Eye (2013) 27, 979–983

www.nature.com/eye

LABORATORY STUDY

Association study of genetic variants on chromosome 7q31 with susceptibility to normal tension glaucoma in a Japanese population

T Kato[1,16], A Meguro[1,16], E Nomura[1], R Uemoto[1], N Nomura[1], M Ota[2], K Kashiwagi[3], F Mabuchi[3], H Iijima[3], K Kawase[4], T Yamamoto[4], M Nakamura[5], A Negi[5], T Sagara[6], T Nishida[6], M Inatani[7], H Tanihara[7], M Aihara[8], M Araie[8], T Fukuchi[9], H Abe[9], T Higashide[10], K Sugiyama[10], T Kanamoto[11], Y Kiuchi[11], A Iwase[12], S Chin[13], S Ohno[14], H Inoko[15] and N Mizuki[1]

Abstract

The caveolin 1 to caveolin 2 (*CAV1–CAV2*) gene region on chromosome 7q31 has been reported to be associated with susceptibility to primary open angle glaucoma (POAG) and normal tension glaucoma (NTG) in previous studies. We investigated whether genetic variants in the *CAV1–CAV2* region are associated with NTG in Japanese patients. Two hundred and ninety-two Japanese patients with NTG and 352 Japanese healthy controls were recruited. We genotyped three single-nucleotide polymorphisms; that is, rs1052990, rs4236601, and rs7795356, in the *CAV1–CAV2* gene region and assessed the allelic diversity among cases and controls. The frequency of the minor allele (G) of rs1052990 was significantly decreased in NTG cases compared with controls ($P = 0.014$, OR = 0.71), whereas NTG or POAG cases had a significantly higher frequency of the allele than controls in previous studies. Conversely, rs7795356 did not show any significant association with NTG cases, and rs4236601 was monomorphic in the Japanese study population. Our findings did not correspond with previous positive results, suggesting that *CAV1–CAV2* variants studied in the present study are not important risk factors for NTG susceptibility in all populations. Further studies are needed to elucidate the possible contribution of the *CAV1–CAV2* region to the development of glaucoma.

Eye (2013) **27,** 979–983; doi:10.1038/eye.2013.123; published online 7 June 2013

Keywords: association study; CAV1; CAV2; normal tension glaucoma; single-nucleotide polymorphism

Introduction

Glaucoma is a degenerative optic neuropathy characterized by the progressive degeneration of retinal ganglion cells and optic nerve axons, including visual field damage, which is usually associated with elevated intraocular pressure (IOP). Primary open angle glaucoma (POAG) is the most common type of glaucoma, and normal tension glaucoma (NTG) is an important subset of POAG. Although many POAG patients have high IOP,[1] IOP in NTG patients is not statistically different from the IOP in healthy individuals.[2–4] NTG is more prevalent in Asian populations, particularly in the Japanese population (92% of Japanese POAG patients have NTG).[5–7] Except for a small percentage of glaucoma patients with Mendelian inheritance patterns including the *MYOC* (myocilin) and *OPTN* (optineurin) genes, glaucoma is generally a multifactorial disorder initiated as the result of several interacting factors, and factors other than IOP are likely to have a role in the pathogenesis of glaucomatous optic neuropathy, particularly in patients with NTG.[8–10]

The caveolin 1 to caveolin 2 (*CAV1–CAV2*) gene region on chromosome 7q31 has been associated with susceptibility to POAG in a recent genome-wide association study (GWAS) in an Icelandic population. The significant genome-wide association was found in rs4236601 and rs1052990, which are located within the same linkage disequilibrium (LD) block between *CAV1* and *CAV2*.[11] As rs1052990 did not show significant association with POAG after adjusting for the observed association with rs4236601, it is suggested that rs4236601 is a primary variant for POAG in *CAV1–CAV2*.[11] The association of rs4236601 and rs1052990 with

[1]Department of Ophthalmology, Yokohama City University School of Medicine, Kanagawa, Japan

[2]Department of Legal Medicine, Shinshu University School of Medicine, Nagano, Japan

[3]Department of Ophthalmology, University of Yamanashi, Faculty of Medicine, Yamanashi, Japan

[4]Department of Ophthalmology, Gifu University Graduate School of Medicine, Gifu, Japan

[5]Department of Surgery, Division of Ophthalmology, Kobe University Graduate School of Medicine, Hyogo, Japan

