC	2	58	1067
Exon-3′ downstream	TACCTTTATTCGCATCATCAGACC	AAGGAAATCACTTCCAAAGGCA	2	65	930

jects (all $P > 0.05$). In the control subjects, the *ATOH7* SNP rs61854782 showed a significant association in the recessive model of the minor allele G (after adjustment for sex and age, $P = 0.004$, $\beta \pm SE = -0.088 \pm 0.030$; Table 3). The same allele of rs61854782 showed the effects of reduced VCDR in NTG and combined POAG, but it did not reach statistical significance ($\beta \pm SE = -0.315 \pm 0.177$, $P = 0.092$, and $\beta \pm SE = -0.316 \pm 0.180$, $P = 0.083$, respectively). For the other three *ATOH7* SNPs and the *RFTN1* SNP rs690037, no significant association with VCDR was observed in either the control or the POAG group ($P > 0.05$).

None of the *ATOH7* SNPs was associated with CCT in the control or the POAG patients ($P > 0.05$, Table 3). *RFTN1* rs690037 was not associated with CCT in the controls ($P > 0.05$). The SNP showed effects of increased CCT in HTG, NTG, and combined POAG, but was only significant in combined POAG in the dominant model ($\beta \pm SE = 24.44 \pm 13.36$, $P = 0.076$; $\beta \pm SE = 47.24 \pm 28.42$, $P = 0.131$; and $\beta \pm SE = 25.66 \pm 11.44$, $P = 0.029$, respectively).

Mutation Screen in *ATOH7*

No mutations in the coding sequences of *ATOH7* were detected in either the HTG or the NTG patients (data not shown). No coding variant was found in the controls.

Single-Gene Association with POAG

None of the genotype frequencies showed any significant difference between the two sexes. The allele frequencies of the four *ATOH7* SNPs were not significantly different between the POAG and control groups (all $P > 0.05$; Table 4). LD analysis revealed relative strong LD among the four *ATOH7* SNPs ($D' \geq 0.78$) in a ~20-kb range. The two SNPs in the 5′-UTR, rs61854782 and rs7916679, formed a 103-bp LD block, and rs1900004 and rs3858145 formed another 11-kb block. None of the haplotypes of these four *ATOH7* SNPs was associated with HTG, NTG, or combined POAG (all $P > 0.05$; Table 5).

Likewise, the alleles of the *RFTN1* rs690037 showed no significant difference in frequency between the POAG and control groups (all $P > 0.05$; Table 4).

Gene–Gene Interaction in Association with POAG

In the case–control analysis, comparison between the full and reduced regression models showed that inclusion of interaction between *ATOH7* and *RFTN1* significantly decreased the model deviance, which thus revealed remarkable interactive effects in POAG associations (Table 6). The most profound interaction in HTG, NTG, and combined POAG was detected, when the additive model of rs3858145 was combined with the dominant model of rs690037 (adjusted $P = 0.069$, 0.026, and 0.013, respectively; Table 6). As shown in Table 7, in the combined POAG, without genotype combination considered, the ORs for rs3858145 AG and GG and rs690037 combined CT+CC genotypes were 0.86, 0.85, and 1.11, respectively. When interaction was considered, rs3858145 GG combined with rs690037 TT exhibited a high risk (OR = 2.69), whereas rs3858145 AG showed an intermediate risk (OR = 1.83). In contrast, when combined with rs690037 CT or CC, rs3858145 GG showed a low risk (OR = 1.15), AG an intermediate risk (OR = 1.45), and AA a high risk (OR = 2.16), with a frequency

TABLE 3. Association of *ATOH7* and *RFTN1* SNPs with VCDR and CCT in Controls and POAG

