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貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

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1. 助成金額： 600,000 円

2. 研究テーマ

開放隅角緑内障、正常眼圧緑内障、発達緑内障の分子遺伝学的解析

3. 成果の概要

緑内障原因遺伝子のスクリーニングを行い、常染色体 2 番の HK2 遺伝

子、NCK2 遺伝子の正常眼圧緑内障との相関を見出した（学位論文）、また発達

緑内障における LTBP2 遺伝子のスクリーニング等と成果は多岐にわたる。

4. 研究業績

(1) 学会における発表 無・有(学会名・演題)

WOC 2012(Abu Dhabi, UAE)

Molecular Genetic Analysis of Primary Open-angle Glaucoma, Normal

Tension Glaucoma, and Developmental Glaucoma for VAV2 and VAV3 Variants in

Japanese

(2) 発表した論文 無・有(雑誌名・題名)

5. 指導責任者の意見(指導責任者がご記入・ご捺印ください)

短期間に次々と成果を上げており、その熱意、才能は他の学生の追隨を

許さない。研究に対する情熱、姿勢、着眼点はすばらしいものがある。

海外の緑内障の遺伝子解析は、明らかに遺伝学的な背景が日本人と異なって

おり、今回の検討は、国内の緑内障分野での遺伝子診断・解析に多大な貢献を

もたらすと考えられる。今後の更なる展開が期待される。

指導責任者署名 布施 昇男



収支報告

## 開放隅角緑内障、正常眼圧緑内障、発達緑内障の分子遺伝学的解析

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### Molecular Genetic Analysis of Primary Open-angle Glaucoma, Normal Tension Glaucoma, and Developmental Glaucoma for VAV2 and VAV3 Variants in Japanese

#### 要旨

**Purpose:** The VAV2 and VAV3 genes have been implicated in primary open angle glaucoma (POAG) in the Japanese. The purpose of this study was to determine whether variants in the vav 2 guanine nucleotide exchange factor (VAV2) and vav 3 guanine nucleotide exchange factor (VAV3) genes are associated with primary open-angle glaucoma (POAG), normal tension glaucoma (NTG), and developmental glaucoma (DG) in the Japanese.

**Methods:** One hundred sixty-eight unrelated Japanese patients with POAG, 163 unrelated patients with NTG, 45 unrelated patients with DG, and 180 ethnically-matched normal controls were studied. Genomic DNA was extracted from leukocytes of the peripheral blood, and variants in the VAV2 and VAV3 genes were amplified by polymerase chain reaction (PCR) and directly sequenced.

**Results:** Two variants were identified; rs2156323 in VAV2 and rs2801219 in VAV3. The allele frequency of rs2156323 in VAV2 in the POAG, NTG, and DG groups was not significantly different from the control group (minor allele frequency 0.051, 0.049, 0.022 vs. 0.036;  $P = 0.35, 0.40$ , and  $0.51$ , respectively). The allele frequency of the rs2801219 in VAV3 was also not significantly higher in the two groups than in the control group (minor allele frequency 0.211, 0.236, 0.244 vs. 0.197;  $P = 0.64, 0.22$  and  $0.32$ ).

**Conclusions:** The variants, rs2156323 in VAV2 and rs2801219 in VAV3 genes and the prevalence of POAG, NTG, and DG in unrelated Japanese patients indicate that they are not involved in the pathogenesis of POAG, NTG, or DG.

**Key Words** POAG, NTG, DG, VAV2, VAV3, Gene screening

#### 緒言

Glaucoma is a complex, heterogeneous disease characterized by a progressive degeneration of the optic nerve axons, and is the second highest cause of blindness affecting approximately 70 million people. The prevalence of NTG is significantly higher among the Japanese than among Caucasians.

Although the precise molecular basis of POAG has not been established, it is probably a genetically heterogeneous disorder caused by the interaction of multiple genes and environmental factors. To date, at least fifteen loci from GLC1A to GLC1O have been linked to POAG, and three genes have been identified.

Recently, the vav 2 guanine nucleotide exchange factor (VAV2) and vav 3 guanine nucleotide exchange factor (VAV3) genes were reported to cause POAG in the Japanese. The authors provided functional evidence suggesting that Vav2 and Vav2/Vav3 deficient mice had a spontaneous glaucoma phenotype resulting in progressive iridocorneal changes and elevated IOPs. In addition, a genome-wide association study (GWAS) screening for glaucoma susceptibility loci using single nucleotide polymorphisms (SNPs) analysis identified intronic SNPs in VAV2 (rs2156323) and VAV3 (rs2801219) as candidates for genes associated with POAG in Japanese patients.