[6]Department of Ophthalmology, Yamaguchi University School of Medicine, Yamaguchi, Japan

[7]Department of Ophthalmology and Visual Science, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

[8]Department of Ophthalmology, University of Tokyo School of Medicine, Tokyo, Japan

[9]Division of Ophthalmology and Visual Science, Graduated School of Medical and Dental Sciences, Niigata University, Niigata, Japan

[10]Department of Ophthalmology and Visual Science, Kanazawa University Graduate School of Medical Science, Ishikawa, Japan

[11]Department of Ophthalmology and Visual Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

[12]Department of Ophthalmology, Tajimi Municipal Hospital, Gifu, Japan

[13]Department of Ophthalmology, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

[14]Department of Ocular Inflammation and Immunology, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

[15]Department of Genetic Information, Division of Molecular Life Science, Tokai University School of Medicine, Kanagawa, Japan

Correspondence:
N Mizuki, Department of Ophthalmology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan
Tel: +81 45 787 2683;
Fax: +81 45 781 9755;
E-mail: mizunobu@med.yokohama-cu.ac.jp

[16]These authors contributed equally to this work.

Received: 15 November 2012
Accepted in revised form: 4 May 2013
Published online: 7 June 2013

POAG was replicated in a Caucasian US population.[12] Conversely, another study investigating rs4236601 with a US population from Iowa showed a lack of association with POAG.[13] A recent GWAS with a Japanese population showed no association of rs1052990 with POAG and no polymorphism of rs4236601, whereas rs7795356 in *CAV1–CAV2* showed a significant association.[14] In the replication study by Wiggs *et al*,[12] stratification by gender and IOP was performed, and the results showed that rs1052990 and rs4236601 were significantly associated with NTG and high tension glaucoma, suggesting that the association between *CAV1–CAV2* single-nucleotide polymorphisms (SNPs) and POAG might be affected by gender.

The aim of the present study was to investigate whether genetic variants in the *CAV1–CAV2* region are associated with NTG in Japanese patients. Here, we assessed the association of SNPs in the *CAV1–CAV2* region with NTG in a Japanese population.

Materials and methods

Participants

We recruited 292 unrelated Japanese patients with NTG and 352 unrelated healthy Japanese controls at Yokohama City University, Yamanashi University, Gifu University, Kobe University, Yamaguchi University, Kumamoto University, Hokkaido University, Tokyo University, Niigata University, Kanazawa University, Hiroshima University, Tajimi Municipal Hospital, and Tokai University in Japan. The criteria used to diagnose NTG have been described previously.[15] Patients exhibiting comparatively early-onset NTG were selected for this study. Advancing age is a major risk factor for glaucoma. Therefore, we did not enroll NTG patients aged 60 years and over to exclude as many patients potentially caused by advancing age as possible, suggesting that the NTG patient group used in this study has a stronger genetic component.

Patient ages ranged between 20 and 59 years (mean age, 46.2 ± 7.7 years); 47.9% of the patients were male and 52.1% were female. The mean refraction value was −4.04 ± 3.01 diopters (D) and the mean deviation (MD) observed in the Humphrey static visual field determination (Carl Zeiss Meditec, Oberkochen, Germany) was −9.19 ± 7.70 decibels.

Controls were healthy volunteers from a geographic region similar to that for the NTG patients. The subjects were not affected by glaucoma or any local or systemic illnesses that could result in optic disc or visual field changes. The criteria for the control group were as follows: vertical cup-disc ratio of less than 0.6, asymmetry of disc cupping less than 0.2, normal neuroretinal rim followed by the Inferior–Superior–Nasal–Temporal rule without notching and undermining, normal blood vessels on disc without bayoneting, and/or no retinal nerve fiber damage, such as nerve fiber layer defect. Furthermore, the controls had no myopia or mild myopia with refractive errors of −3.00 D or less. The controls were age-matched (mean age, 47.9 ± 6.1 years; range, 38–62 years) to the patients, and 54.8% of the cases were male and 45.2% were female. This study was approved by the ethics committee of each participating institute, and complied with the guidelines of the Declaration of Helsinki. All study details were explained to all patients and controls before obtaining their consent for genetic screening.

Myopia has a complex genetic basis. The mean refraction value of the patients was −4.04 ± 3.01 D, while controls with refractive errors of −3.00 D or less were recruited. As the difference in refraction value between the patient and control group was slight, we believe that there is little possibility that the difference leads to biased results in this study.

