High frequency of open-angle glaucoma in Japanese patients with Alzheimer's disease

Hiroki Tamura[a], Hideshi Kawakami[b], Takashi Kanamoto[a], Tomoko Kato[a], Tomoko Yokoyama[a], Ken Sasaki[c], Yuishin Izumi[d], Masayasu Matsumoto[e], Hiromu K. Mishima[a]

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

[b]*Department of Epidemiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Japan*

[c]*Kinoko Espoir Hospital, Kasaoka, Okayama, Japan*

[d]*Vihara Hananosato Hospital, Miyoshi, Hiroshima, Japan*

[e]*Department of Clinical Neuroscience and Therapeutics, Graduate School of Biomedical Sciences, Hiroshima University, Japan*

Correspondence to Hideshi Kawakami

1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Tel: +81-82-257-5201

Fax: +81-82-505-0490

E-mail: hkawakam@hiroshima-u.ac.jp

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Abstract

The clinical and genetic relationships between Alzheimer's disease (AD) and glaucoma remain obscure. The aim of this study was to determine the prevalence of open-angle glaucoma (OAG) in patients with AD and whether the apolipoprotein E (*APOE*) 4 allele is associated with AD, with or without OAG, in Japanese. The groups consisted of 172 patients with the diagnostic criteria of AD and 176 age-matched controls. Ophthalmic examinations were conducted, and genomic analysis was performed by PCR and digestion of products with an enzyme. OAG was found in 41 (23.8%) of the AD patients, which was a significantly (p=0.0002) higher prevalence than that in the controls (9.9%). Furthermore, there was no significant difference between intraocular pressures (IOPs) in AD patients with OAG and without OAG. The percentage of AD patients who carried an *APOE* ε4 allele (29.5%) was significantly (p=0.0007) higher than that of the controls (9.1%). However, the percentage of AD patients with OAG who carried an *APOE* ε4 allele (35.7%) was not significantly different than that of AD patients without OAG (27.7%, p=0.42). In summary, the prevalence of OAG is high in Japanese patients with AD, suggesting that common factors other than APOE may contribute to the two diseases.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that is the most common cause of dementia in the elderly. AD is characterized neuropathologically by large extracellular beta-amyloid plaques and tau-containing intraneuroral neurofibrillary tangles. Early-onset AD may be explained by highly penetrate mutations in the genes encoding amyloid precursor protein, presenilin 1 and presenilin 2, and the majority of cases (90-95%) are late-onset AD (LOAD) in which several factors have been implicated. The ε4 allele of the gene encoding apolipoprotein E (*APOE*) is a major risk factor for LOAD in the general population [1-4].

Glaucoma is the second or third leading cause of visual loss worldwide. Open-angle glaucoma (OAG) is the most common type of glaucoma in many populations [5-8]. The pathogenesis of OAG is not yet known, but there are several known risk factors [9-11], one of which is elevated intraocular pressure (IOP). Lowering IOP is the current standard therapy for many kinds of glaucoma and effectively slows down disease progression [12]. However, even after pressure reduction, many patients have ongoing visual loss. In addition, 30%~50% of OAG patients suffer from 'normal-tension' glaucoma (NTG) in which there is optic nerve degeneration without elevation in IOP [8,12,13].

AD and glaucoma have common features. Both become more severe with advance of age, and they both occur more frequently in women than in men [5, 14]. Neurons are affected in the brain of AD patients and the eye of glaucoma patients. In addition, it has been reported that patients with AD demonstrate widespread axonal degeneration of the optic nerves and loss of retinal cells, especially ganglion cells [7, 15-17]. Moreover, the ε4 allele of *APOE* has been established as a genetic risk factor of AD. Some studies have shown that ε4 is a genetic risk factor of OAG, though the contribution of the ε4 allele remains controversial [18-21]. Therefore, AD and glaucoma might have some common risk factors, mechanisms, or pathways.

To clarify the relationship between AD and glaucoma, we investigated the frequency of OAG in AD patients and we evaluated APOE as a common risk factor for AD and OAG in Japanese.

2. Materials and Methods

2.1. Prevalence of open-angle glaucoma in patients with AD

A total of 172 patients with sporadic AD (age, 80.9 ± 8.4 years, mean ± SD; 35 men, 137 women) who were institutionalized residents in four Japanese hospitals or who visited those hospitals for treatment of AD were recruited into this study. The diagnosis of probable AD was based on clinical findings according to the National Institute of Neurological and Communicative Disorders Association (NINCDS-ADRDA) criteria. A total of 176 individuals (age, 81. 9 ± 8.8 years, mean ± SD; 42 men, 134 women) who were institutionalized residents without AD in another three Japanese hospitals or who visited these hospitals for treatment of diseases other than AD were randomly chosen to be control subjects. These patients were matched for age and sex (Table 1).

