BMJ Open

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022987
Article Type:	Research
Date Submitted by the Author:	17-Mar-2018
Complete List of Authors:	CHEN, YU-YEN; Taichung Veterans General Hospital, Department of Ophthalmology Lai, Yun-ju; Puli Branch of Taichung Veterans General Hospital, Department of Internal Medicine Yen, Yong-Fong ; Taipei City Hospital, Section of Infectious Diseases Shen, Ying-Cheng; Taichung Veterans General Hospital, Department of Ophthalmology Wang, Chun-Yuan; Taichung Veterans General Hospital, Department of Ophthalmology Liang, Chiao-Ying; Taichung Veterans General Hospital, Department of Ophthalmology Lin, Keng-Hung ; Taichung Veterans General Hospital, Department of Ophthalmology Fan, Lir-Wan; University of Mississippi Medical Center
Keywords:	normal tension glaucoma, Alzheimer's disease, risk factors, National Health Insurance Research Database, cohort study

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study

Yu-Yen Chen,[1,2,3] Yun-Ju Lai,[2,4,5] Yong-Fong Yen,[2,6,] Ying-Cheng Shen,[1] Chun-Yuan Wang,[1] Chiao-Ying Liang,[1] Keng-Hung Lin,[1] Lir-Wan Fan[7*]

[1] Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan.

[2] School of Medicine, National Yang-Ming University, Taipei, Taiwan.

[3] Community Medicine Research Center and Institute of Public Health, National Yang-Ming University, Taipei, Taiwan.

[4] Division of Endocrinology and Metabolism, Department of Internal Medicine, Puli Branch of Taichung Veterans General Hospital, Nantou, Taiwan

[5] Department of Exercise Health Science, National Taiwan University of Sport, Taichung, Taiwan

[5] Department of Exercise Health Science, National Taiwan University of Sport, Taichung, Taiwan

[6] Section of Infectious Diseases, Taipei City Hospital, Taipei City Government, Taipei, Taiwan

[7] Department of Pediatrics; Division of Newborn Medicine, University of Mississippi Medical Center, Mississippi, USA

***Correspondence to**:

Yu-Yen Chen, MD., Ph.D.

Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan.

1650 Taiwan Boulevard Sect. 4, Taichung, Taiwan 40705

Tel：886-4-23592525 press 9
Fax：886-4-23595046
E-mail address: yuyenchen.phd@gmail.com

Word count: 2266

Author Contributors

Conceptualization: YYC, YJL, YFY, YCS, and LWF; Formal analysis: YYC, YJL, CYW and LWF; Investigation: YYC, CYW, CYL, KHL and LWF; Methodology: YYC, YJL, YFY, HYH, and YCS; Validation: YYC, YFY, and KHL; Writing the original draft: YYC, and LWF.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest

None.

Ethics approval

This study has been approved by the institutional review board of National Yang-Ming University (2015A018).

Data sharing statement

Data are available from the National Health Insurance Research Database (NHIRD) published by Taiwan National Health Insurance (NHI) Bureau. The data utilized in this study cannot be made available in the manuscript, the supplemental files, or in a public repository due to the Personal Information Protection Act executed by Taiwan's government, starting from 2012. Requests for data can be sent as a formal proposal to the NHIRD (http://nhird.nhri.org.tw) or by email to wt.gro.irhn@drihn.

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study

Abstract

Objectives: To investigate a possible association between normal tension glaucoma (NTG) and an increased risk of developing Alzheimer's disease (AD).

Design: Retrospective cohort study.

Setting: NTG group and the comparison group were retrieved from the whole population of the Taiwan National Health Insurance Research Database (NHIRD) from January 1, 2001, to December 31, 2013.

Participants: A total of 15,317 subjects with NTG were enrolled in the NTG group, and 61,268 age- and gender-matched subjects without glaucoma were enrolled in the comparison group.

Primary and secondary outcome measures: Kaplan-Meier curves were generated to compare the cumulative hazard of AD between the two groups. A multivariable Cox regression analysis was used to estimate the adjusted hazard ratios (HRs) of AD, adjusted for diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke. Furthermore, risks factors for developing AD among the NTG group were investigated.

Results: The mean age of the cohort was 62.1±12.5 yes. NTG patients had significantly higher proportions of diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke than the comparisons. NTG patients had a significantly

higher cumulative hazard for AD than the comparisons (p<0.0001). In the multivariable Cox regression after adjustment for confounders, the NTG group had a significantly higher risk of AD (adjusted HR=1.52; 95% CI: 1.41 to 1.63). Moreover, in the NTG group, when we compared the effects of different types of glaucoma eye drops, none of the eye drops used were significant risk factors or protective factors for AD.

Conclusions: People with NTG are at a significantly greater risk of developing AD compared with individuals without glaucoma. Among NTG patients, none of the glaucoma eye drops used significantly changed the risk of subsequent AD.

Keywords: normal tension glaucoma, Alzheimer's disease, risk factors, National Health Insurance Research Database, cohort study

Article Summary

Strengths and limitations of this study

- The study utilized the comprehensive, whole national population database with a long study period to investigate the relationship between normal tension glaucoma and Alzheimer's disease.
- The cohort study design and survival analysis would elucidate the association between normal tension glaucoma and subsequent risk of developing Alzheimer's disease.
- The diagnoses in the study were based on the generally accepted diagnosis classification system and were confirmed by psychiatrist/neurologist and the National Health Administration.
- Possible confounders were adjusted in the Cox regression to derive the more

real correlation between normal tension glaucoma and Alzheimer's disease.

Introduction

Glaucoma is a chronic, progressive disease with characteristics of retinal ganglion cell loss, retinal nerve fiber layer (RNFL) thinning, and optic nerve atrophy.[1-3] Elevated intraocular pressure (IOP) plays an important role in the development of glaucomatous optic neuropathy. However, 30 to 92 percent of primary open-angle glaucoma (POAG) have IOP within the normal range and are classified as normal tension glaucoma (NTG).[2, 4-7] NTG occurs because of a fragile optic nerve that can be damaged despite a normal IOP, or because of mechanisms other than an elevated IOP (e.g., insufficient ocular perfusion pressure or autonomic dysfunction).[8-13] Without treatment, NTG leads to optic nerve atrophy, progressive visual field loss and even blindness. The mainstay treatment for NTG, similar to the therapy for other types of glaucoma, is aimed at reducing IOP with glaucoma eye drops.

Alzheimer's disease (AD) is the most common form of dementia that is characterized by a progressive loss of memory and cognition and changes in personalities and behavior, as well as by an impaired ability to perform daily activities.[14-15] In the brains of AD patients, amyloid plaques and neurofibrillary tangles (aggregation of abnormal tau proteins) lead to gross atrophy of the brain.[15]

NTG and AD have common features. Both of them are chronic, progressive neurodegenerative diseases with an age-related and female-predominant incidence.[16] In structural and pathological studies, AD patients exhibit RGC loss and RNFL thinning, similar to NTG patients.[17-20] In genetic studies, the epsilon 4 allele of apolipoprotein E (APOE), which is a risk factor for late-onset AD, has been observed in the pathogenesis of NTG.[21] The optineurin gene has also been found to be

associated with both AD and NTG.[22-23] In addition, in clinical, cross-sectional studies, Bayer and Tamura et al. have found an increased prevalence of glaucoma in AD patients.[24-26] All of these may imply that glaucoma and AD share similar pathophysiological or underlying mechanisms.

However, population-based cohort studies evaluating the association between glaucoma and AD have revealed inconsistent findings. The Three-City-Bordeaux-Alienor study conducted in France showed that open-angle glaucoma (OAG) patients were four times more likely to develop dementia during the 3-year period.[27] In a Taiwan registry study, Lin et al. also found a significantly higher risk of developing AD among the POAG patients.[28] In contrast, there was no increased risk of developing AD among the NTG patients in a Danish registry study and among the POAG patients in a population-based study in Sweden.[29-30] The discrepancies may be due to the insufficient statistical power, non-equivalent study designs, and different diagnostic criteria.

The objective of our study was to investigate whether patients with NTG have a higher risk of developing AD than controls using the National Health Insurance Research Database (NHIRD) in Taiwan. To eliminate the limitations of previous studies, we utilized the whole population database; therefore, we had sufficient statistical power. In addition, the NHIRD adopted the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes. Thus, the diagnostic criteria used in our study are generally accepted worldwide.

Materials and Methods

Data source

The National Health Insurance (NHI) program of Taiwan covers the healthcare services of greater than 99% of Taiwan's 23 million residents. The NHIRD is maintained by the National Health Research Institutes of Taiwan and includes inpatient and outpatient medical benefit claims. To ensure confidentiality, the identification of all patients in the database was encrypted prior to releasing data for research purposes. Therefore, according to the rules of the Institutional Review Board, written informed consent was waived. Based on the healthcare claims of the entire population, we sought to compare the hazard of AD in subjects with and without NTG during the 13-year period. This study was approved by the ethical committee of Yang-Ming University Hospital (2015A018).

Patient involvement

Patients were not directly involved in the design of this study.

Inclusion and exclusion criteria

Using the Taiwan NHIRD from 1996 to 2013, we performed a retrospective cohort study. We first selected patients with NTG (ICD-9-CM codes 365.12) from January 1, 2001, to December 31, 2013. Patients with NTG diagnoses from January 1, 1996, to December 31, 2000, were excluded to ensure that our NTG patients were newly diagnosed. The date of the first NTG claim was defined as the index date. Those who had never received a diagnosis of glaucoma were randomly selected as a comparison group at a ratio of 1:4 and matched with the NTG group on age, gender, and index year (the year of the index date or enrollment). The two groups were followed up to see whether they developed subsequent AD. Follow-up time was calculated from the index date (enrollment date) to the date of AD diagnosis, death,

or the end of 2013, whichever occurred first. The diagnosis of AD (ICD-9-CM codes: 331.0) was confirmed by neurologists or psychiatrists through a well-acknowledged, standard diagnostic protocol. Subjects who received a diagnosis of AD or dementia before the index date or enrollment were excluded to ensure that the occurrence of AD was newly diagnosed.

Statistical analysis

After investigating the two groups descriptively by age, gender, and comorbidities, the group differences were analyzed by the two-sample t-test (for continuous variables) and chi-square test (for categorical variables). Survival analysis using the Kaplan-Meier method with the log-rank test was applied to describe and compare the cumulative incidence curves of AD. A Cox proportional hazard model was used to estimate the hazard ratio (HR) for the occurrence of AD according to each variable in the univariate and multivariate analyses. Variables included in the regression analysis were age, gender and comorbidities, including diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke. Comorbidities were regarded as time-dependent covariates.

Subsequently, we focused on the NTG group. The Cox regression was used to identify the risk factors associated with AD among NTG patients. Variables included for analysis were age, gender, comorbidities, and different types of glaucoma eye drops. Glaucoma eye drops were identified and classified by the National Drug Code and the Anatomic Therapeutic Chemical (ATC) code. According to the classification system, types of glaucoma eye drops include α-agonists, parasympathomimetics (pilocarpine), carbonic anhydrase inhibitors, β-blockers, prostaglandin analogs, and fixed combinations. All statistical operations were performed using SAS statistical package, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Demographic characteristics of the study sample

A total of 15,317 NTG patients and 61,268 matched controls were enrolled in the study. Table 1 displays the demographics of the two groups. The mean age in both groups was 62.1 years. The NTG group had a significantly higher proportion of diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke than the comparison group. During the 13-year study period, the cumulative incidence of Alzheimer's disease was significantly higher in the NTG group (1023/15317; 6.7%) than in the comparison group (2574/61268; 4.2%).

Table 1. Characteristics of the study subjects

Variable	NTG group *n*=15317	Comparison group *n*= 61268	*p*-value
	n (%)	n (%)	
Age, year, (mean±SD)	62.1±12.5	62.1±12.5	1.000
Age, categorical			1.000
<55	4778 (31.2)	19112 (31.2)	
55-65	3889 (25.4)	15556 (25.4)	
65-75	3627 (23.7)	14508 (23.7)	
≥75	3023 (19.7)	12092 (19.7)	
Gender			1.000
Male	8372 (54.7)	33488 (54.7)	
Female	6945 (45.3)	27780 (45.3)	
Comorbidities			
Diabetes	5110 (33.4)	15671 (25.6)	<0.0001
Hypertension	9148 (59.7)	32790 (53.5)	<0.0001
Hyperlipidemia	7590 (49.6)	21628 (35.3)	<0.0001
Coronary artery disease	5627 (36.7)	16323 (26.6)	<0.0001
Stroke	1572 (10.3)	6518 (10.6)	0.18
Follow-up period, year (mean±SD)	4.92±3.29	4.96±3.27	0.18
Occurrence of AD during the follow-up period	1023 (6.7)	2574 (4.2)	<0.0001

NTG indicates normal tension glaucoma

Cumulative hazard curves by the Kaplan-Meier method

Figure 1 illustrates the cumulative hazard curves for AD in the NTG group and the comparison group. A log-rank test revealed a statistically significant difference between the hazard curves of the two groups (*p*-value < 0.0001).