The development of an accurate diagnostic test for pre-symptomatic individuals at risk for glaucoma is needed, and the screening of the VAV2 and VAV3 genes may identify pre-symptomatic cases in the general population. Thus, the purpose of this study was to determine whether variants in the VAV2 and VAV3 genes contribute to POAG, NTG, and developmental glaucoma (DG) in Japanese patients.

### 対象と方法

#### **Patients**

168 unrelated Japanese patients with POAG, 163 unrelated Japanese patients with NTG, and 45 unrelated Japanese patients with DG, who were diagnosed in the ophthalmological clinic at the Tohoku University Hospital, Sendai, Japan, were studied.

#### **Sample Preparation and Variant Screening**

Genomic DNA was extracted from leukocytes of peripheral blood and purified with the Qiagen QIAamp Blood Kit (Qiagen, USA). SNPs rs2156323 (VAV2) and rs2801219 (VAV3) and their flanking regions were amplified by a polymerase chain reaction (PCR) using 0.5  $\mu$ M intronic primers in the amplification mixture (25  $\mu$ l) containing 0.2 mM dNTPs and 0.5 U Ex Taq polymerase (Takara) with 30 ng template DNA at an annealing temperature of 60° C. Oligonucleotides for amplification and sequencing were selected using Primer3 software, ([http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi/](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi/) provided in the public domain by the Massachusetts Institute of Technology, Cambridge, MA).

The PCR fragments were purified with ExoSAP-IT (USB, Cleveland, Ohio, USA), sequenced by the BigDye<sup>TM</sup> Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, California, USA) on an automated DNA sequencer (ABI PRISM<sup>TM</sup> 3100 Genetic Analyzer, Perkin-Elmer).

### 結果

#### **Allelic frequencies for rs2156323 SNP in VAV2 and rs2801219 SNP in VAV3**

Two variants were identified: rs2156323 in VAV2 and rs2801219 in VAV3. The allele frequencies of rs2156323 in VAV2 in the POAG, the NTG, and the DG groups were not significantly different from that in the control group. The allele frequency of the rs2801219 in VAV3 was also not significantly higher in the two groups than in the control group.

#### **Genotype frequencies for rs2156323 SNP in VAV2 and rs2801219 SNP in VAV3**

For the rs2156323 in VAV2, the genotype frequency was not statistically higher in the POAG, the NTG, and the DG groups than in the control group. For the rs2801219 in VAV3, the genotype

frequency was not statistically higher in the POAG, the NTG, and DG groups than in the control group.

#### **Dominant and recessive model for rs2156323 SNP in VAV2 and rs2801219 SNP in VAV3**

The homozygotes for rs2156323 SNP A/A were 0% in the glaucoma subjects, and 0.6% in the control subjects. We analyzed the dominant and recessive model for rs2801219 SNP in VAV3. There was also no significant difference between the subgroups of glaucoma and SNP rs2801219 in VAV3. However, in NTG group, the  $P = 0.06$  for the dominant model.

#### **考察**

Obtaining evidence that candidate genes and gene variants are significantly associated with a specific disease is biologically more meaningful when they are found in different ethnic populations. The significant associations would then indicate that these genes play a role in the pathogenesis of the disease. Our findings showed that the risk alleles rs2156323 (VAV2) and rs2801219 (VAV3) were not significantly associated with POAG in Japanese patients. These risk alleles were also found to be not significantly associated with either POAG or primary angle closure glaucoma (PACG) in an Indian cohort. The investigators found that the genotype frequencies at these loci were not significantly different among the POAG, PACG, and controls subjects among their Indian cohorts.

The finding that Vav2-deficiency alone resulted in a glaucoma phenotype in mice suggested that the absence of Vav2 is associated with the development of glaucoma in mice. However, our findings showed there was no significant association between the VAV2 SNP and POAG, NTG, and DG. In addition, the VAV2 SNP rs2156323 was not associated with these glaucoma phenotypes. Functionally, Vav2/Vav3-deficient (Vav2<sup>-/-</sup>Vav3<sup>-/-</sup>) mice have buphthalmos along with iridocorneal changes that altered the aqueous outflow leading to the elevated intraocular pressures. The optic nerve head cupping resembled that in developmental glaucoma and PACG. Thus, we suggest that VAV2 and VAV3 could be major candidate genes for developmental glaucoma in humans. But our results showed that not only DG but also POAG and NTG were not associated with alleles rs2156323 (VAV2) and rs2801219 (VAV3).