SNP	Minor Allele	Control			HTG			NTG			Combined POAG (HTG + NTG)		
		β	SE	P*	β	SE	P*	β	SE	P*	β	SE	P*
VCDR													
ATOH7													
rs61854782	C	−0.088	0.030	**0.004**§	−0.011	0.045	0.800†	−0.315	0.177	0.092§	−0.316	0.180	0.083§
rs7916697	T	0.006	0.009	0.497‡	0.101	0.067	0.139§	0.076	0.081	0.362‡	0.089	0.059	0.138§
rs1900004	T	−0.003	0.009	0.762‡	0.070	0.064	0.276§	0.058	0.081	0.483‡	0.065	0.057	0.254§
rs3858145	G	0.006	0.009	0.499‡	0.051	0.069	0.462§	0.054	0.059	0.371†	0.052	0.058	0.372§
RFTN1													
rs690037	C	−0.013	0.01	0.201‡	−0.041	0.041	0.324‡	0.057	0.123	0.651§	−0.039	0.037	0.299‡
CCT													
ATOH7													
rs61854782	C	−1.041	4.292	0.808§	8.546	13.88	0.542†	47.62	30.14	0.149†	12.6	11.84	0.292†
rs7916697	T	−4.559	4.364	0.297‡	−5.902	9.514	0.539†	19.61	12.36	0.147†	5.638	16.8	0.739§
rs1900004	T	−7.179	6.404	0.263§	−14.23	11.86	0.238‡	19.61	12.36	0.147†	−6.359	9.629	0.512‡
rs3858145	G	−4.417	6.493	0.497§	−13.81	11.77	0.248‡	19.61	12.36	0.147†	13.51	17.63	0.447§
RFTN1													
rs690037	C	−8.002	5.388	0.139§	24.44	13.36	0.076‡	47.24	28.42	0.131‡	25.66	11.44	**0.029**‡

* The lowest *P* value after adjusted to sex and age is shown, and bold denotes $P < 0.05$.

†, ‡, § *P* calculated using additive, dominant, and recessive models of the minor allele, respectively.

TABLE 4. Allelic Association between SNPs in the Current Study and POAG

			Minor Allele Frequency					
			Control		Patients			
	SNP	M/m	*n*	%	*n*	%	OR (95% CI)	*P**
HTG								
ATOH7	rs61854782	A/C	87	15.1	31	13.4	0.87 (0.55–1.35)	0.613
	rs7916697	C/T	204	35.3	79	34.1	0.95 (0.69–1.30)	0.800
	rs1900004	C/T	202	34.9	75	32.1	0.88 (0.63–1.21)	0.480
	rs3858145	A/G	199	34.4	73	31.7	0.89 (0.64–1.23)	0.517
RFTN1	rs690037	T/C	264	45.7	112	47.9	1.09 (0.81–1.48)	0.625
NTG								
ATOH7	rs61854782	A/C	87	15.1	9	18.0	1.25 (0.55–2.57)	0.726
	rs7916697	C/T	204	35.3	17	34.0	0.95 (0.50–1.73)	0.976
	rs1900004	C/T	202	34.9	16	32.0	0.88 (0.46–1.61)	0.791
	rs3858145	A/G	199	34.4	16	33.3	0.96 (0.50–1.77)	0.996
RFTN1	rs690037	T/C	264	45.7	23	46.0	1.01 (0.56–1.82)	0.917
Combined POAG (HTG + NTG)								
ATOH7	rs61854782	A/C	87	15.1	40	14.2	0.94 (0.62–1.39)	0.815
	rs7916697	C/T	204	35.3	96	34.0	0.95 (0.70–1.28)	0.775
	rs1900004	C/T	202	34.9	91	32.0	0.88 (0.65–1.19)	0.441
	rs3858145	A/G	199	34.4	89	32.0	0.90 (0.66–1.22)	0.533
RFTN1	rs690037	T/C	264	45.7	135	47.5	1.08 (0.81–1.43)	0.658

M/m, major/minor allele.
* *P*-values were derived from χ^2 tests.

of 9.2% higher in the combined POAG compared with the control groups (39.6% vs. 30.4%). Interestingly, similar patterns of two-locus genetic risk were also found in NTG and HTG (Fig. 2). Combined with rs690037 TT, rs3858145 AG showed an intermediate risk, and rs3858145 GG exhibited a high risk when compared to rs3858145 AA. When combined with rs690037 CT or CC, the order of genotypic risk was inverted, with rs3858145 AA exhibiting a high risk, rs3858145 AG an intermediate risk and rs3858145 GG a low risk. In addition, the interactive effects were more evident in NTG compared with those in HTG.