Univariate and multivariate analyses by the Cox regression model

The unadjusted HR for AD was 1.66 times greater in the NTG group than in the comparison group (95% CI: 1.55-1.79) (Table 2). After adjusting for covariates, the

significantly greater hazard ratio for AD in the NTG group remained (adjusted HR= 1.52; 95% CI: 1.41-1.63). Age was a significant risk factor for AD in both the univariate and multivariate analyses. The adjusted HR for AD in patients over 75 years old reached 38.85 when compared with those patients younger than 55 years old. Males were less likely to develop AD than females (adjusted HR = 0.92; 95% CI: 0.86-0.98). In the univariate analysis, patients with diabetes, hypertension, hyperlipidemia, coronary artery disease, or stroke had a significantly higher risk of developing AD. After adjustment for the covariates, only stroke remained a significant risk factor for AD (adjusted HR = 1.73; 95% CI: 1.61-1.87).

Table 2. Analysis of Risk Factors for Alzheimer's disease in patients with and without normal tension glaucoma

Predictive variables	Univariate analysis		Multivariate analysis	
	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
NTG (Yes vs. No)	1.66 (1.55–1.79)	<0.0001	1.52 (1.41–1.63)	<0.0001
Age				
<55	Reference		Reference	
55-65	5.40 (4.30–6.78)	<0.0001	4.97 (3.95–6.24)	0.0002
65-75	20.25 (16.45-24.94)	<0.0001	17.33 (14.02–21.42)	<0.0001
≥75	47.85 (38.92–58.82)	<0.0001	38.85 (31.43–48.02)	<0.0001
Gender (Male vs. Female)	0.81 (0.76–0.87)	<0.0001	0.92 (0.86–0.98)	0.01
Comorbidities				
Diabetes	1.56 (1.45-1.66)	<0.0001	1.05 (0.98–1.13)	0.20
Hypertension	2.98 (2.74–3.23)	<0.0001	1.07 (0.97-1.17)	0.17
Hyperlipidemia	1.30 (1.21-1.39)	<0.0001	1.01 (0.94-1.09)	0.79
Coronary artery disease	2.27 (2.13-2.42)	<0.0001	1.06 (0.99-1.14)	0.10
Stroke	3.39 (3.16–3.64)	<0.0001	1.73 (1.61–1.87)	<0.0001

NTG indicates normal tension glaucoma; HR indicates hazard ratio; CI indicates confidence interval;
In the multivariate analysis, all the other variables listed in the table were included for adjustment.

Risk factors for depression among NTG patients

Table 3 displays the risk factors for AD among NTG patients. Older age, female gender, and stroke significantly increased the risk of developing AD among NTG patients in univariate as well as multivariate cox regression analyses. Moreover, when we compared the effects of different types of glaucoma eye drops, none of them were significant risk factors or protective factors for AD.

Table 3. Analysis of risk factors for AD among patients with normal tension glaucoma

Predictive variables	Univariate analysis		Multivariate analysis	
	Unadjusted HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
Age				
<55	Reference		Reference	
55-65	5.14 (3.48-7.59)	<0.0001	4.74 (3.20-7.02)	<0.0001
65-75	14.65 (10.20-21.04)	<0.0001	12.83 (8.85-18.60)	<0.0001
≥75	34.06 (23.83-48.68)	<0.0001	29.01 (20.04-42.00)	<0.0001
Gender (Male vs. Female)	0.93 (0.83–1.06)	0.28	0.81 (0.71-0.92)	0.0009
Comorbidities				
Diabetes	1.36 (1.20-1.54)	<0.0001	1.01 (0.88-1.15)	0.91
Hypertension	2.99 (2.54–3.52)	<0.0001	1.11 (0.93-1.32)	0.27
Hyperlipidemia	1.21 (1.07-1.37)	0.002	1.08 (0.95-1.23)	0.23
Coronary artery disease	2.21 (1.95-2.50)	<0.0001	1.14 (0.99-1.30)	0.06
Stroke	2.55 (2.21–2.94)	<0.0001	1.38 (1.19-1.60)	<0.0001
Types of glaucoma eye drops				
α-agonist (Yes vs. No)	1.42 (1.20-1.69)	<0.0001	0.93 (0.82-1.06)	0.25
Carbonic anhydrase inhibitors (Yes vs. No)	0.80 (0.61-1.04)	0.09	0.89 (0.74-1.06)	0.19
β-blocker (Yes vs. No)	1.19 (0.83-1.69)	0.34	0.85 (0.70-1.04)	0.12
Parasympathomimetics (pilocarpine)	1.26 (0.52-3.02)	0.61	0.96 (0.68-1.36)	0.83
Prostaglandin analogs (Yes vs. No)	1.03 (0.88-1.21)	0.72	1.07 (0.93-1.23)	0.37

NTD indicates New Taiwan Dollar; HR indicates hazard ratio; CI indicates confidence interval; AC indicates angle closure. In the multivariate analysis, all the other variables listed in the table were included for adjustment.

Discussion

We conducted a 13-year cohort study on population-based data from the Taiwan NHIRD. Compared with those without glaucoma, patients with NTG had a significantly higher risk (HR = 1.52) of developing AD after adjustment for age, gender, and comorbidities. Among NTG patients, older age, female gender, and stroke were significant risk factors for developing AD. However, the types of glaucoma eye drops were not risk factors or protective factors for AD among NTG patients.

Both NTG and high-tension glaucoma (HTG) belong to POAG. They present a continuum of POAG, in which the underlying mechanism shifts from predominantly elevated IOP in HTG to hemodynamic changes in NTG. Lee et al. conducted a cross-sectional study in South Korea and found that NTG patients had a significantly higher prevalence of hypertension, hyperlipidemia, ischemic heart disease, and metabolic syndrome.[31] These findings are compatible with our findings in Table 1. Compared with non-NTG patients, we also found that NTG patients tended to be older and female predominant. These findings are consistent with the previous literature.[16]

Previous studies regarding the relationship between glaucoma and AD have mostly investigated the association between POAG and AD.[27-28, 32-34] Nevertheless, POAG includes NTG and HTG, which are distinctive disease entities. NTG has different features compared to HTG, such as IOP-independent mechanisms and characteristic patterns of structural/functional damage.[31, 35] Therefore, it is not clear enough to regard NTG and HTG as one to evaluate the subsequent risk of AD. One strength of our study is that we specified the association between NTG and AD. To the best of our knowledge, only one previous population-based study focused on the

relationship between NTG and AD.[29] Bach-Holm et al. tracked 69 NTG patients in Denmark for a mean follow-up period of 12.7 years and did not find any significantly higher risk of developing dementia/AD compared with the general population. In fact, none of those 69 NTG patients subsequently developed AD. Statistically, the case numbers were too small to perform analyses effectively. It is another strength of our study that we included over 15,000 NTG patients from our nationwide database. Thus, we have sufficient statistical power to draw convincing conclusions.

Another strength of our study is the adjustment for possible confounders in the Cox regression. Table 2 shows that the unadjusted HR for AD is 1.66 (95% CI: 1.55 to 1.79) in the NTG group compared with the comparison group. Age, female gender, and vascular/metabolic comorbidities have been reported to be risk factors of both NTG and AD;[16, 31, 36-37] thus, these should be adjusted for as confounders to address the association between NTG and AD. Due to the completeness of our database, we obtained inpatient and outpatient medical records of the whole population. After adjustment for confounders, the association between NTG and AD was still significant (HR= 1.52; 95% CI: 1.41 to 1.63), providing evidence that the significant association between NTG and AD is a real phenomenon. In addition, in our database, the diagnoses of NTG, AD, and confounding comorbidities (i.e., diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke) were accurate and were verified by the National Health Administration (NHA). In our health care system, the NHA not only checks the consistencies between the claimed data and the charts but also makes sure the patient received a standard protocol of examinations to confirm the diagnosis. Furthermore, in our database, we adopted the commonly used diagnosis classification system of ICD-9-CM codes. Thus, our results can be clearly interpreted and compared to further studies in other countries.

Still another strength is that we concentrated on the NTG group to investigate

the risk factors for AD. Table 3 shows that among NTG patients, age, female gender, and stroke significantly increased the risk of subsequent AD. To the best of our knowledge, this is the first study to find factors that were associated with an increased risk of subsequent AD among NTG patients. This finding reminds us to be alert when we identify these high-risk factors among patients with NTG.

Previous animal and in vitro studies have revealed that Betaxolol and Brimonidine eye drops are neuroprotective.[38-42] However, in our study, none of the glaucoma eye drops were identified as risk factors or protective factors for AD among the NTG patients. Further studies are warranted to investigate the issue.

A limitation of our study is that NHIRD does not provide information regarding the severity of visual field defects or visual acuity. Therefore, we could not evaluate whether the risk of AD was positively correlated with the severity of NTG or visual impairment. Future studies including chart review will be conducted to consider these factors.

The findings from our study have both clinical and public health implications. Clinically, when treating NTG patients, ophthalmologists need to focus not only on the medical aspects of NTG but must also on changes in cognitive function or memory. NTG patients at a higher risk for AD, such as older patients, female patients, and patients with stroke, should be referred to a neurologist or psychiatrist if early signs of AD become apparent. From a public health perspective, policy makers are encouraged to enforce screening for AD risk in patients with NTG and to provide more substantial and integrated care.

References

1. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Archives of ophthalmology* 2002;120(6):714-20.
2. Sommer A, Tielsch JM, Katz J, et al. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. *Archives of ophthalmology* 1991;109(8):1090-5.
3. Weih LM, Nanjan M, McCarty CA, et al. Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. *Ophthalmology* 2001;108(11):1966-72.
4. Bonomi L, Marchini G, Marraffa M, et al. Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study. *Ophthalmology* 1998;105(2):209-15.
5. Cho HK, Kee C. Population-based glaucoma prevalence studies in Asians. *Survey of ophthalmology* 2014;59(4):434-47.
6. Klein BE, Klein R, Sponsel WE, et al. Prevalence of glaucoma. The Beaver Dam Eye Study. *Ophthalmology* 1992;99(10):1499-504.
7. Rotchford AP, Johnson GJ. Glaucoma in Zulus: a population-based cross-sectional survey in a rural district in South Africa. *Archives of ophthalmology* 2002;120(4):471-8.
8. Caprioli J, Coleman AL. Blood pressure, perfusion pressure, and glaucoma. *Am J Ophthalmol* 2010;149(5):704-12.
9. Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. *The EPMA journal* 2013;4(1):14.
10. Mozaffarieh M, Flammer J. New insights in the pathogenesis and treatment of normal tension glaucoma. *Current opinion in pharmacology* 2013;13(1):43-9.
11. Park HY, Park SH, Park CK. Central visual field progression in normal-tension glaucoma patients with autonomic dysfunction. *Investigative ophthalmology & visual science* 2014;55(4):2557-63.
12. Siaudvytyte L, Januleviciene I, Daveckaite A, et al. Literature review and meta-analysis of translaminar pressure difference in open-angle glaucoma. *Eye* 2015;29(10):1242-50.
13. Mroczkowska S, Benavente-Perez A, Negi A, et al. Primary open-angle glaucoma vs normal-tension glaucoma: the vascular perspective. *JAMA ophthalmology* 2013;131(1):36-43.
14. Burns A, Iliffe S. Alzheimer's disease. *BMJ* 2009;338:b158.
15. Tiraboschi P, Hansen LA, Thal LJ, et al. The importance of neuritic plaques and

tangles to the development and evolution of AD. *Neurology* 2004;62(11):1984-9.