These patients received ophthalmic examinations including estimation of width of the angle of the anterior chamber, and indirect ophthalmoscopy was performed in dilated pupils in all eyes to evaluate optic nerve head cup-to-disc ratios. At the same time as the ophthalmic examinations, the IOP of each of the AD patients was measured three times and that of each of the control subjects was measured once or several times. An electric impression tonometer (Tono-pen®) using the principle of

the Mackay-Marg tonometer, which can measure IOP with the patients in any position, was used for measurement of the IOP in AD patients because measurement in sitting position was difficult for many of the AD patients. The IOP of the control subjects was measured using a Goldmann applanation tonometer, which is a tonometer that operates on the basis of Imbert-Fick's law and is suitable for outpatients. However, in almost all AD patients, no reliable data of visual field could be obtained. Probable OAG was diagnosed by width of the angle of the anterior chamber > grade 2 (Van Herick method [22]), a vertical cup-to-disc ratio of the optic nerve head > 0.7 and/or difference between the vertical cup-to-disc ratio in the eyes > 0.2 with characteristic glaucomatous disc change. In no instance was the diagnosis of OAG dependent on the level of IOP. Ophthalmic examination was performed and diagnosis was made by two glaucoma specialists who were not blinded to the clinical diagnosis (dementia/control).

2. 2. APOE genotyping

All of the AD patients and control subjects in the prevalence study who gave informed consent for the purpose and all procedures of this study, including collection of blood samples, were enrolled in the study. Blood samples were collected from 122 patients with sporadic AD (age, 81.6 ± 8.6 years, mean ± SD; 21 men, 101 women) and 77 patients without AD as controls (age, 79.7 ± 8.9 years, mean ± SD; 17 men, 60 women). They were examined in the same Japanese hospitals as those in which patients in the prevalence study were examined (Table 2). The study protocol was in accordance with the Ethical Principles for Human Genome Research of the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare and the Ministry of Economy, Trade and Industry of Japan.

Genomic DNA was extracted from peripheral blood leukocytes using commercially available kits (QIAmpDNA Blood Maxi kit, Qiagen, Germany). *APOE* isoforms were amplified by polymerase chain reaction (PCR) using oligonucleotide primers F4 (5'-AGAGAATTCGCGCCGGCCTGGTACAC-3') and F6 (5'-TAAGCTTGGCACGGCTGTCCAAGGA-3') as described by Hixon et al.[23]. Each amplification reaction mixure contained 1 μl leukocyte DNA, 0.5 pmol/μl of each primer, 10% dimethyl sulfoxide, and 0.025 units/μl of Taq polymerase (Expand

High Fidelity PCR System; Roche Diagnostics, Swiss) in a final volume of 30 µl. Each reaction mixture was heated at 95°C for 3 min for denaturation and subjected to 30 cycles of amplification by primer annealing (62°C for 1 min), extension (72°C for 2 min), and denaturation (95°C for 1 min). After PCR amplification, 5 units of HhaI (New England Biolabs, USA) were added directly to each reaction mixture for digestion of the APOE sequences (3 h and 30 min at 37°C). This process required purification of the PCR products. Each reaction mixture was loaded onto an 8% polyacrylamide non-denaturing gel and subjected to electrophoresis for 2 h under a constant current (45 mA). After electrophoresis, the gel was treated with ethidium bromide (0.1 mg/l) for 15 min, and DNA fragments were visualized by UV illumination. The size of the HhaI fragments was estimated by comparison with known size markers (20-bp DNA ladder marker; Takara Bio Inc, Japan).

2.3. Statistical methods

Age and IOP were compared between patients and controls and between AD cases with OAG and AD cases without OAG by means of Mann-Whitney's U test. Sex was compared by means of the chi-square test for independence or Fisher's exact test as appropriate. Differences in genotype and allele frequency distributions among the AD cases and controls and among AD cases with OAG and AD cases without open-angle glaucoma were assessed with the chi-square test for independence or Fisher's exact test as appropriate. These analyses were performed with standard statistical software (Stat View 5.0; SAS Institute, USA), and significance level was set at 0.05.