DISCUSSION

Our results provided evidence for interactive effects of *ATOH7* and *RFTN1* polymorphisms that resulted in increased risk to POAG. Either gene alone, however, did not significantly contribute to risk for POAG in the Southern Chinese population.

In a GWAS, SNPs rs3858145, rs1900004, and rs7916697 in *ATOH7* were significantly associated with optic disc area.[19] SNP rs3858145 was associated with optic cup area in a meta-analysis of Australian and U.K. cohorts. The association of *ATOH7* with optic disc area was also found in GWAS in the Netherlands[20] and in Asian populations.[21] With respect to VCDR association, inconsistent findings were reported. The most significant association was reported at rs190004 by the Netherlands GWAS. However, none of these three SNPs was associated with VCDR in Asians[21] or American Caucasians.[14] In the present study in the Chinese, no significant association was observed between these three SNPs and VCDR. All these SNPs showed a low β with a relatively large SE in association analysis

TABLE 5. Association of *ATOH7* Haplotypes with POAG

	Frequency								*P*		
	Control		HTG		NTG		Combined POAG (HTG + NTG)				
Haplotype	*n*	%	*n*	%	*n*	%	*n*	%	HTG	NTG	Combined POAG (HTG + NTG)
ACCA	351	60.7	147	64.5	31	64.55	178	64.5	0.400	0.747	0.380
ACCG	6	1.1	0	0.0	1	2.1	2	0.7	0.384	0.564	0.612
ACTA	7	1.3	0	0.0	0	0.0	0	0.0	0.093	0.431	0.064
ACTG	7	1.2	2	0.9	0	0.0	2	0.8	0.674	0.456	0.513
ATCG	6	1.1	1	0.4	0	0.0	1	0.4	0.396	0.498	0.306
ATTA	6	1.0	2	1.0	0	0.0	2	0.8	0.968	0.482	0.823
ATTG	104	18.0	45	19.6	8	16.7	52	18.7	0.755	0.738	0.869
CTCA	4	0.7	3	1.3	0	0.0	3	1.1	0.391	1.000	0.554
CTTA	6	1.1	3	1.2	1	2.1	4	1.4	0.937	0.547	0.768
CTTG	70	12.2	22	9.8	7	14.6	30	10.9	0.412	0.671	0.579

* Alleles of rs61854782, rs7916697, rs1900004, and rs3858145 are shown according to their chromosomal positions. Only haplotypes with frequency ≥1% were analyzed.

TABLE 6. *P* Values of Interaction between *ATOH7* and *RFTN1* Genotypes in POAG

		HTG			NTG			Combined POAG (HTG + NTG)		
		RFTN1 rs690037			*RFTN1* rs690037			*RFTN1* rs690037		
ATOH7		ADD	DOM	REC	ADD	DOM	REC	ADD	DOM	REC
rs61854782	ADD	0.767	0.540	0.842	0.050	0.055	0.132	0.570	0.691	0.610
	DOM	0.556	0.308	0.896	0.120	0.158	0.153	0.888	0.880	0.683
	REC	0.328	0.363	0.749	0.075	0.063	0.737	0.124	0.133	0.665
rs7916697	ADD	0.825	0.317	0.498	0.108	0.099	0.279	0.460	0.124	0.683
	DOM	0.762	0.357	0.605	0.122	0.097	0.305	0.373	0.123	0.853
	REC	0.982	0.487	0.529	0.301	0.330	0.436	0.829	0.379	0.577
rs1900004	ADD	0.452	0.134	0.727	0.074	0.063	0.266	0.216	**0.043**	0.907
	DOM	0.400	0.173	0.940	0.073	**0.045**	0.298	0.150	**0.041**	0.843
	REC	0.747	0.273	0.543	0.325	0.361	0.432	0.652	0.231	0.594
rs3858145	ADD	0.364	0.069	0.650	**0.033**	**0.026**	0.197	0.113	**0.013**	0.934
	DOM	0.482	0.133	0.643	0.078	0.063	0.265	0.186	**0.034**	0.928
	REC	0.404	0.125	0.793	0.087	0.084	0.334	0.191	**0.045**	0.970