16. Mallick J, Devi L, Malik PK, et al. Update on Normal Tension Glaucoma. *Journal of ophthalmic & vision research* 2016;11(2):204-8.
17. Berisha F, Feke GT, Trempe CL, et al. Retinal abnormalities in early Alzheimer's disease. *Investigative ophthalmology & visual science* 2007;48(5):2285-9.
18. Guo L, Duggan J, Cordeiro MF. Alzheimer's disease and retinal neurodegeneration. *Current Alzheimer research* 2010;7(1):3-14.
19. Kesler A, Vakhapova V, Korczyn AD, et al. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. *Clinical neurology and neurosurgery* 2011;113(7):523-6.
20. Sadun AA, Bassi CJ. Optic nerve damage in Alzheimer's disease. *Ophthalmology* 1990;97(1):9-17.
21. Vickers JC, Craig JE, Stankovich J, et al. The apolipoprotein epsilon4 gene is associated with elevated risk of normal tension glaucoma. *Molecular vision* 2002;8:389-93.
22. Liu YH, Tian T. Hypothesis of optineurin as a new common risk factor in normal-tension glaucoma and Alzheimer's disease. *Medical hypotheses* 2011;77(4):591-2.
23. Osawa T, Mizuno Y, Fujita Y, et al. Optineurin in neurodegenerative diseases. *Neuropathology : official journal of the Japanese Society of Neuropathology* 2011;31(6):569-74.
24. Tamura H, Kawakami H, Kanamoto T, et al. High frequency of open-angle glaucoma in Japanese patients with Alzheimer's disease. *Journal of the neurological sciences* 2006;246(1-2):79-83.
25. Bayer AU, Ferrari F, Erb C. High occurrence rate of glaucoma among patients with Alzheimer's disease. *European neurology* 2002;47(3):165-8.
26. Bayer AU, Keller ON, Ferrari F, et al. Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. *American Journal of Ophthalmology* 2002;133(1):135-37.
27. Helmer C, Malet F, Rougier MB, et al. Is there a link between open-angle glaucoma and dementia? The Three-City-Alienor cohort. *Annals of neurology* 2013;74(2):171-9.
28. Lin IC, Wang YH, Wang TJ, et al. Glaucoma, Alzheimer's disease, and Parkinson's disease: an 8-year population-based follow-up study. *PloS one* 2014;9(9):e108938.
29. Bach-Holm D, Kessing SV, Mogensen U, et al. Normal tension glaucoma and

Alzheimer disease: comorbidity? *Acta ophthalmologica* 2012;90(7):683-5.

30. Ekstrom C, Kilander L. Open-angle glaucoma and Alzheimer's disease: a population-based 30-year follow-up study. *Acta ophthalmologica* 2017;95(2):e157-e58.
31. Lee SH, Kim GA, Lee W, et al. Vascular and metabolic comorbidities in open-angle glaucoma with low- and high-teen intraocular pressure: a cross-sectional study from South Korea. 2017;95(7):e564-e74.
32. Kessing LV, Lopez AG, Andersen PK, et al. No increased risk of developing Alzheimer disease in patients with glaucoma. *Journal of glaucoma* 2007;16(1):47-51.
33. Ou Y, Grossman DS, Lee PP, et al. Glaucoma, Alzheimer disease and other dementia: a longitudinal analysis. *Ophthalmic epidemiology* 2012;19(5):285-92.
34. Keenan TD, Goldacre R, Goldacre MJ. Associations between primary open angle glaucoma, Alzheimer's disease and vascular dementia: record linkage study. *The British journal of ophthalmology* 2015;99(4):524-7.
35. Kim KE, Park KH. Update on the Prevalence, Etiology, Diagnosis, and Monitoring of Normal-Tension Glaucoma. *Asia-Pacific journal of ophthalmology (Philadelphia, Pa)* 2016;5(1):23-31.
36. Hersi M, Irvine B, Gupta P, et al. Risk factors associated with the onset and progression of Alzheimer's disease: A systematic review of the evidence. *Neurotoxicology* 2017;61:143-87.
37. Stefanova E, Pavlovic A, Jovanovic Z, et al. Vascular risk factors in Alzheimer's disease - preliminary report. *Journal of the neurological sciences* 2012;322(1-2):166-9.
38. Baptiste DC, Hartwick AT, Jollimore CA, et al. Comparison of the neuroprotective effects of adrenoceptor drugs in retinal cell culture and intact retina. *Investigative ophthalmology & visual science* 2002;43(8):2666-76.
39. Lee D, Kim KY, Noh YH, et al. Brimonidine blocks glutamate excitotoxicity-induced oxidative stress and preserves mitochondrial transcription factor a in ischemic retinal injury. *PloS one* 2012;7(10):e47098.
40. Osborne NN, DeSantis L, Bae JH, et al. Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the retina. *Experimental eye research* 1999;69(3):331-42.
41. Tatton W, Chen D, Chalmers-Redman R, et al. Hypothesis for a common basis for neuroprotection in glaucoma and Alzheimer's disease: anti-apoptosis by alpha-2-adrenergic receptor activation. *Survey of ophthalmology* 2003;48 Suppl 1:S25-37.

42. Wood JP, DeSantis L, Chao HM, et al. Topically applied betaxolol attenuates ischaemia-induced effects to the rat retina and stimulates BDNF mRNA. *Experimental eye research* 2001;72(1):79-86.

Figure Legends

Fig. 1. Kaplan-Meier curves for Alzheimer's disease among NTG patients and the comparison group. The black line represents the NTG group and the gray line represents the comparison group.

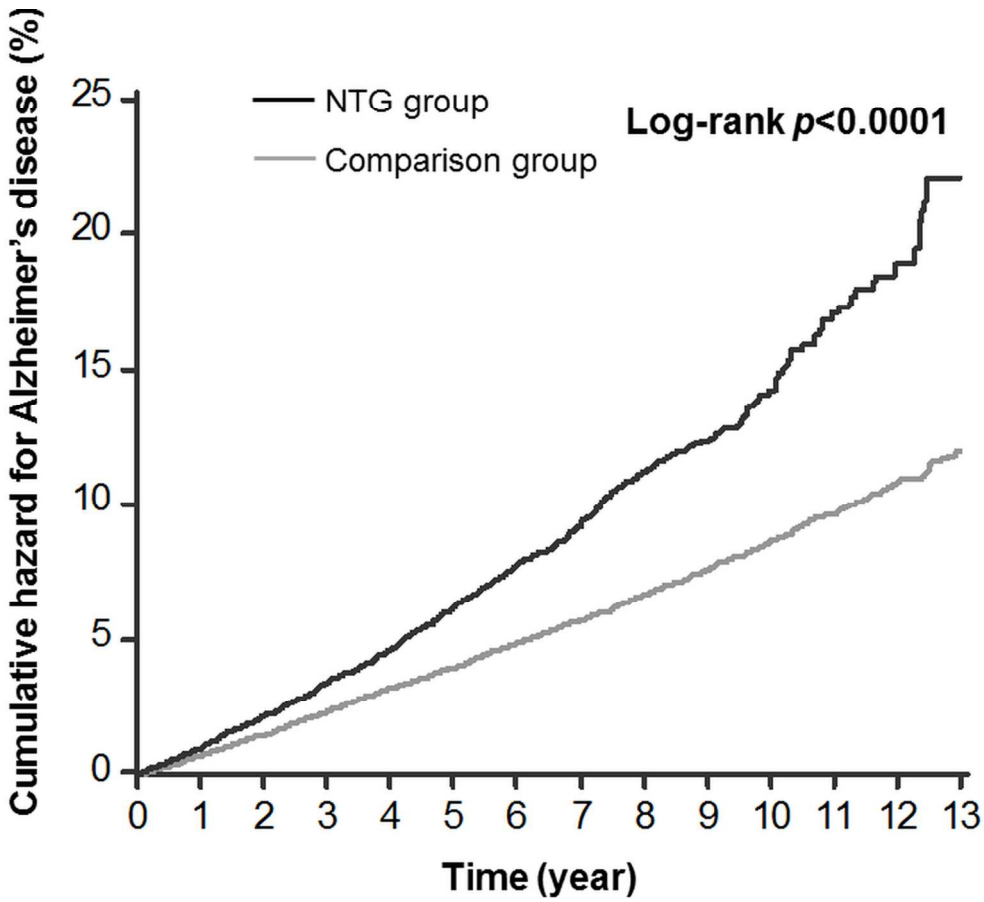

Fig. 1.Kaplan-Meier curves for Alzheimer's disease among NTG patients and the comparison group. The black line represents the NTG group and the gray line represents the comparison group.

135x120mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8-9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8*
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	11
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	13
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022987.R1
Article Type:	Research
Date Submitted by the Author:	01-Jun-2018
Complete List of Authors:	CHEN, YU-YEN; Taichung Veterans General Hospital, Department of Ophthalmology Lai, Yun-ju; Puli Branch of Taichung Veterans General Hospital, Department of Internal Medicine Yen, Yong-Fong ; Taipei City Hospital, Section of Infectious Diseases Shen, Ying-Cheng; Taichung Veterans General Hospital, Department of Ophthalmology Wang, Chun-Yuan; Taichung Veterans General Hospital, Department of Ophthalmology Liang, Chiao-Ying; Taichung Veterans General Hospital, Department of Ophthalmology Lin, Keng-Hung ; Taichung Veterans General Hospital, Department of Ophthalmology Fan, Lir-Wan; University of Mississippi Medical Center
Primary Subject Heading:	Ophthalmology
Secondary Subject Heading:	Epidemiology, Neurology
Keywords:	normal tension glaucoma, Alzheimer's disease, risk factors, National Health Insurance Research Database, cohort study

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan

Yu-Yen Chen,[1,2,3] Yun-Ju Lai,[2,4,5] Yong-Fong Yen,[2,6,] Ying-Cheng Shen,[1] Chun-Yuan Wang,[1] Chiao-Ying Liang,[1] Keng-Hung Lin,[1] Lir-Wan Fan[7*]

[1] Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan.

[2] School of Medicine, National Yang-Ming University, Taipei, Taiwan.

[3] Community Medicine Research Center and Institute of Public Health, National Yang-Ming University, Taipei, Taiwan.

[4] Division of Endocrinology and Metabolism, Department of Internal Medicine, Puli Branch of Taichung Veterans General Hospital, Nantou, Taiwan

[5] Department of Exercise Health Science, National Taiwan University of Sport, Taichung, Taiwan

[5] Department of Exercise Health Science, National Taiwan University of Sport, Taichung, Taiwan

[6] Section of Infectious Diseases, Taipei City Hospital, Taipei City Government, Taipei, Taiwan

[7] Department of Pediatrics; Division of Newborn Medicine, University of Mississippi Medical Center, Mississippi, USA

***Correspondence to**:

Yu-Yen Chen, MD., Ph.D.

Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan.

1650 Taiwan Boulevard Sect. 4, Taichung, Taiwan 40705

Tel：886-4-23592525
Fax：886-4-23595046
E-mail address: yuyenchen.phd@gmail.com

Word count: 2814

Author Contributors

Conceptualization: YYC, YJL, YFY, YCS, and LWF; Formal analysis: YYC, YJL, CYW and LWF; Investigation: YYC, CYW, CYL, KHL and LWF; Methodology: YYC, YJL, YFY, HYH, and YCS; Validation: YYC, YFY, and KHL; Writing the original draft: YYC, and LWF.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest

None.

Ethics approval

This study has been approved by the institutional review board of National Yang-Ming University (2015A018).

Data sharing statement

Data are available from the National Health Insurance Research Database (NHIRD) published by Taiwan National Health Insurance (NHI) Bureau. The data utilized in this study cannot be made available in the manuscript, the supplemental files, or in a public repository due to the Personal Information Protection Act executed by Taiwan's government, starting from 2012. Requests for data can be sent as a formal proposal to the NHIRD (http://nhird.nhri.org.tw) or by email to wt.gro.irhn@drihn.

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan

Abstract

Objectives: To investigate a possible association between normal tension glaucoma (NTG) and an increased risk of developing Alzheimer's disease (AD).

Design: Retrospective cohort study.

Setting: NTG group and the comparison group were retrieved from the whole population of the Taiwan National Health Insurance Research Database (NHIRD) from January 1, 2001, to December 31, 2013.

Participants: A total of 15,317 subjects with NTG were enrolled in the NTG group, and 61,268 age- and gender-matched subjects without glaucoma were enrolled in the comparison group.

Primary and secondary outcome measures: Kaplan-Meier curves were generated to compare the cumulative hazard of AD between the two groups. A multivariable Cox regression analysis was used to estimate the adjusted hazard ratios (HRs) of AD, adjusted for diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke. Furthermore, risks factors for developing AD among the NTG group were investigated.