Genotyping and statistical analysis

The QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) was used to collect peripheral blood lymphocytes and extract genomic DNA from peripheral blood cells. Procedures were performed under standardized conditions to prevent variation in DNA quality.

We evaluated three SNPs in the *CAV1–CAV2* gene region; that is, rs1052990, rs4236601, and rs7795356, which have previously been reported to be associated with POAG (Table 1).[11,12,14] Genotyping of all the SNPs was performed by TaqMan 5′ exonuclease assay using primers supplied by ABI (Foster City, CA, USA). The probe fluorescence signal was detected using the TaqMan Assay for Real-Time PCR (7500 Real-Time PCR System, Applied Biosystems, Carlsbad, CA, USA) using the manufacturer's instructions.

Hardy–Weinberg equilibrium was tested for each SNP among the controls. Differences in allele frequencies between cases and controls were assessed by a χ^2 test.

Table 1 Information on the *CAV1–CAV2* variants used in this study

SNP	*Alleles*	*Physical map location (build 37.1)*	*Related gene*	*SNP type*
rs1052990	G/T	Chr7: 116,148,370	*CAV2*	3′UTR
rs4236601	A/G	Chr7: 116,162,729	*CAV1*	5′UTR
rs7795356	C/T	Chr7: 116,217,029	*CAV1*	3′UTR

Abbreviations: SNP, single-nucleotide polymorphism; CAV1, caveolin 1; CAV2, caveolin 2.

Table 2 Allele frequencies of the *CAV1–CAV2* variants among NTG patients and controls

SNP	*Alleles (1/2)*	*Total*				*Males*				*Females*			
		MAF		P-*value*	*OR (95% CI)*	*MAF*		P-*value*	*OR (95% CI)*	*MAF*		P-*value*	*OR (95% CI)*
		Cases (n = *292*)	*Controls* (n = *352*)			*Cases* (n = *140*)	*Controls* (n = *193*)			*Cases* (n = *152*)	*Controls* (n = *159*)		
rs1052990	T/G	0.179	0.236	0.014	0.71 (0.54–0.93)	0.192	0.223	0.331	0.83 (0.56–1.21)	0.168	0.250	0.012	0.60 (0.41–0.90)
rs4236601	G/A	0.000	0.000	—	—								
rs7795356	C/T	0.052	0.058	0.592	0.88 (0.54–1.42)	0.040	0.052	0.461	0.75 (0.36–1.60)	0.063	0.066	0.841	0.94 (0.49–1.78)

1, major allele; 2, minor allele; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.
P-values were calculated using a χ^2-test 2×2 contingency table.

The software Haploview 4.1 software (Broad Institute of MIT and Harvard, Cambridge, MA, USA) was used to compute pairwise LD statistics.[16]

Results

Three SNPs in the *CAV1–CAV2* region were genotyped. The observed and expected frequencies of each genotype for the three SNPs in the NTG case and control participants were in Hardy–Weinberg equilibrium (data not shown).

The allele frequencies of the *CAV1–CAV2* SNPs in NTG cases and controls are shown in Table 2. rs4236601 was monomorphic in the Japanese population. The frequency of minor allele G of rs1052990 showed a significant decrease in NTG cases as compared with controls (17.9 *vs* 23.6%, $P = 0.014$, OR = 0.71). On the other hand, the frequency of minor allele T of rs7795356 in the cases was slightly lower than in the controls, but no statistically significant association was observed (5.2 *vs* 5.8%, $P = 0.59$, OR = 0.88). rs1052990 and rs7795356 were in weak LD ($D' = 1$, $r^2 = 0.01$).

Since a previous study has suggested that the association between *CAV1–CAV2* SNPs and POAG might be affected by gender,[12] we performed a gender-stratification analysis for rs1052990 and rs7795356 (Table 2). There was a greater difference in the minor allele frequency of rs1052990 between the cases and controls in females, and rs1052990 was significantly associated with NTG only in females (16.8 *vs* 25.0%, $P = 0.012$, OR = 0.60). In rs7795356, the difference in allele frequency between cases and controls increased in males but not in females, however, the difference did not reach significance.