P values were adjusted to age and sex. Bold denotes $P < 0.05$, indicating that the inclusion of interaction significantly reduced the model deviance in regression. ADD, additive model; DOM, dominant model; REC, recessive model.

of VCDR ($|\beta| \leq 0.006$). Instead, significant association was found in controls and possibly in POAG at another SNP rs61854782 located at an *ATOH7* 5′-UTR, 104 bp downstream of rs7916697. It had a lower minor allele frequency compared with the other three *ATOH7* SNPs (15.1% in controls) and exerted a much larger effect of 0.088 reduction on VCDR in controls. Furthermore, rs61854782 was in high LD with the other three *ATOH7* SNPs, suggesting that it probably is the true VCDR-associated SNP in *ATOH7*. Although this SNP has been detected in Australian GWAS, its effects on optic disc parameters had not been investigated. This is the first report thus far of the association of this *ATOH7* SNP with VCDR.

ATOH7 is a highly conserved gene, sharing high similarity to its *Drosophila* homolog.[22] *ATOH7* mutations have been reported in optic nerve hypoplasia.[19] We did not detect any *ATOH7* mutation in our Chinese POAG patients. In addition, in an association analysis, all *ATOH7* SNPs showed similar allele frequency between the control and POAG groups, with allelic ORs close to 1, suggesting that these SNPs were not associated with POAG. In the GWAS in the Netherlands, *ATOH7* was marginally associated with open-angle glaucoma (rs1900004; $P = 0.04$). However, in a GWAS involving 1,263 POAG patients and 34,877 controls in Iceland that identified caveolin-1 (*CAV1*) and caveolin-2 (*CAV2*) as putative POAG susceptibility genes, *ATOH7* was not significantly associated with POAG at a genome-wide significance level ($P > 10^{-4}$). In addition, no association was shown between *ATOH7* and POAG in a recent study of American Caucasians.[14]

TABLE 7. Interaction Analysis between *ATOH7* rs3858145 and *RFTN1* rs690037 Genotype in Combined POAG (HTG + NTG)

A. Genotype Distribution

	RFTN1 rs690037 Genotype							
	Combined POAG (HTG + NTG) (n = 142)				Control (n = 289)			
ATOH7 rs3858145 Genotype	TT		CT + CC		TT		CT + CC	
AA	11	7.9%	55	39.6%	38	13.1%	88	30.4%
AG	18	12.9%	39	28.1%	34	11.8%	93	32.2%
GG	7	5.0%	9	6.5%	9	3.1%	27	9.3%

B. Joint ORs and 95% CI

ATOH7 rs3858145	*RFTN1* rs690037 Genotype	
Main Effects	TT	CT + CC
OR_{RFTN1}	1 (Ref)	1.11 (0.70–1.76)
OR_{ATOH7}	Joint Effects	
AA	1 (Ref)	2.16 (1.02–4.57)
AG	0.86 (0.56–1.32)	1.83 (0.76–4.42)	1.45 (0.67–3.12)
GG	0.85 (0.44–1.64)	2.69 (0.81–8.87)	1.15 (0.42–3.16)