Results: The mean age of the cohort was 62.1±12.5 years. NTG patients had significantly higher proportions of diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke than the comparisons. NTG patients had a significantly

higher cumulative hazard for AD than the comparisons (p<0.0001). In the multivariable Cox regression after adjustment for confounders, the NTG group had a significantly higher risk of AD (adjusted HR=1.52; 95% CI: 1.41 to 1.63). Moreover, in the NTG group, when we compared the effects of different types of glaucoma eye drops, none of the eye drops used were significant risk factors or protective factors for AD.

Conclusions: People with NTG are at a significantly greater risk of developing AD compared with individuals without glaucoma. Among NTG patients, none of the glaucoma eye drops used significantly changed the risk of subsequent AD.

Keywords: normal tension glaucoma, Alzheimer's disease, risk factors, National Health Insurance Research Database, cohort study

Article Summary

Strengths and limitations of this study

- The study utilized the comprehensive, whole national population database with a long study period to investigate the relationship between normal tension glaucoma and Alzheimer's disease.
- The cohort study design and survival analysis would elucidate the association between normal tension glaucoma and subsequent risk of developing Alzheimer's disease.
- Possible confounders were adjusted in the Cox regression to derive the more real correlation between normal tension glaucoma and Alzheimer's disease.

Introduction

Glaucoma is a chronic, progressive disease with characteristics of retinal ganglion cell loss, retinal nerve fiber layer (RNFL) thinning, and optic nerve atrophy.[1-3] Elevated intraocular pressure (IOP) plays an important role in the development of glaucomatous optic neuropathy. However, 30 to 92 percent of primary open-angle glaucoma (POAG) have IOP within the normal range and are classified as normal tension glaucoma (NTG).[2, 4-7] NTG occurs because of a fragile optic nerve that can be damaged despite a normal IOP, or because of mechanisms other than an elevated IOP (e.g., insufficient ocular perfusion pressure or autonomic dysfunction).[8-13] Without treatment, NTG leads to optic nerve atrophy, progressive visual field loss and even blindness. The mainstay treatment for NTG, similar to the therapy for other types of glaucoma, is aimed at reducing IOP with glaucoma eye drops.

Alzheimer's disease (AD) is the most common form of dementia that is characterized by a progressive loss of memory and cognition and changes in personalities and behavior, as well as by an impaired ability to perform daily activities.[14-15] In the brains of AD patients, amyloid plaques and neurofibrillary tangles (aggregation of abnormal tau proteins) lead to gross atrophy of the brain.[15]

NTG and AD have common features. Both of them are chronic, progressive neurodegenerative diseases with an age-related and female-predominant incidence.[16] In structural and pathological studies, AD patients exhibit RGC loss and RNFL thinning, similar to NTG patients.[17-20] In genetic studies, the epsilon 4 allele of apolipoprotein E (APOE), which is a risk factor for late-onset AD, has been observed in the pathogenesis of NTG.[21] The optineurin gene has also been found to be

associated with both AD and NTG.[22-23] In addition, in clinical, cross-sectional studies, Bayer and Tamura et al. have found an increased prevalence of glaucoma in AD patients.[24-26] All of these may imply that glaucoma and AD share similar pathophysiological or underlying mechanisms.

However, population-based cohort studies evaluating the association between glaucoma and AD have revealed inconsistent findings. The Three-City-Bordeaux-Alienor study conducted in France showed that open-angle glaucoma (OAG) patients were four times more likely to develop dementia during the 3-year period.[27] In a Taiwan registry study, Lin et al. also found a significantly higher risk of developing AD among the POAG patients.[28] In contrast, there was no increased risk of developing AD among the NTG patients in a Danish registry study and among the POAG patients in a population-based study in Sweden.[29-30] The discrepancies may be due to the insufficient statistical power, non-equivalent study designs, and different diagnostic criteria.

The objective of our study was to investigate whether patients with NTG have a higher risk of developing AD than controls using the National Health Insurance Research Database (NHIRD) in Taiwan. We utilized the whole population database, therefore, had large numbers of patients and a high level of statistical power. In addition, the NHIRD adopted the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes, which are generally accepted worldwide. Thus, our results can be clearly interpreted and compared to further studies in other countries.

Materials and Methods

Data source

The National Health Insurance (NHI) program of Taiwan covers the healthcare services of greater than 99% of Taiwan's 23 million residents. The NHIRD is maintained by the National Health Research Institutes of Taiwan and includes inpatient and outpatient medical benefit claims. In NHIRD, the diagnoses were accurate and were verified by the National Health Administration (NHA). The NHA not only checks the consistencies between the claimed data and the charts but also makes sure the patient received a standard protocol of examinations to confirm the diagnoses. To ensure confidentiality, the identification of all patients in the database was encrypted prior to releasing data for research purposes. Therefore, according to the rules of the Institutional Review Board, written informed consent was waived. Based on the healthcare claims of the entire population, we sought to compare the hazard of AD in subjects with and without NTG during the 13-year period. This study was approved by the ethical committee of Yang-Ming University Hospital (2015A018).

Patient involvement

Patients were not directly involved in the design of this study.

Inclusion and exclusion criteria

Using the Taiwan NHIRD from 1996 to 2013, we performed a retrospective cohort study. We first selected patients with NTG (ICD-9-CM codes 365.12) from January 1, 2001, to December 31, 2013. Patients with NTG diagnoses from January 1, 1996, to December 31, 2000, were excluded to ensure that our NTG patients were newly diagnosed. The date of the first NTG claim was defined as the index date. Those who had never received a diagnosis of glaucoma were randomly selected as a

comparison group at a ratio of 1:4 and matched with the NTG group on age, gender, and index year (the year of the index date or enrollment). The two groups were followed up to see whether they developed subsequent AD. Follow-up time was calculated from the index date (enrollment date) to the date of AD diagnosis, death, or the end of 2013, whichever occurred first. The diagnosis of AD (ICD-9-CM codes: 331.0) was confirmed by neurologists or psychiatrists through a well-acknowledged, standard diagnostic protocol. Subjects who received a diagnosis of AD or dementia before the index date or enrollment were excluded to ensure that the occurrence of AD was newly diagnosed.

Statistical analysis

After investigating the two groups descriptively by age, gender, and comorbidities, the group differences were analyzed by the two-sample t-test (for continuous variables) and chi-square test (for categorical variables). Survival analysis using the Kaplan-Meier method with the log-rank test was applied to describe and compare the cumulative incidence curves of AD. A Cox proportional hazard model was used to estimate the hazard ratio (HR) for the occurrence of AD according to each variable in the univariate and multivariate analyses. Variables included in the regression analysis were age, gender and comorbidities, including diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke. Comorbidities were regarded as time-dependent covariates.

Subsequently, we focused on the NTG group. The Cox regression was used to identify the risk factors associated with AD among NTG patients. Variables included for analysis were age, gender, comorbidities, and different types of glaucoma eye drops. Glaucoma eye drops were identified and classified by the National Drug Code and the Anatomic Therapeutic Chemical (ATC) code. According to the classification

system, types of glaucoma eye drops include α-agonists, parasympathomimetics (pilocarpine), carbonic anhydrase inhibitors, β-blockers, prostaglandin analogs, and fixed combinations. All statistical operations were performed using SAS statistical package, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Demographic characteristics of the study sample

A total of 15,317 NTG patients and 61,268 matched controls were enrolled in the study. Table 1 displays the demographics of the two groups. The mean age in both groups was 62.1 years. The NTG group had a significantly higher proportion of diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke than the comparison group. During the 13-year study period, the cumulative incidence of AD was significantly higher in the NTG group (1023/15317; 6.7%) than in the comparison group (2574/61268; 4.2%). Age of AD onset was 73.8±8.1 years.

Table 1. Characteristics of the study subjects

Variable	NTG group *n*=15317	Comparison group *n*= 61268	*p*-value
	n (%)	n (%)	
Age, year, (mean±SD)	62.1±12.5	62.1±12.5	1.000
Age, categorical			1.000
<55	4778 (31.2)	19112 (31.2)	
55-65	3889 (25.4)	15556 (25.4)	
65-75	3627 (23.7)	14508 (23.7)	
≥75	3023 (19.7)	12092 (19.7)	
Gender			1.000
Male	8372 (54.7)	33488 (54.7)	
Female	6945 (45.3)	27780 (45.3)	
Comorbidities			
Diabetes	5110 (33.4)	15671 (25.6)	<0.0001
Hypertension	9148 (59.7)	32790 (53.5)	<0.0001
Hyperlipidemia	7590 (49.6)	21628 (35.3)	<0.0001
Coronary artery disease	5627 (36.7)	16323 (26.6)	<0.0001
Stroke	1572 (10.3)	6518 (10.6)	0.18
Follow-up period, year (mean±SD)	4.92±3.29	4.96±3.27	0.18
Occurrence of AD during the follow-up period	1023 (6.7)	2574 (4.2)	<0.0001

NTG indicates normal tension glaucoma

Cumulative hazard curves by the Kaplan-Meier method

Figure 1 illustrates the cumulative hazard curves for AD in the NTG group and the comparison group. A log-rank test revealed a statistically significant difference between the hazard curves of the two groups (*p*-value < 0.0001).

Univariate and multivariate analyses by the Cox regression model

The unadjusted HR for AD was 1.66 times greater in the NTG group than in the comparison group (95% CI: 1.55-1.79) (Table 2). After adjusting for covariates, the

significantly greater hazard ratio for AD in the NTG group remained (adjusted HR= 1.52; 95% CI: 1.41-1.63). Age was a significant risk factor for AD in both the univariate and multivariate analyses. The adjusted HR for AD in patients over 75 years old reached 38.85 when compared with those patients younger than 55 years old. Males were less likely to develop AD than females (adjusted HR = 0.92; 95% CI: 0.86-0.98). In the univariate analysis, patients with diabetes, hypertension, hyperlipidemia, coronary artery disease, or stroke had a significantly higher risk of developing AD. After adjustment for the covariates, only stroke remained a significant risk factor for AD (adjusted HR = 1.73; 95% CI: 1.61-1.87). In supplementary Table 1, subgroup analyses were also presented among individuals with older and younger age separately. Among subjects younger than 65, the adjusted HR for AD was 1.98 times greater in the NTG group than in the comparison group (95% CI: 1.63-2.42); the other independent, significant risk factors for AD were female and stroke. Among subjects older than 65, in addition to these risk factors, diabetes also significantly increased the risk for AD.

Table 2. Analysis of risk factors for Alzheimer's disease in patients with and without normal tension glaucoma

Predictive variables	Univariate analysis		Multivariate analysis	
	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
NTG (Yes vs. No)	1.66 (1.55–1.79)	<0.0001	1.52 (1.41–1.63)	<0.0001
Age				
<55	Reference		Reference	
55-65	5.40 (4.30–6.78)	<0.0001	4.97 (3.95–6.24)	0.0002
65-75	20.25 (16.45-24.94)	<0.0001	17.33 (14.02–21.42)	<0.0001
≥75	47.85 (38.92–58.82)	<0.0001	38.85 (31.43–48.02)	<0.0001
Gender (Male vs. Female)	0.81 (0.76–0.87)	<0.0001	0.92 (0.86–0.98)	0.01
Comorbidities				
Diabetes	1.56 (1.45-1.66)	<0.0001	1.05 (0.98–1.13)	0.20
Hypertension	2.98 (2.74–3.23)	<0.0001	1.07 (0.97-1.17)	0.17
Hyperlipidemia	1.30 (1.21-1.39)	<0.0001	1.01 (0.94-1.09)	0.79
Coronary artery disease	2.27 (2.13-2.42)	<0.0001	1.06 (0.99-1.14)	0.10
Stroke	3.39 (3.16–3.64)	<0.0001	1.73 (1.61–1.87)	<0.0001

NTG indicates normal tension glaucoma; HR indicates hazard ratio; CI indicates confidence interval;
In the multivariate analysis, all the other variables listed in the table were included for adjustment.

Risk factors for AD among NTG patients

Table 3 displays the risk factors for AD among NTG patients. Older age, female gender, and stroke significantly increased the risk of developing AD among NTG patients in univariate as well as multivariate cox regression analyses. Moreover, when we compared the effects of different types of glaucoma eye drops, none of them were significant risk factors or protective factors for AD. Furthermore, NTG patients were divided into younger and older subgroups. The same cox regression analyses were applied separately to the two subgroups (supplementary Table 2). Among NTG patients younger than 65, female and stroke were found to be significant risk factors for AD. Among NTG patients older than 65, the significant risk factors for AD were female, stroke, and diabetes.