There is a possibility that a lack of significant associations at these loci in our POAG cases could have been due to clinical heterogeneity.

Another possibility for the lack of significant associations is the sample size.

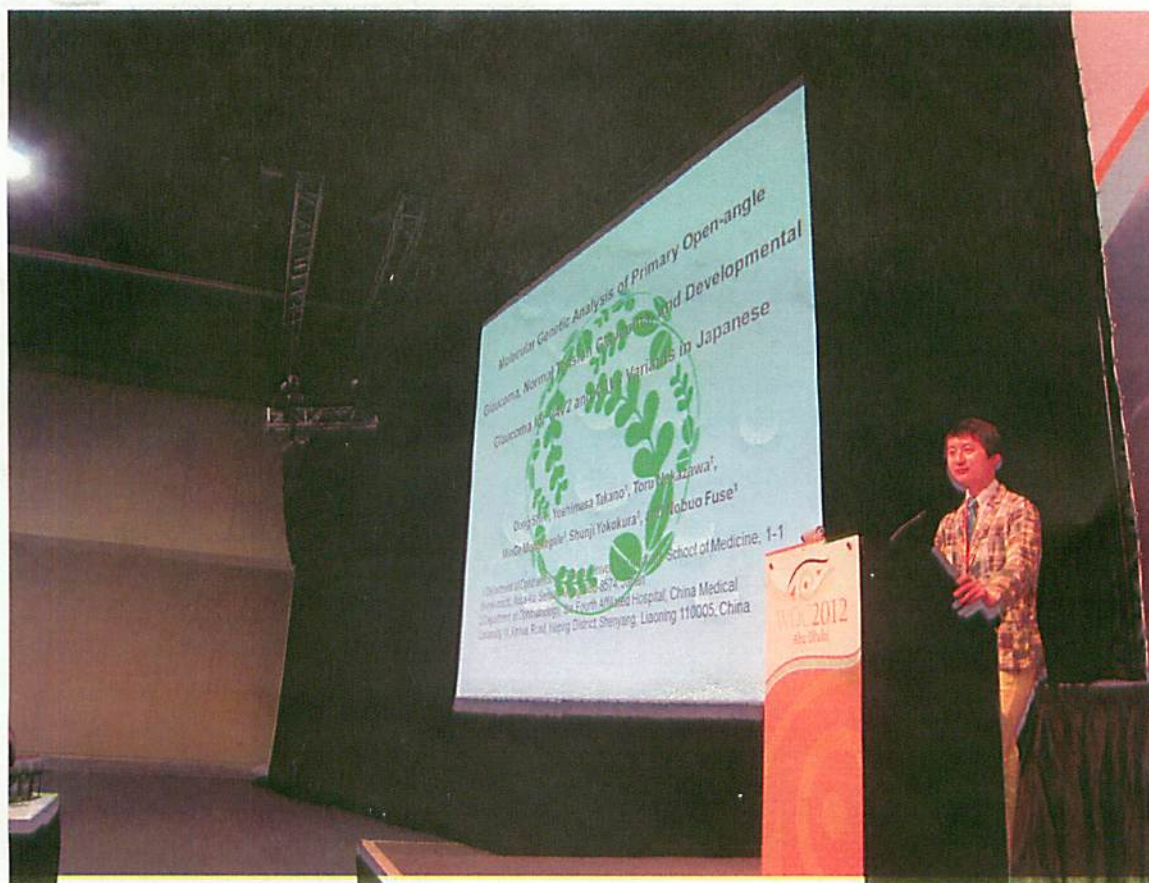
In summary, variants rs2156323 in VAV2 and rs2801219 in VAV3 genes do not appear to be major risk factors for the pathogenesis of glaucoma in the Japanese. However, Vav2/Vav3-deficient mice can still serve as a useful model of spontaneous glaucoma, and investigations of the development of their phenotype may provide information on the pathogenesis of glaucoma in humans.

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