Discussion

An Icelandic GWAS by Thorleifsson *et al* identified two genome-wide association signals, rs4236601 ($P = 5.0 \times 10^{-10}$, OR = 1.36) and rs1052990 ($P = 1.1 \times 10^{-9}$, OR = 1.32), in the *CAV1–CAV2* region on chromosome 7q31 as a POAG susceptibility variant and rs4236601, but not rs1052990 showed a primary association with the disease.[11] The replicated association for rs4236601 was observed in a European cohort from the Swedish, UK and Australian populations, whereas rs4236601 had a smaller or no effect on disease risk in each replication population (Swedish: $P = 0.092$, OR = 1.33; Leicester, UK: $P = 0.2$, OR = 1.14; Southampton, UK: $P = 0.75$, OR = 1.04; Australian: $P = 0.0063$, OR = 1.23) compared with the Icelandic GWAS population.[11] The association for rs4236601 was also replicated in two Chinese populations, however, the minor allele A of rs4236601was rare in the Chinese populations.[11] The replicated association for rs1052990

was also observed in a European cohort from the Swedish and Australian populations although rs1052990 had a smaller risk for the disease in the replication cohort ($P = 0.0055$, OR = 1.20).[11] Wiggs *et al* confirmed significant associations for several *CAV1–CAV2* SNPs with POAG and NTG in a Caucasian US population (the strongest association was observed in rs1052990 ($P = 0.0003$, OR = 1.30) and the second was observed in rs4236601($P = 0.0014$, OR = 1.28), and presented that the associations are significant mostly in females.[12] However, Kuehn *et al* reported a lack of association between rs4236601 and POAG in a US Iowa population[13], and a recent Japanese GWAS by Osman *et al* showed no association of rs4236601 and rs1052990 with POAG, whereas a significant association for rs7795356 in the *CAV1–CAV2* region was observed.[14]

In this study, we found a significant difference in the allele frequency of rs1052990 between NTG cases and controls, showing a significant decrease in the G allele frequency in the NTG cases; the G allele served a protective role in the Japanese NTG. However, the result indicates the opposite of previous studies, in which NTG or POAG cases had a significantly higher frequency of the allele G than controls; the G allele served a risk role in NTG or POAG cases of previous studies.[11,12] After stratification by gender, the difference between NTG cases and controls in rs1052990 allele frequency increased in females; however, the odds ratio was still in contrast with previous studies.[11,12] The odds ratio for rs7795356 in this study was also in contrast with that obtained from a previous study with Japanese patients with POAG showing a significant increase in the T-allele frequency in POAG cases,[14] whereas in an Icelandic population, the T-allele frequency was decreased in POAG cases.[11] rs4236601, which showed that the strongest association signal in the original GWAS study was not polymorphic in the Japanese population, which is in agreement with previous study.[14] The allele frequency of rs4236601 is known to vary dramatically among ethnic groups, and the minor allele (A) frequency is zero or nearly zero in east Asian populations.[11,14]

Our results showed the different risk-associated allele from the previous studies suggesting that the *CAV1–CAV2* variants contribute to NTG or POAG risk. Glaucoma is not a single condition and it encompasses a wide variety of different pathogeneses. Therefore, it is assumed that the differences in disease type and/or severity lead to the different results among genetic studies on glaucoma. Our current study applied more strict criteria for NTG diagnosis compared with general criteria. Moreover, in this study, we used early-onset NTG (mean age, 46.2 years) because early onset suggests stronger involvement of genetic factors. Conversely, the mean age of the POAG patients including NTG was 64.6 years in Wiggs *et al*'s study.[12] Thus, pathological conditions in our NTG patients may be different from the patient population of Wiggs *et al*, suggesting that the phenotypic differences observed between each patient group may lead to detect the different genetic risk factors. In addition, the differences in genetic and environmental backgrounds between ethnic populations could also affect association levels of genetic factors with glaucoma as it has been suggested that a genetic factor may be involved in the development of glaucoma by interactions with other genetic and/or environmental factors. Therefore, the genetic factor in *CAV1–CAV2* may have a stronger effect on glaucoma risk in some ethnic populations than others. Moreover, we need to consider the third reason for the different results between the present and previous studies; there is a possibility that another *CAV1–CAV2* variant is a true genetic factor, and that the associations observed in the present and previous studies resulted secondarily from a strong LD with the true *CAV1–CAV2* variant. Variable LD patterns among different ethnic groups could explain the conflicting results; the true risk-associated allele in *CAV1–CAV2* may be linked with G allele of rs1052990 in Caucasian populations, whereas T allele of rs1052990 in our Japanese population. The association for rs4236601 observed in the Caucasian populations may also result from a LD with the true risk-associated allele. To clarify this hypothesis, it will be necessary to further explore the LD region of rs1052990 by a resequencing analysis.