Table 3. Analysis of risk factors for AD among patients with normal tension glaucoma

Predictive variables	Univariate analysis		Multivariate analysis	
	Unadjusted HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
Age				
<55	Reference		Reference	
55-65	5.14 (3.48-7.59)	<0.0001	4.74 (3.20-7.02)	<0.0001
65-75	14.65 (10.20-21.04)	<0.0001	12.83 (8.85-18.60)	<0.0001
≥75	34.06 (23.83-48.68)	<0.0001	29.01 (20.04-42.00)	<0.0001
Gender (Male vs. Female)	0.93 (0.83–1.06)	0.28	0.81 (0.71-0.92)	0.0009
Comorbidities				
Diabetes	1.36 (1.20-1.54)	<0.0001	1.01 (0.88-1.15)	0.91
Hypertension	2.99 (2.54–3.52)	<0.0001	1.11 (0.93-1.32)	0.27
Hyperlipidemia	1.21 (1.07-1.37)	0.002	1.08 (0.95-1.23)	0.23
Coronary artery disease	2.21 (1.95-2.50)	<0.0001	1.14 (0.99-1.30)	0.06
Stroke	2.55 (2.21–2.94)	<0.0001	1.38 (1.19-1.60)	<0.0001
Types of glaucoma eye drops				
α-agonist (Yes vs. No)	1.42 (1.20-1.69)	<0.0001	0.93 (0.82-1.06)	0.25
Carbonic anhydrase inhibitors (Yes vs. No)	0.80 (0.61-1.04)	0.09	0.89 (0.74-1.06)	0.19
β-blocker (Yes vs. No)	1.19 (0.83-1.69)	0.34	0.85 (0.70-1.04)	0.12
Parasympathomimetics (pilocarpine)	1.26 (0.52-3.02)	0.61	0.96 (0.68-1.36)	0.83
Prostaglandin analogs (Yes vs. No)	1.03 (0.88-1.21)	0.72	1.07 (0.93-1.23)	0.37

HR indicates hazard ratio; CI indicates confidence interval; AC indicates angle closure. In the multivariate analysis, all the other variables listed in the table were included for adjustment.

Discussion

We conducted a 13-year cohort study on population-based data from the Taiwan NHIRD. Compared with those without glaucoma, patients with NTG had a significantly higher risk (HR = 1.52) of developing AD after adjustment for age, gender, and comorbidities. Among NTG patients, older age, female gender, and stroke were significant risk factors for developing AD. However, the types of glaucoma eye drops were not risk factors or protective factors for AD among NTG patients.

Both NTG and high-tension glaucoma (HTG) belong to POAG. They present a continuum of POAG, in which the underlying mechanism shifts from predominantly elevated IOP in HTG to hemodynamic changes in NTG. Lee et al. conducted a cross-sectional study in South Korea and found that NTG patients had a significantly higher prevalence of hypertension, hyperlipidemia, ischemic heart disease, and metabolic syndrome.[31] These findings are compatible with our findings in Table 1. Compared with non-NTG patients, we also found that NTG patients tended to be older and female predominant. These findings are consistent with the previous literature.[16]

Previous studies regarding the relationship between glaucoma and AD have mostly investigated the association between POAG and AD.[27-28, 32-34] Nevertheless, POAG includes NTG and HTG, which are distinctive disease entities. NTG has different features compared to HTG, such as IOP-independent mechanisms and characteristic patterns of structural/functional damage.[31, 35] Therefore, it is not clear enough to regard NTG and HTG as one to evaluate the subsequent risk of AD. One strength of our study is that we specified the association between NTG and AD. To the best of our knowledge, only one previous population-based study focused on the

relationship between NTG and AD.[29] Bach-Holm et al. tracked 69 NTG patients in Denmark for a mean follow-up period of 12.7 years and did not find any significantly higher risk of developing dementia/AD compared with the general population. In fact, none of those 69 NTG patients subsequently developed AD. Statistically, the case numbers were too small to perform analyses effectively. It is another strength of our study that we included over 15,000 NTG patients from our nationwide database. Thus, we have a higher statistical power to draw convincing conclusions.

Another strength of our study is the adjustment for possible confounders in the Cox regression. Table 2 shows that the unadjusted HR for AD is 1.66 (95% CI: 1.55 to 1.79) in the NTG group compared with the comparison group. Age, female gender, and vascular/metabolic comorbidities have been reported to be risk factors of both NTG and AD;[16, 31, 36-37] thus, these should be adjusted for as confounders to address the association between NTG and AD. Due to the completeness of our database, we obtained inpatient and outpatient medical records of the whole population. After adjustment for confounders, the association between NTG and AD was still significant (HR= 1.52; 95% CI: 1.41 to 1.63), providing evidence that the significant association between NTG and AD is a real phenomenon.

Another strength is that we concentrated on the NTG group to investigate the risk factors for AD. Table 3 shows that among NTG patients, age, female gender, and stroke significantly increased the risk of subsequent AD. To the best of our knowledge, this is the first study to find factors that were associated with an increased risk of subsequent AD among NTG patients. This finding reminds us to be alert when we identify these high-risk factors among patients with NTG.

Previous animal and in vitro studies have revealed that Betaxolol and Brimonidine eye drops are neuroprotective.[38-42] However, in our study, none of the glaucoma eye drops were identified as risk factors or protective factors for AD

among the NTG patients. Further studies are warranted to investigate the issue.

We also performed subgroup analyzes according to age in the cox regression (Supplementary Table 1, Supplementary Table 2). In Table 2 and Table 3, diabetes was not a significant risk factor for AD among all the enrolled subjects (adjusted HR=1.05, 95% CI: 0.98-1.13) and among NTG patients (adjusted HR=1.01, 95% CI: 0.88-1.15). However, previous studies found diabetes to be a significant risk factors for AD among the elderly.[43-45] To unravel the possible interaction effect of age and diabetes on AD, we performed the cox regression in subgroups according to younger age and older age. Among those over 65 years, diabetes significantly increased the risk of AD, which was compatible with the results of previous studies.[43-45]

In our next studies investigating the association between HTG and AD, as well as the relationship between NTG and dementia other than AD, the adjusted HR is 1.12 (95% CI: 0.89-1.36) and 1.21 (95% CI: 0.90-1.49), respectively (the detailed results will be presented in the future). Thus, the significantly positive association was specifically exhibited between NTG and AD. One of the possible explanation is the common pathogenesis of neurotoxic substances in NTG and AD. Abnormal hyperphosphorylated tau and ß-amyloid, are linked to retinal ganglion cell death in glaucoma and contribute to neuronal apoptosis in AD.[46] Another pathological explanation may be the low intracranial pressure (ICP) in both NTG and AD. Low ICP leads to cerebrospinal fluid (CSF) circulatory failure and accumulation of neurotoxins in CSF as well as along the optic nerve, thus playing a role in NTG and AD.[47-50]

From our study, we can not conclude that NTG or its treatment causes AD, because the association of the two diseases may be resulted from their common pathogenesis. In Figure 1, the two lines converge from the outset without any latent period, suggesting the same risk factors that influence NTG also influence AD.

Further studies are warranted to elucidate the explanations of relationship between NTG and AD.

A limitation of our study is that NHIRD does not provide information regarding the severity of visual field defects or visual acuity. Therefore, we could not evaluate whether the risk of AD was positively correlated with the severity of NTG or visual impairment. Future studies including chart review will be conducted to consider these factors. Another limitation is NTG or AD may be under-diagnosed in database studies. Besides, those with NTG may have more healthcare visits, leading to a higher chance of being diagnosed AD. Fortunately, in our healthcare system, NHI covers the fee of the comprehensive, regular health checkup of all beneficiaries. Individuals over 40 years are compelled to receive health checkup once per 3 years and those over 65 years should have once per year. The high accessibility of healthcare ensures the similar chance of diagnosis in NTGs and comparisons if they had AD. It is proved by the similar frequencies of healthcare professionals contacts (excluding ophthalmologists contacts) in NTG and control subjects (10.2±7.6 vs. 10.0±7.7 times per year; p=0.08). Even if NTG and AD are under-diagnosed, the misclassification is non-differential and causes toward-the-null bias. Therefore, the positive association between NTG and AD is true and more prominent in real situation.

The findings from our study have both clinical and public health implications. Clinically, when treating NTG patients, ophthalmologists need to focus not only on the medical aspects of NTG but also on changes in cognitive function or memory. NTG patients at a higher risk for AD, such as older patients, female patients, and patients with stroke, should be referred to a neurologist or psychiatrist if early signs of AD become apparent. From a public health perspective, policy makers are encouraged to enforce screening for AD risk in patients with NTG and to provide more substantial and integrated care.

References

1. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Archives of ophthalmology* 2002;120(6):714-20.
2. Sommer A, Tielsch JM, Katz J, et al. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. *Archives of ophthalmology* 1991;109(8):1090-5.
3. Weih LM, Nanjan M, McCarty CA, et al. Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. *Ophthalmology* 2001;108(11):1966-72.
4. Bonomi L, Marchini G, Marraffa M, et al. Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study. *Ophthalmology* 1998;105(2):209-15.
5. Cho HK, Kee C. Population-based glaucoma prevalence studies in Asians. *Survey of ophthalmology* 2014;59(4):434-47.
6. Klein BE, Klein R, Sponsel WE, et al. Prevalence of glaucoma. The Beaver Dam Eye Study. *Ophthalmology* 1992;99(10):1499-504.
7. Rotchford AP, Johnson GJ. Glaucoma in Zulus: a population-based cross-sectional survey in a rural district in South Africa. *Archives of ophthalmology* 2002;120(4):471-8.
8. Caprioli J, Coleman AL. Blood pressure, perfusion pressure, and glaucoma. *Am J Ophthalmol* 2010;149(5):704-12.
9. Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. *The EPMA journal* 2013;4(1):14.
10. Mozaffarieh M, Flammer J. New insights in the pathogenesis and treatment of normal tension glaucoma. *Current opinion in pharmacology* 2013;13(1):43-9.
11. Park HY, Park SH, Park CK. Central visual field progression in normal-tension glaucoma patients with autonomic dysfunction. *Investigative ophthalmology & visual science* 2014;55(4):2557-63.
12. Siaudvytyte L, Januleviciene I, Daveckaite A, et al. Literature review and meta-analysis of translaminar pressure difference in open-angle glaucoma. *Eye* 2015;29(10):1242-50.
13. Mroczkowska S, Benavente-Perez A, Negi A, et al. Primary open-angle glaucoma vs normal-tension glaucoma: the vascular perspective. *JAMA ophthalmology* 2013;131(1):36-43.
14. Burns A, Iliffe S. Alzheimer's disease. *BMJ* 2009;338:b158.
15. Tiraboschi P, Hansen LA, Thal LJ, et al. The importance of neuritic plaques and

tangles to the development and evolution of AD. *Neurology* 2004;62(11):1984-9.