3. Results

Clinical characteristics of the AD patients and control subjects are shown in Table 3. Among the 172 patients with AD, 41 (23.8%) had OAG. In the control group, 16 subjects (9.9%) had OAG. The percentage of AD patients with OAG (23.8%) was significantly higher than the percentage of control subjects with OAG (9.9%; p=0.0002). There was no variation between the study group and control group as the difference between slit-lamp biomicroscopy and indirect ophthalmoscopy which could represent a source of error in diagnosis of OAG. A statistically significant difference was found between the mean age of the AD patients with OAG and that of AD patients without OAG (p=0.025). There was no significant difference between groups in male: female ratio. Although almost entire exam was not compromised by non-cooperation, IOP was not measured in 38 of the 172 patients with AD because of non-cooperation. We could not directly compare IOP of the AD patients with that of the control subjects because of the different methods used to measure IOP. However IOPs in the two groups were not greatly different. IOP of the subjects with OAG was not significantly higher than that of the subjects without OAG in both the AD group (p=0.09) and the control group (p=0.64) (Table 3). Moreover, almost all subjects with OAG showed normal IOP (<21 mmHg).

The distribution of *APOE* genotyping is shown in Table 4. The ε3/ε3 genotype was found most frequently in all study groups. The relative proportion of the ε3/ε3 genotype was significantly lower in AD patients in both groups (p=0.027) than in the controls, and the relative proportion of the ε3/ε4 genotype was significantly higher in the AD patients (p=0.0009) than in the controls. Moreover, the percentage of AD patients with the ε4 allele (29.5%) was significantly greater than that of control subjects (9.1%, p=0.0007). However, the percentage of AD patients with OAG who had the ε3/ε3 genotype (57.1%) was not significantly different from that of AD patients without OAG who had the ε3/ε3 genotype (68.0%, p=0.29), and the percentage of AD patients with OAG who had the ε3/ε4 genotype (28.6%) was not significantly different than that of AD patients without OAG who had ε3/ε4 genotype (26.6%, p=0.84). The percentage of AD patients with OAG who had an *APOE* ε4 allele (35.7%) was not significantly different from that of AD patients without OAG who had an *APOE* ε4 allele (27.7%, p=0.42). The other genotyping distributions of these patients were not significantly different from each other.

The allele frequencies of the *APOE* gene are shown in Table 5. The ε3 allele frequency was not significantly lower in the AD patients (81.1%) than in the controls (87.7%, p=0.086). The ε4 allele frequency was higher in the AD patients (15.2%) than

in the controls (4.5%, p=0.001). However, the ε4 allele frequency in AD patients with OAG (19.6%) was not significantly higher than that in AD patients without OAG (13.8%, p=0.29). The other allele frequency distributions in these patients were not significantly different from each other.

4. Discussion

The prevalence of OAG in the AD patients was 23.8%, which was significantly higher than in that of the control subjects (9.9%). In the Tajimi study [24] (a study carried out to assess a population-based prevalence of glaucoma among residents aged 40 years or older in Tajimi City, located in central Japan, by simple random sampling without stratification, which was used to select 4000 subjects from the 54165 residents in Tajimi City), the prevalence of OAG increased with age from 2.0% for subjects aged 40-49 years to 7.6% for those aged 70 years or older. In our study, the mean age of patients was almost 80 years. The prevalence of probable OAG in our control group was almost the same as that in the Tajimi study. In Germany, the prevalences of probable OAG were reported to be 25.9 % in AD patients and 5.2% in controls, with a high prevalence of glaucoma in patients with AD [25]. Our results are consistent with those of the study in Germany. Therefore, it is thought that AD and glaucoma have some common mechanisms or risk factors of disease onset.

Furthermore, there was no significant difference between IOPs in AD patients with OAG and without OAG, and almost all AD patients with OAG showed normal tension. These results may be contributed that approximately 90% of OAG patients in Japan suffer from NTG [5, 24]. Moreover, relationships of damage to

abnormalities of the optic nerve in blood viscosity, to vasospasm and immunoreactive tendencies also have been reported [9-11]. Considering these findings, factors other than IOP might contribute to the optic nerve degeneration in AD patients.

APOE ε4 allele carriers accounted for a significantly high ratio (29.5%) in *APOE* genotyping of AD patients (with and without OAG), and the *APOE* ε4 allele accounted for a significantly high ratio (15.2%) in *APOE* allele frequency of AD patients. These findings are similar to results of previous studies [3, 4]. *APOE* genotyping of AD patients showed that the rate of *APOE* ε4 allele carriers was significantly greater among AD patients. However, there was no significant difference among the three AD groups (all AD cases, AD cases with OAG and AD cases without OAG), although a very large percentage of AD patients with OAG carried at least one *APOE* ε4 allele compared to the percentage of carriers in the control group. Therefore, the results of this study did not suggest that AD accompanied by OAG occurs with high frequency in *APOE* ε4 allele carriers. As another study, we also investigated *APOE* ε4 carriers in OAG patients without AD. The percentage of *APOE* ε4 carriers in these patients was high, but the difference was not significant. The ratio of *APOE* ε4 carriers was not different in AD patients with OAG and in non-AD controls with OAG in Japanese [Tamura et al., unpubl, results]. Therefore, *APOE*ε4 was not a sufficient risk

factor of OAG in our Japanese study and the results suggested that *APOE*ε4 polymorphism may not be a common risk factor for both AD and OAG.