In conclusion, we found that the *CAV1–CAV2* variant, rs1052990 in chromosome 7q31 is associated with NTG in our Japanese population, whereas the disease risk-associated allele differs between the present and the previous studies. Our findings suggest that the *CAV1–CAV2* variants studied in the present study are not an important risk factor for NTG susceptibility in all populations. However, as another *CAV1–CAV2* variant may potentially affect the risk of glaucoma, further genetic studies are needed to clarify the contribution of the *CAV1–CAV2* region in the development of glaucoma.

Summary

What was known before

- The *CAV1–CAV2* gene region on chromosome 7q31 has been reported to be associated with susceptibility to POAG and NTG in previous studies.

What this study adds

- The CAV1–CAV2 variants may be not an important risk factor for NTG susceptibility in all populations.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by a grant from the Ministry of Health, Labour, and Welfare, Japan.

References

1 Quigley HA. Open-angle glaucoma. *N Engl J Med* 1993; **328**: 1097–1106.
2 Hitchings RA, Anderson SA. A comparative study of visual field defects seen in patients with low-tension glaucoma and chronic simple glaucoma. *Br J Ophthalmol* 1983; **67**: 818–821.
3 Hitchings RA. Low-tension glaucoma—its place in modern glaucoma practice. *Br J Ophthalmol* 1992; **76**: 494–496.
4 Werner EB. Normal-tension glaucoma. In: Ritch R, Shields MB, Krupin T (eds). *The Glaucomas*. 2nd edn. Mosby: St Louis, MO, USA, 1996, pp 769–797.
5 Iwase A, Suzuki Y, Araie M, Yamamoto T, Abe H, Shirato S *et al*. The prevalence of primary open-angle glaucoma in Japanese: the Tajimi Study. *Ophthalmology* 2004; **111**: 1641–1648.
6 Oku Y, Oku H, Park M, Hayashi K, Takahashi H, Shouji T *et al*. Long axial length as risk factor for normal tension glaucoma. *Graefes Arch Clin Exp Ophthalmol* 2009; **247**: 781–787.
7 Shiose Y, Kitazawa Y, Tsukahara S, Akamatsu T, Mizokami K, Futa R *et al*. Epidemiology of glaucoma in Japan—a nationwide glaucoma survey. *Jpn J Ophthalmol* 1991; **35**: 133–155.
8 Wiggs JL. Genetic etiologies of glaucoma. *Arch Ophthalmol* 2007; **125**: 30–37.
9 Osborne NN, Lascaratos G, Bron AJ, Chidlow G, Wood JP. A hypothesis to suggest that light is a risk factor in glaucoma and the mitochondrial optic neuropathies. *Br J Opthalmol* 2006; **90**: 237–241.
10 Fuse N. Genetic bases for glaucoma. *Tohoku J Exp Med* 2010; **221**: 1–10.
11 Thorleifsson G, Walters GB, Hewitt AW, Masson G, Helgason A, DeWan A *et al*. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet* 2010; **42**: 906–909.
12 Wiggs JL, Kang JH, Yaspan BL, Mirel DB, Laurie C, Crenshaw A *et al*. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma in Caucasians from the USA. *Hum Mol Genet* 2011; **20**: 4707–4713.
13 Kuehn MH, Wang K, Roos B, Stone EM, Kwon YH, Alward WL *et al*. Chromosome 7q31 POAG locus: ocular expression of caveolins and lack of association with POAG in a US cohort. *Mol Vis* 2011; **17**: 430–435.
14 Osman W, Low SK, Takahashi A, Kubo M, Nakamura Y. A genome-wide association study in the Japanese population confirms 9p21 and 14q23 as susceptibility loci for primary open angle glaucoma. *Hum Mol Genet* 2012; **21**: 2836–2842.
15 Shibuya E, Meguro A, Ota M, Kashiwagi K, Mabuchi F, Iijima H *et al*. Association of Toll-like receptor 4 gene polymorphisms with normal tension glaucoma. *Invest Ophthalmol Vis Sci* 2008; **49**: 4453–4457.
16 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.