16. Mallick J, Devi L, Malik PK, et al. Update on Normal Tension Glaucoma. *Journal of ophthalmic & vision research* 2016;11(2):204-8.
17. Berisha F, Feke GT, Trempe CL, et al. Retinal abnormalities in early Alzheimer's disease. *Investigative ophthalmology & visual science* 2007;48(5):2285-9.
18. Guo L, Duggan J, Cordeiro MF. Alzheimer's disease and retinal neurodegeneration. *Current Alzheimer research* 2010;7(1):3-14.
19. Kesler A, Vakhapova V, Korczyn AD, et al. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. *Clinical neurology and neurosurgery* 2011;113(7):523-6.
20. Sadun AA, Bassi CJ. Optic nerve damage in Alzheimer's disease. *Ophthalmology* 1990;97(1):9-17.
21. Vickers JC, Craig JE, Stankovich J, et al. The apolipoprotein epsilon4 gene is associated with elevated risk of normal tension glaucoma. *Molecular vision* 2002;8:389-93.
22. Liu YH, Tian T. Hypothesis of optineurin as a new common risk factor in normal-tension glaucoma and Alzheimer's disease. *Medical hypotheses* 2011;77(4):591-2.
23. Osawa T, Mizuno Y, Fujita Y, et al. Optineurin in neurodegenerative diseases. *Neuropathology : official journal of the Japanese Society of Neuropathology* 2011;31(6):569-74.
24. Tamura H, Kawakami H, Kanamoto T, et al. High frequency of open-angle glaucoma in Japanese patients with Alzheimer's disease. *Journal of the neurological sciences* 2006;246(1-2):79-83.
25. Bayer AU, Ferrari F, Erb C. High occurrence rate of glaucoma among patients with Alzheimer's disease. *European neurology* 2002;47(3):165-8.
26. Bayer AU, Keller ON, Ferrari F, et al. Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. *American Journal of Ophthalmology* 2002;133(1):135-37.
27. Helmer C, Malet F, Rougier MB, et al. Is there a link between open-angle glaucoma and dementia? The Three-City-Alienor cohort. *Annals of neurology* 2013;74(2):171-9.
28. Lin IC, Wang YH, Wang TJ, et al. Glaucoma, Alzheimer's disease, and Parkinson's disease: an 8-year population-based follow-up study. *PloS one* 2014;9(9):e108938.
29. Bach-Holm D, Kessing SV, Mogensen U, et al. Normal tension glaucoma and

Alzheimer disease: comorbidity? *Acta ophthalmologica* 2012;90(7):683-5.
30. Ekstrom C, Kilander L. Open-angle glaucoma and Alzheimer's disease: a population-based 30-year follow-up study. *Acta ophthalmologica* 2017;95(2):e157-e58.
31. Lee SH, Kim GA, Lee W, et al. Vascular and metabolic comorbidities in open-angle glaucoma with low- and high-teen intraocular pressure: a cross-sectional study from South Korea. 2017;95(7):e564-e74.
32. Kessing LV, Lopez AG, Andersen PK, et al. No increased risk of developing Alzheimer disease in patients with glaucoma. *Journal of glaucoma* 2007;16(1):47-51.
33. Ou Y, Grossman DS, Lee PP, et al. Glaucoma, Alzheimer disease and other dementia: a longitudinal analysis. *Ophthalmic epidemiology* 2012;19(5):285-92.
34. Keenan TD, Goldacre R, Goldacre MJ. Associations between primary open angle glaucoma, Alzheimer's disease and vascular dementia: record linkage study. *The British journal of ophthalmology* 2015;99(4):524-7.
35. Kim KE, Park KH. Update on the Prevalence, Etiology, Diagnosis, and Monitoring of Normal-Tension Glaucoma. *Asia-Pacific journal of ophthalmology (Philadelphia, Pa)* 2016;5(1):23-31.
36. Hersi M, Irvine B, Gupta P, et al. Risk factors associated with the onset and progression of Alzheimer's disease: A systematic review of the evidence. *Neurotoxicology* 2017;61:143-87.
37. Stefanova E, Pavlovic A, Jovanovic Z, et al. Vascular risk factors in Alzheimer's disease - preliminary report. *Journal of the neurological sciences* 2012;322(1-2):166-9.
38. Baptiste DC, Hartwick AT, Jollimore CA, et al. Comparison of the neuroprotective effects of adrenoceptor drugs in retinal cell culture and intact retina. *Investigative ophthalmology & visual science* 2002;43(8):2666-76.
39. Lee D, Kim KY, Noh YH, et al. Brimonidine blocks glutamate excitotoxicity-induced oxidative stress and preserves mitochondrial transcription factor a in ischemic retinal injury. *PloS one* 2012;7(10):e47098.
40. Osborne NN, DeSantis L, Bae JH, et al. Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the retina. *Experimental eye research* 1999;69(3):331-42.
41. Tatton W, Chen D, Chalmers-Redman R, et al. Hypothesis for a common basis for neuroprotection in glaucoma and Alzheimer's disease: anti-apoptosis by alpha-2-adrenergic receptor activation. *Survey of ophthalmology* 2003;48 Suppl 1:S25-37.

42. Wood JP, DeSantis L, Chao HM, et al. Topically applied betaxolol attenuates ischaemia-induced effects to the rat retina and stimulates BDNF mRNA. *Experimental eye research* 2001;72(1):79-86.
43. Ahtiluoto S, Polvikoski T, Peltonen M, et al. Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. *Neurology* 2010;75(13):1195-202. doi: 10.1212/WNL.0b013e3181f4d7f8 [published Online First: 2010/08/27]
44. Ohara T, Doi Y, Ninomiya T, et al. Glucose tolerance status and risk of dementia in the community: the Hisayama study. *Neurology* 2011;77(12):1126-34. doi: 10.1212/WNL.0b013e31822f0435 [published Online First: 2011/09/21]
45. Qiu C, Xu W, Winblad B, et al. Vascular risk profiles for dementia and Alzheimer's disease in very old people: a population-based longitudinal study. *Journal of Alzheimer's disease : JAD* 2010;20(1):293-300. doi: 10.3233/jad-2010-1361 [published Online First: 2010/02/19]
46. Jain S, Aref AA. Senile Dementia and Glaucoma: Evidence for a Common Link. *Journal of ophthalmic & vision research* 2015;10(2):178-83. doi: 10.4103/2008-322x.163766 [published Online First: 2015/10/02]
47. Wostyn P, De Groot V, Van Dam D, et al. Senescent changes in cerebrospinal fluid circulatory physiology and their role in the pathogenesis of normal-tension glaucoma. *Am J Ophthalmol* 2013;156(1):5-14.e2. doi: 10.1016/j.ajo.2013.03.003 [published Online First: 2013/04/24]
48. Serot JM, Zmudka J, Jouanny P. A possible role for CSF turnover and choroid plexus in the pathogenesis of late onset Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2012;30(1):17-26. doi: 10.3233/jad-2012-111964 [published Online First: 2012/02/22]
49. Preston JE. Ageing choroid plexus-cerebrospinal fluid system. *Microscopy research and technique* 2001;52(1):31-7. doi: 10.1002/1097-0029(20010101)52:1<31::aid-jemt5>3.0.co;2-t [published Online First: 2001/01/03]
50. Silverberg GD, Mayo M, Saul T, et al. Alzheimer's disease, normal-pressure hydrocephalus, and senescent changes in CSF circulatory physiology: a hypothesis. *The Lancet Neurology* 2003;2(8):506-11. [published Online First: 2003/07/25]

Figure Legends

Fig. 1. Kaplan-Meier curves for Alzheimer's disease among NTG patients and the comparison group. The black line represents the NTG group and the gray line represents the comparison group.

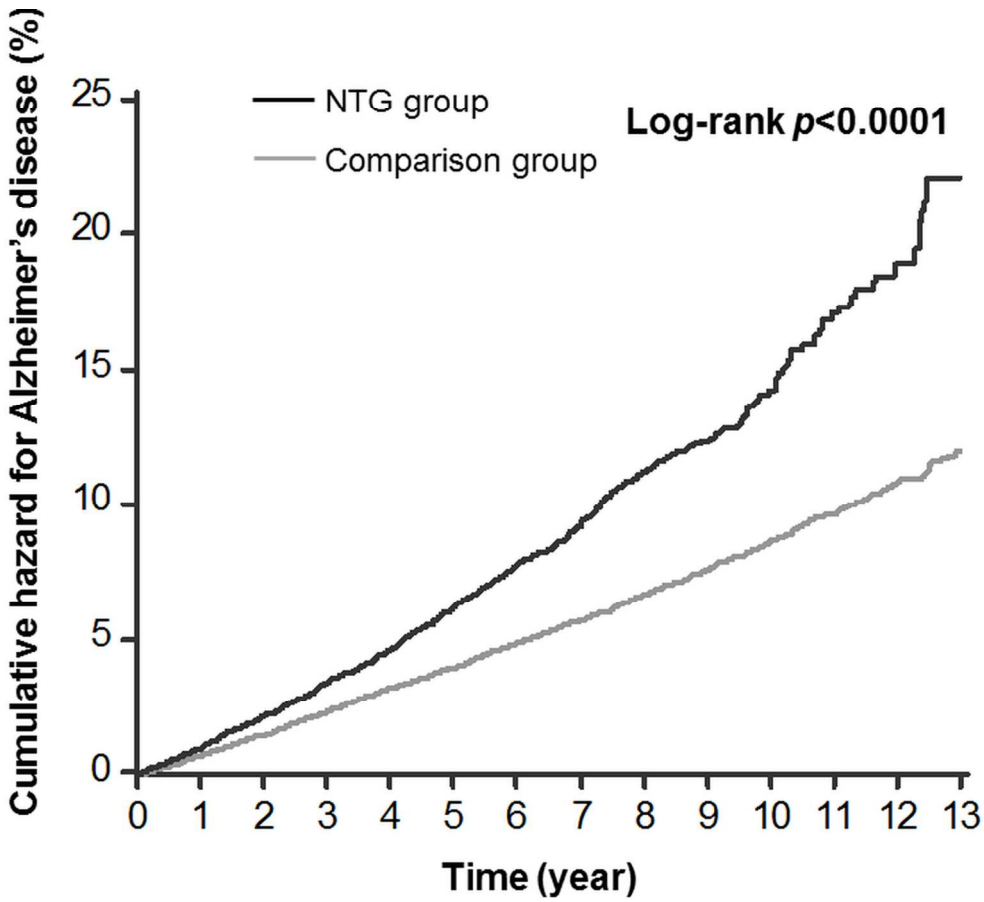

Fig. 1.Kaplan-Meier curves for Alzheimer's disease among NTG patients and the comparison group. The black line represents the NTG group and the gray line represents the comparison group.

135x120mm (300 x 300 DPI)

Supplementary Table 1. Subgroup analysis of risk factors for Alzheimer's disease in patients with and without normal tension glaucoma, stratified by age

Predictive variables	Age<65		Age≥65	
	Adjusted HR (95% CI)	*P value*	Adjusted HR (95% CI)	*P* value
NTG (Yes vs. No)	1.98 (1.63–2.42)	<0.0001	1.50 (1.39–1.63)	<0.0001
Gender (Male vs. Female)	0.68 (0.56–0.82)	<0.0001	0.93 (0.86–0.99)	0.03
Comorbidities				
Diabetes	1.19 (0.96-1.47)	0.12	1.02 (1.01–1.12)	0.03
Hypertension	1.05 (0.84–1.31)	0.69	1.09 (0.98-1.21)	0.10
Hyperlipidemia	1.22 (0.93-1.59)	0.15	1.01 (0.99-1.18)	0.08
Coronary artery disease	1.26 (0.94-1.65)	0.12	1.07 (0.99-1.13)	0.08
Stroke	4.19 (3.30–5.30)	<0.0001	1.73 (1.60–1.87)	<0.0001

NTG indicates normal tension glaucoma; HR indicates hazard ratio; CI indicates confidence interval; all the other variables listed in the table were included for adjustment.

Supplementary Table 2. Subgroup analysis of risk factors for AD among patients with normal tension glaucoma, stratified by age

Predictive variables	Age<65		Age≥65	
	Adjusted HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
Gender (Male vs. Female)	0.57 (0.41–0.80)	0.001	0.83 (0.72-0.94)	0.005
Comorbidities				
Diabetes	1.01 (0.70-1.46)	0.97	1.17 (1.05-1.12)	<0.0001
Hypertension	1.04 (0.72–1.22)	0.6	1.24 (0.99-1.62)	0.06
Hyperlipidemia	1.45 (0.94-2.18)	0.09	1.07 (0.94-1.22)	0.30
Coronary artery disease	1.37 (0.91-2.03)	0.14	1.13 (0.98-1.30)	0.10
Stroke	2.91 (1.87–4.54)	<0.0001	1.35 (1.16-1.58)	0.0002
Types of glaucoma eye drops				
α-agonist (Yes vs. No)	0.98 (0.79-1.56)	0.53	0.91 (0.79-1.04)	0.17
Carbonic anhydrase inhibitors (Yes vs. No)	0.87 (0.53-1.42)	0.57	0.90 (0.75-1.10)	0.30
β-blocker (Yes vs. No)	0.71 (0.43-1.17)	0.18	0.90 (0.77-1.02)	0.09
Parasympathomimetics (pilocarpine)	0.78 (0.29-2.13)	0.63	1.04 (0.72-1.30)	0.83
Prostaglandin analogs (Yes vs. No)	1.04 (0.71-1.51)	0.84	1.08 (0.94-1.22)	0.30

HR indicates hazard ratio; CI indicates confidence interval; all the other variables listed in the table were included for adjustment.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8-9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8*
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	11
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	13
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022987.R2
Article Type:	Research
Date Submitted by the Author:	27-Jul-2018
Complete List of Authors:	CHEN, YU-YEN; Taichung Veterans General Hospital, Department of Ophthalmology Lai, Yun-ju; Puli Branch of Taichung Veterans General Hospital, Department of Internal Medicine Yen, Yong-Fong ; Taipei City Hospital, Section of Infectious Diseases Shen, Ying-Cheng; Taichung Veterans General Hospital, Department of Ophthalmology Wang, Chun-Yuan; Taichung Veterans General Hospital, Department of Ophthalmology Liang, Chiao-Ying; Taichung Veterans General Hospital, Department of Ophthalmology Lin, Keng-Hung ; Taichung Veterans General Hospital, Department of Ophthalmology Fan, Lir-Wan; University of Mississippi Medical Center
Primary Subject Heading:	Ophthalmology
Secondary Subject Heading:	Epidemiology, Neurology
Keywords:	normal tension glaucoma, Alzheimer's disease, risk factors, National Health Insurance Research Database, cohort study

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan

Yu-Yen Chen,[1,2,3] Yun-Ju Lai,[2,4,5] Yong-Fong Yen,[2,6,] Ying-Cheng Shen,[1] Chun-Yuan Wang,[1] Chiao-Ying Liang,[1] Keng-Hung Lin,[1] Lir-Wan Fan[7*]

[1] Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan.