In this study, we revealed a clinical correlation between the two diseases, but we could not show the common risk factors of the diseases. There are several candidates in common risk factors. For example, genotypes of the *APOE* promoter were also reported to modify OAG [26, 27] and they may be candidates. As well as genetic factors, chronic or repetitive intermittent intracranial pressure elevations may be common risk factors of AD and glaucoma [28].

In summary, we found that the prevalence of OAG was high in Japanese patients with AD. We expect that the two diseases have common causative factors other than APOEε4. Therefore, careful attention should be given to the potential for OAG in AD patients not to give the impression to non-ophthalmic trained physicians or clinicians that OAG would be manifest through visual disturbance.

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Table 1. Age and sex of the prevalence study subjects

	AD (n=172)	Controls (n=176)	All subjects (n=348)	P-value
Age (yr)[a]	80.9 ± 8.4	81.9 ± 8.8	81.4 ± 8.7	0.11
Sex[b]				
Male	35	42	77	0.43
Female	137	134	271	

[a] Mann-Whitney's U test

[b] Chi-square test for independence

Table 2. Age and sex of the genomic study subjects

	AD (n=122)	Controls (n=77)	All subjects (n=199)	P-value
Age (yr) [a]	81.6±8.6	79.7±8.9	80.9±8.8	0.23
Sex [b]				
Male	21	17	38	0.40
Female	101	60	161	

[a] Mann-Whitney's U test

[b] Chi-square test for independence

Table 3. Clinical characteristics of patients with Alzheimer's disease and controls

	A D (n=172)		Controls (n=176)	
	No Glaucoma	Glaucoma	No Glaucoma	Glaucoma
No.of Patients[a]	131	41 *	160	16
Age (yr)[b]	80.0 ± 11.0	84.0 ±7.6**	81.6 ± 8.9	80.6 ± 7.5
Sex [c]				
Male	26	9	38	4
Female	105	32	122	12
IOP (mmHg)[b]	15.5 ± 3.0	16.4 ± 3.2	14.8 ± 3.9	15.2 ± 3.6

[a]Chi-square test for independence: AD vs. Controls
[b]Mann-Whitney's U test: No Glaucoma vs. Glaucoma
[c]Chi-square test for independence: No Glaucoma vs. Glaucoma
*P=0.0002
**P=0.025

Table 4. Apolipoprotein E genotype in patients with Alzheimer's disease and controls

	AD			Controls
	Glaucoma(－)	Glaucoma(＋)	Total	
	n=94	n=28	n=122	n=77
APOE genotype	No. (%)	No. (%)	No. (%)	No. (%)
ε2/ε2	1 (1.1)	0 (0.0)	1 (0.8)	2 (2.6)
ε2/ε3	3 (3.2)	2 (7.1)	5 (4.1)	7 (9.1)
ε3/ε3	64 (68.0)	16 (57.1)	80 (65.6)*	61 (79.2)
ε3/ε4	25 (26.6)	8 (28.6)	33 (27.1)*	6 (7.8)
ε2/ε4	1 (1.1)	1 (3.6)	2 (1.6)	1 (1.3)
ε4/ε4	0 (0.0)	1(3.6)	1(0.8)	0 (0.0)
ε4 carrier	26 (27.7)	10 (35.7)	36 (29.5)*	7 (9.1)

*$p<0.05$, chi-square test for independence

Table 5. Apolipoprotein E allele frequency in patients with Alzheimer's disease and controls

APOE allele frequency	AD			Controls
	Glaucoma(－) No. (%)	Glaucoma(＋) No. (%)	Total No. (%)	No. (%)
ε2	6 (3.2%)	3 (5.4)	9 (3.7)	12 (7.8)
ε3	156 (83.0)	42 (75.0)	198 (81.1)	135 (87.7)
ε4	26 (13.8)	11 (19.6)	37 (15.2)*	7 (4.5)
Total	188 (100)	56 (100)	244 (100)	154 (100)

*$p<0.05$, chi-square test for independence