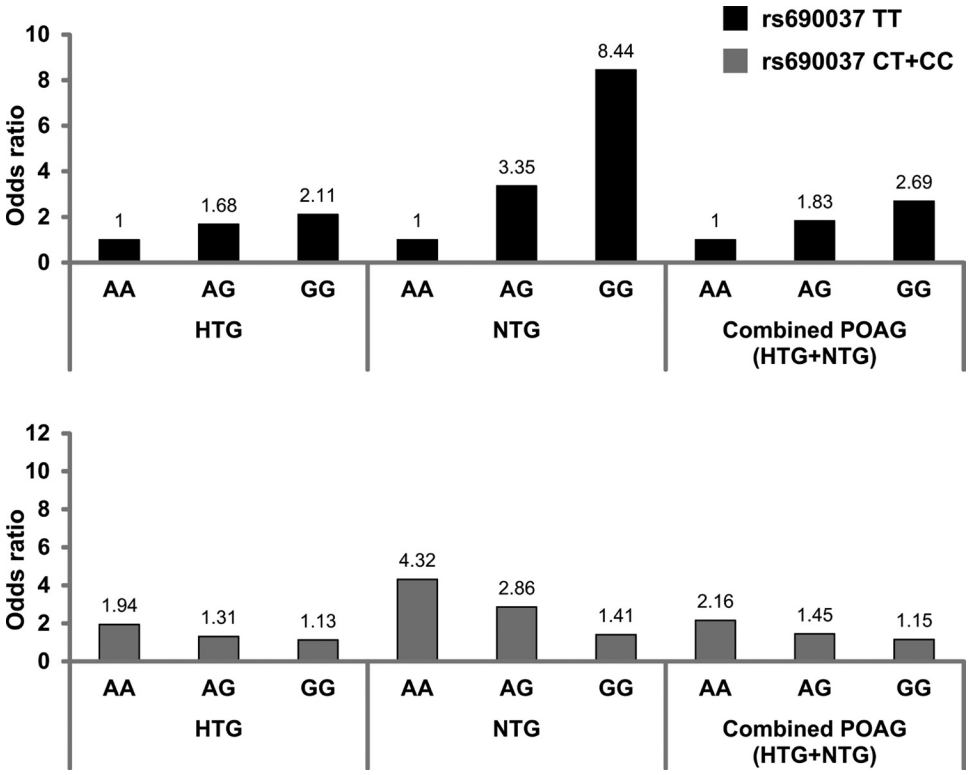

FIGURE 2. Two-locus (*ATOH7* and *RFTN1*) genotype-specific POAG risk. With combination of rs690037 TT/rs3858145 AA as the reference (OR = 1), ORs are calculated for different genotype combinations. (**A**) Joint effect analysis with rs3858145 genotypes combined with the rs690037 TT genotype and (**B**) with rs3858145 genotypes combined with the rs690037 CT or CC genotype.

The role of *RFTN1* in neurons remains unclear. However, it has recently been reported to associate with Alzheimer's disease,[28] another degenerative neuronal disease. Interestingly, other lipid-raft molecules have been shown to interact with myocilin (*MYOC*) and *CAV1*.[34,35] *RFTN1* rs690037 was associated with cup area and VCDR in Australian twin and U.K. cohorts.[19] In our study, the association of rs690037 with VCDR was implicated in the controls. Moreover, in HTG, NTG, and combined POAG rs690037 was associated with CCT. Although no association of rs690037 with POAG was found in our study, these findings still suggest that *RFTN1* is a modifier in POAG etiology. Interestingly, the C allele was found to have a lower frequency in our Chinese cohort than in Caucasians (45.7% in our controls vs. 53.5% in the HapMap CEU subjects).

Results of our gene–gene interaction analysis indicated significant interaction between *ATOH7* and *RFTN1*. The effects of different *ATOH7* genotypes could be reverted by combination of different *RFTN1* genotypes. This could be an explanation to the absence of single-gene effect when either gene was analyzed separately. Furthermore, in a recent report by Ghiasvand et al.,[25] a 6.5-kb deletion located 21.7 to 15.2 kb upstream of the *ATOH7* transcription start site, led to nonsyndromic congenital retinal nonattachment. The deletion spanned an evolutionarily conserved, remote secondary enhancer required for normal expression of *ATOH7* in neurogenesis. It was noted that the deletion also spanned rs3858145, which probably implicated the possible functional link of this SNP. The rs3858145 T allele was reported to have effects of increased optic disc and cup areas in Australian twin and U.K. cohorts.[19] Furthermore, similar patterns of two-locus genotypic risk were observed in both HTG and NTG compared with combined POAG, underlining a potential common role of the interaction in glaucomatous optic neuropathy. In addition, the interactive effects were more evident in NTG than in HTG. Combination of *ATOH7* and *RNTN1* genotypes conferred a greater risk for glaucoma in NTG than in HTG.

In conclusion, our results showed association between *ATOH7* and VCDR. There were significant interactive effects of the two optic disc parameter–related genes but no single-gene effect in association with adult-onset POAG in our Chinese cohort. Interaction between the *ATOH7* and *RFTN1* genotypes conferred risk for POAG. Our findings reflect the complexity and underline the importance of gene–gene interactions in POAG genetics.

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