[2] School of Medicine, National Yang-Ming University, Taipei, Taiwan.

[3] Community Medicine Research Center and Institute of Public Health, National Yang-Ming University, Taipei, Taiwan.

[4] Division of Endocrinology and Metabolism, Department of Internal Medicine, Puli Branch of Taichung Veterans General Hospital, Nantou, Taiwan

[5] Department of Exercise Health Science, National Taiwan University of Sport, Taichung, Taiwan

[5] Department of Exercise Health Science, National Taiwan University of Sport, Taichung, Taiwan

[6] Section of Infectious Diseases, Taipei City Hospital, Taipei City Government, Taipei, Taiwan

[7] Department of Pediatrics; Division of Newborn Medicine, University of Mississippi Medical Center, Mississippi, USA

***Correspondence to**:

Yu-Yen Chen, MD., Ph.D.

Department of Ophthalmology, Taichung Veterans General Hospital, Taichung, Taiwan.

1650 Taiwan Boulevard Sect. 4, Taichung, Taiwan 40705

Tel：886-4-23592525
Fax：886-4-23595046
E-mail address: yuyenchen.phd@gmail.com

Word count: 2949

Author Contributors

Conceptualization: YYC, YJL, YFY, YCS, and LWF; Formal analysis: YYC, YJL, CYW and LWF; Investigation: YYC, CYW, CYL, KHL and LWF; Methodology: YYC, YJL, YFY, and YCS; Validation: YYC, YFY, and KHL; Writing the original draft: YYC, and LWF.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest

None.

Ethics approval

This study has been approved by the institutional review board of National Yang-Ming University (2015A018).

Data sharing statement

Data are available from the National Health Insurance Research Database (NHIRD) published by Taiwan National Health Insurance (NHI) Bureau. The data utilized in this study cannot be made available in the manuscript, the supplemental files, or in a public repository due to the Personal Information Protection Act executed by Taiwan's government, starting from 2012. Requests for data can be sent as a formal proposal to the NHIRD (http://nhird.nhri.org.tw) or by email to wt.gro.irhn@drihn.

The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan

Abstract

Objectives: To investigate a possible association between normal tension glaucoma (NTG) and an increased risk of developing Alzheimer's disease (AD).

Design: Retrospective cohort study.

Setting: NTG group and the comparison group were retrieved from the whole population of the Taiwan National Health Insurance Research Database (NHIRD) from January 1, 2001, to December 31, 2013.

Participants: A total of 15,317 subjects with NTG were enrolled in the NTG group, and 61,268 age- and gender-matched subjects without glaucoma were enrolled in the comparison group.

Primary and secondary outcome measures: Kaplan-Meier curves were generated to compare the cumulative hazard of AD between the two groups. A multivariable Cox regression analysis was used to estimate the adjusted hazard ratios (HRs) of AD, adjusted for diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke. Furthermore, risks factors for developing AD among the NTG group were investigated.

Results: The mean age of the cohort was 62.1±12.5 years. NTG patients had significantly higher proportions of diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke than the comparisons. NTG patients had a significantly

higher cumulative hazard for AD than the comparisons (p<0.0001). In the multivariable Cox regression after adjustment for confounders, the NTG group had a significantly higher risk of AD (adjusted HR=1.52; 95% CI: 1.41 to 1.63). Moreover, in the NTG group, when we compared the effects of different types of glaucoma eye drops, none of the eye drops used were significant risk factors or protective factors for AD.

Conclusions: People with NTG are at a significantly greater risk of developing AD compared with individuals without glaucoma. Among NTG patients, none of the glaucoma eye drops used significantly changed the risk of subsequent AD.

Keywords: normal tension glaucoma, Alzheimer's disease, risk factors, National Health Insurance Research Database, cohort study

Article Summary

Strengths and limitations of this study

- The study utilized the comprehensive, whole national population database with a long study period to investigate the relationship between normal tension glaucoma and Alzheimer's disease.
- The cohort study design and survival analysis would elucidate the association between normal tension glaucoma and subsequent risk of developing Alzheimer's disease.
- Possible confounders were adjusted in the Cox regression to derive the more real correlation between normal tension glaucoma and Alzheimer's disease.

Introduction

Glaucoma is a chronic, progressive disease with characteristics of retinal ganglion cell loss, retinal nerve fiber layer (RNFL) thinning, and optic nerve atrophy.[1-3] Elevated intraocular pressure (IOP) plays an important role in the development of glaucomatous optic neuropathy. However, 30 to 92 percent of primary open-angle glaucoma (POAG) have IOP within the normal range and are classified as normal tension glaucoma (NTG).[2, 4-7] NTG occurs because of a fragile optic nerve that can be damaged despite a normal IOP, or because of mechanisms other than an elevated IOP (e.g., insufficient ocular perfusion pressure or autonomic dysfunction).[8-13] Without treatment, NTG leads to optic nerve atrophy, progressive visual field loss and even blindness. The mainstay treatment for NTG, similar to the therapy for other types of glaucoma, is aimed at reducing IOP with glaucoma eye drops.

Alzheimer's disease (AD) is the most common form of dementia that is characterized by a progressive loss of memory and cognition and changes in personalities and behavior, as well as by an impaired ability to perform daily activities.[14-15] In the brains of AD patients, amyloid plaques and neurofibrillary tangles (aggregation of abnormal tau proteins) lead to gross atrophy of the brain.[15]

NTG and AD have common features. Both of them are chronic, progressive neurodegenerative diseases with an age-related and female-predominant incidence.[16] In structural and pathological studies, AD patients exhibit RGC loss and RNFL thinning, similar to NTG patients.[17-20] In genetic studies, the epsilon 4 allele of apolipoprotein E (APOE), which is a risk factor for late-onset AD, has been observed in the pathogenesis of NTG.[21] The optineurin gene has also been found to be

associated with both AD and NTG.[22-23] In addition, in clinical, cross-sectional studies, Bayer and Tamura et al. have found an increased prevalence of glaucoma in AD patients.[24-26] All of these may imply that glaucoma and AD share similar pathophysiological or underlying mechanisms.

However, population-based cohort studies evaluating the association between glaucoma and AD have revealed inconsistent findings. The Three-City-Bordeaux-Alienor study conducted in France showed that open-angle glaucoma (OAG) patients were four times more likely to develop dementia during the 3-year period.[27] In a Taiwan registry study, Lin et al. also found a significantly higher risk of developing AD among the POAG patients.[28] In contrast, there was no increased risk of developing AD among the NTG patients in a Danish registry study and among the POAG patients in a population-based study in Sweden.[29-30] The discrepancies may be due to the insufficient statistical power, non-equivalent study designs, and different diagnostic criteria.

The objective of our study was to investigate whether patients with NTG have a higher risk of developing AD than controls using the National Health Insurance Research Database (NHIRD) in Taiwan. We utilized the whole population database, therefore, had large numbers of patients and a high level of statistical power. In addition, the NHIRD adopted the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes, which are generally accepted worldwide. Thus, our results can be clearly interpreted and compared to further studies in other countries.

Materials and Methods

Data source

The National Health Insurance (NHI) program of Taiwan covers the healthcare services of greater than 99% of Taiwan's 23 million residents. The NHIRD is maintained by the National Health Research Institutes of Taiwan and includes inpatient and outpatient medical benefit claims. In NHIRD, the diagnoses were accurate and were verified by the National Health Administration (NHA). The NHA not only checks the consistencies between the claimed data and the charts but also makes sure the patient received a standard protocol of examinations to confirm the diagnoses. To ensure confidentiality, the identification of all patients in the database was encrypted prior to releasing data for research purposes. Therefore, according to the rules of the Institutional Review Board, written informed consent was waived. Based on the healthcare claims of the entire population, we sought to compare the hazard of AD in subjects with and without NTG during the 13-year period. This study was approved by the ethical committee of Yang-Ming University Hospital (2015A018).

Patient involvement

Patients were not directly involved in the design of this study.

Inclusion and exclusion criteria

Using the Taiwan NHIRD from 1996 to 2013, we performed a retrospective cohort study. We first selected patients with NTG (ICD-9-CM codes 365.12) from January 1, 2001, to December 31, 2013. Patients with NTG diagnoses from January 1, 1996, to December 31, 2000, were excluded to ensure that our NTG patients were newly diagnosed. The date of the first NTG claim was defined as the index date. Those who had never received a diagnosis of glaucoma were randomly selected as a

comparison group at a ratio of 1:4 and matched with the NTG group on age, gender, and index year (the year of the index date or enrollment). The two groups were followed up to see whether they developed subsequent AD. Follow-up time was calculated from the index date (enrollment date) to the date of AD diagnosis, death, or the end of 2013, whichever occurred first. The diagnosis of AD (ICD-9-CM codes: 331.0) was confirmed by neurologists or psychiatrists through a well-acknowledged, standard diagnostic protocol. Subjects who received a diagnosis of AD or dementia before the index date or enrollment were excluded to ensure that the occurrence of AD was newly diagnosed.

Statistical analysis

After investigating the two groups descriptively by age, gender, and comorbidities, the group differences were analyzed by the two-sample t-test (for continuous variables) and chi-square test (for categorical variables). Survival analysis using the Kaplan-Meier method with the log-rank test was applied to describe and compare the cumulative incidence curves of AD. A Cox proportional hazard model was used to estimate the hazard ratio (HR) for the occurrence of AD according to each variable in the univariate and multivariate analyses. Variables included in the regression analysis were age, gender and comorbidities, including diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke. Comorbidities were regarded as time-dependent covariates.

We additionally performed stratified analyses according to age, in order to evaluate the risk factors for AD among different age subgroups. Then, using Cox proportional hazard model, we also explored the relationship between high-tension glaucoma (HTG) and AD, as well as the relationship between NTG and all dementia/dementia other than AD.

Subsequently, we focused on the NTG group. The Cox regression was used to identify the risk factors associated with AD among NTG patients. Variables included for analysis were age, gender, comorbidities, and different types of glaucoma eye drops. Glaucoma eye drops were identified and classified by the National Drug Code and the Anatomic Therapeutic Chemical (ATC) code. According to the classification system, types of glaucoma eye drops include α-agonists, parasympathomimetics (pilocarpine), carbonic anhydrase inhibitors, β-blockers, prostaglandin analogs, and fixed combinations. All statistical operations were performed using SAS statistical package, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Demographic characteristics of the study sample

A total of 15,317 NTG patients and 61,268 matched controls were enrolled in the study. Table 1 displays the demographics of the two groups. The mean age in both groups was 62.1 years. The NTG group had a significantly higher proportion of diabetes, hypertension, hyperlipidemia, coronary artery disease, and stroke than the comparison group. During the 13-year study period, the cumulative incidence of AD was significantly higher in the NTG group (1023/15317; 6.7%) than in the comparison group (2574/61268; 4.2%). Age of AD onset was 73.8±8.1 years.

Table 1. Characteristics of the study subjects

Variable	NTG group *n*=15317	Comparison group *n*= 61268	*p*-value
	n (%)	n (%)	
Age, year, (mean±SD)	62.1±12.5	62.1±12.5	1.000
Age, categorical			1.000
<55	4778 (31.2)	19112 (31.2)	
55-65	3889 (25.4)	15556 (25.4)	
65-75	3627 (23.7)	14508 (23.7)	
≥75	3023 (19.7)	12092 (19.7)	
Gender			1.000
Male	8372 (54.7)	33488 (54.7)	
Female	6945 (45.3)	27780 (45.3)	
Comorbidities			
Diabetes	5110 (33.4)	15671 (25.6)	<0.0001
Hypertension	9148 (59.7)	32790 (53.5)	<0.0001
Hyperlipidemia	7590 (49.6)	21628 (35.3)	<0.0001
Coronary artery disease	5627 (36.7)	16323 (26.6)	<0.0001
Stroke	1572 (10.3)	6518 (10.6)	0.18
Follow-up period, year (mean±SD)	4.92±3.29	4.96±3.27	0.18
Occurrence of AD during the follow-up period	1023 (6.7)	2574 (4.2)	<0.0001

NTG indicates normal tension glaucoma

Cumulative hazard curves by the Kaplan-Meier method

Figure 1 illustrates the cumulative hazard curves for AD in the NTG group and the comparison group. A log-rank test revealed a statistically significant difference between the hazard curves of the two groups (*p*-value < 0.0001).

Univariate and multivariate analyses by the Cox regression model

The unadjusted HR for AD was 1.66 times greater in the NTG group than in the comparison group (95% CI: 1.55-1.79) (Table 2). After adjusting for covariates, the

significantly greater hazard ratio for AD in the NTG group remained (adjusted HR= 1.52; 95% CI: 1.41-1.63). Age was a significant risk factor for AD in both the univariate and multivariate analyses. The adjusted HR for AD in patients over 75 years old reached 38.85 when compared with those patients younger than 55 years old. Males were less likely to develop AD than females (adjusted HR = 0.92; 95% CI: 0.86-0.98). In the univariate analysis, patients with diabetes, hypertension, hyperlipidemia, coronary artery disease, or stroke had a significantly higher risk of developing AD. After adjustment for the covariates, only stroke remained a significant risk factor for AD (adjusted HR = 1.73; 95% CI: 1.61-1.87). In supplementary Table 1, subgroup analyses were also presented among individuals with older and younger age separately. Among subjects younger than 65, the adjusted HR for AD was 1.98 times greater in the NTG group than in the comparison group (95% CI: 1.63-2.42); the other independent, significant risk factors for AD were female and stroke. Among subjects older than 65, in addition to these risk factors, diabetes also significantly increased the risk for AD (adjusted HR=1.02; 95% CI: 1.01-1.12).

Table 2. Analysis of risk factors for Alzheimer's disease in patients with and without normal tension glaucoma

Predictive variables	Univariate analysis		Multivariate analysis	
	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
NTG (Yes vs. No)	1.66 (1.55–1.79)	<0.0001	1.52 (1.41–1.63)	<0.0001
Age				
<55	Reference		Reference	
55-65	5.40 (4.30–6.78)	<0.0001	4.97 (3.95–6.24)	0.0002
65-75	20.25 (16.45-24.94)	<0.0001	17.33 (14.02–21.42)	<0.0001
≥75	47.85 (38.92–58.82)	<0.0001	38.85 (31.43–48.02)	<0.0001
Gender (Male vs. Female)	0.81 (0.76–0.87)	<0.0001	0.92 (0.86–0.98)	0.01
Comorbidities				
Diabetes	1.56 (1.45-1.66)	<0.0001	1.05 (0.98–1.13)	0.20
Hypertension	2.98 (2.74–3.23)	<0.0001	1.07 (0.97-1.17)	0.17
Hyperlipidemia	1.30 (1.21-1.39)	<0.0001	1.01 (0.94-1.09)	0.79
Coronary artery disease	2.27 (2.13-2.42)	<0.0001	1.06 (0.99-1.14)	0.10
Stroke	3.39 (3.16–3.64)	<0.0001	1.73 (1.61–1.87)	<0.0001

NTG indicates normal tension glaucoma; HR indicates hazard ratio; CI indicates confidence interval;
In the multivariate analysis, all the other variables listed in the table were included for adjustment.

Regarding the association between HTG and AD, the HR was non-significant (adjusted HR=1.12; 95% CI: 0.89-1.36). In Table 3, the relationship between NTG and dementia other than AD was also non-significant (adjusted HR=1.21; 95% CI: 0.90-1.49). However, diabetes significantly increased the risk for dementia other than AD (adjusted HR=2.16; 95% CI: 2.03-2.26).

Table 3. Analyses of risk factors for all dementia and dementia other than Alzheimer's disease

Predictive variables	Outcome variables			
	All dementia		Dementia other than AD	
	Adjusted HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
NTG (Yes vs. No)	1.39(1.25-1.46)	<0.0001	1.21(0.90-1.49)	0.25
Diabetes	1.51(1.47-1.54)	<0.0001	2.16(2.03-2.26)	<0.0001

HR indicates hazard ratio; CI indicates confidence interval; AD indicates Alzheimer's disease; NTG indicates normal tension glaucoma; Variables included in the mulrivariate cox regression: NTG, age, gender, diabetes, hyperlipidemia, coronary artery disease, stroke.

Risk factors for AD among NTG patients

Table 4 displays the risk factors for AD among NTG patients. Older age, female gender, and stroke significantly increased the risk of developing AD among NTG patients in univariate as well as multivariate cox regression analyses. Moreover, when we compared the effects of different types of glaucoma eye drops, none of them were significant risk factors or protective factors for AD.

Table 4. Analysis of risk factors for AD among patients with normal tension glaucoma

Predictive variables	Univariate analysis		Multivariate analysis	
	Unadjusted HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
Age				
<55	Reference		Reference	
55-65	5.14 (3.48-7.59)	<0.0001	4.74 (3.20-7.02)	<0.0001
65-75	14.65 (10.20-21.04)	<0.0001	12.83 (8.85-18.60)	<0.0001
≥75	34.06 (23.83-48.68)	<0.0001	29.01 (20.04-42.00)	<0.0001
Gender (Male vs. Female)	0.93 (0.83–1.06)	0.28	0.81 (0.71-0.92)	0.0009
Comorbidities				
Diabetes	1.36 (1.20-1.54)	<0.0001	1.01 (0.88-1.15)	0.91
Hypertension	2.99 (2.54–3.52)	<0.0001	1.11 (0.93-1.32)	0.27
Hyperlipidemia	1.21 (1.07-1.37)	0.002	1.08 (0.95-1.23)	0.23
Coronary artery disease	2.21 (1.95-2.50)	<0.0001	1.14 (0.99-1.30)	0.06
Stroke	2.55 (2.21–2.94)	<0.0001	1.38 (1.19-1.60)	<0.0001
Types of glaucoma eye drops				
α-agonist (Yes vs. No)	1.42 (1.20-1.69)	<0.0001	0.93 (0.82-1.06)	0.25
Carbonic anhydrase inhibitors (Yes vs. No)	0.80 (0.61-1.04)	0.09	0.89 (0.74-1.06)	0.19
β-blocker (Yes vs. No)	1.19 (0.83-1.69)	0.34	0.85 (0.70-1.04)	0.12
Parasympathomimetics (pilocarpine)	1.26 (0.52-3.02)	0.61	0.96 (0.68-1.36)	0.83
Prostaglandin analogs (Yes vs. No)	1.03 (0.88-1.21)	0.72	1.07 (0.93-1.23)	0.37

HR indicates hazard ratio; CI indicates confidence interval; AC indicates angle closure. In the multivariate analysis, all the other variables listed in the table were included for adjustment.

Discussion

We conducted a 13-year cohort study on population-based data from the Taiwan NHIRD. Compared with those without glaucoma, patients with NTG had a significantly higher risk (HR = 1.52) of developing AD after adjustment for age, gender, and comorbidities. Among NTG patients, older age, female gender, and stroke were significant risk factors for developing AD. However, the types of glaucoma eye drops were not risk factors or protective factors for AD among NTG patients.

Both NTG and HTG belong to POAG. They present a continuum of POAG, in which the underlying mechanism shifts from predominantly elevated IOP in HTG to hemodynamic changes in NTG. Lee et al. conducted a cross-sectional study in South Korea and found that NTG patients had a significantly higher prevalence of hypertension, hyperlipidemia, ischemic heart disease, and metabolic syndrome.[31] These findings are compatible with our findings in Table 1. Compared with non-NTG patients, we also found that NTG patients tended to be older and female predominant. These findings are consistent with the previous literature.[16]

Previous studies regarding the relationship between glaucoma and AD have mostly investigated the association between POAG and AD.[27-28, 32-34] Nevertheless, POAG includes NTG and HTG, which are distinctive disease entities. NTG has different features compared to HTG, such as IOP-independent mechanisms and characteristic patterns of structural/functional damage.[31, 35] Therefore, it is not clear enough to regard NTG and HTG as one to evaluate the subsequent risk of AD. One strength of our study is that we specified the association between NTG and AD. To the best of our knowledge, only one previous population-based study focused on the relationship between NTG and AD.[29] Bach-Holm et al. tracked 69 NTG patients in

Denmark for a mean follow-up period of 12.7 years and did not find any significantly higher risk of developing dementia/AD compared with the general population. In fact, none of those 69 NTG patients subsequently developed AD. Statistically, the case numbers were too small to perform analyses effectively. It is another strength of our study that we included over 15,000 NTG patients from our nationwide database. Thus, we have a higher statistical power to draw convincing conclusions.

Another strength of our study is the adjustment for possible confounders in the Cox regression. Table 2 shows that the unadjusted HR for AD is 1.66 (95% CI: 1.55 to 1.79) in the NTG group compared with the comparison group. Age, female gender, and vascular/metabolic comorbidities have been reported to be risk factors of both NTG and AD.[16, 31, 36-37] thus, these should be adjusted for as confounders to address the association between NTG and AD. Due to the completeness of our database, we obtained inpatient and outpatient medical records of the whole population. After adjustment for confounders, the association between NTG and AD was still significant (HR= 1.52; 95% CI: 1.41 to 1.63), providing evidence that the significant association between NTG and AD is a real phenomenon.

Another strength is that we concentrated on the NTG group to investigate the risk factors for AD. Table 4 shows that among NTG patients, age, female gender, and stroke significantly increased the risk of subsequent AD. To the best of our knowledge, this is the first study to find factors that were associated with an increased risk of subsequent AD among NTG patients. This finding reminds us to be alert when we identify these high-risk factors among patients with NTG.

Previous animal and in vitro studies have revealed that Betaxolol and Brimonidine eye drops are neuroprotective.[38-42] However, in our study, none of the glaucoma eye drops were identified as risk factors or protective factors for AD among the NTG patients. Further studies are warranted to investigate the issue.

In our study, Table 3 shows diabetes is a significant risk factor for dementia other than AD (mostly are vascular dementia). This finding is consistent with the previous hospital-based study in Taiwan, which revealed a significant association between diabetes and vascular dementia.[43] On the other hand, our study shows diabetes is not a significant risk factor for AD among all the enrolled subjects (adjusted HR=1.05, 95% CI: 0.98-1.13). However, previous studies found diabetes to be a significant risk factor for AD among the elderly.[44-46] To unravel the possible interaction effect of age and diabetes on AD, we performed the cox regression in subgroups according to younger age and older age. Among those over 65 years, diabetes significantly increased the risk of AD, which was compatible with the results of previous studies.[44-46] Even so, the HR in our study (1.02) was lower than the previous population-based study in Taiwan, which had the study period from 1997 to 2007 and revealed a HR of 1.76 (95% 1.50-2.07).[47] The weaker association might result from the better diabetes care in recent years.[48] Since poor-controlled fasting plasma glucose and HbA_{1c} are significant predictors of AD,[49] the better diabetes care might possibly reduce the development of AD. Our study had a more recent study period (from 2001-2013), therefore the association between diabetes and AD is weaker. The postulation should be investigated in future studies.

In our investigation regarding the association between HTG and AD, as well as the relationship between NTG and dementia other than AD, the adjusted HR is 1.12 (95% CI: 0.89-1.36) and 1.21 (95% CI: 0.90-1.49), respectively. Thus, the significantly positive association was specifically exhibited between NTG and AD. One of the possible explanation is the common pathogenesis of neurotoxic substances in NTG and AD. AD is characterized by abnormal hyperphosphorylated tau and ß-amyloid in the brain. These abnormal protein are also related to retinal ganglion cell death in

glaucoma.[50] Another pathological explanation may be the low intracranial pressure (ICP) in both NTG and AD. Low ICP leads to cerebrospinal fluid (CSF) circulatory failure and accumulation of neurotoxins in CSF as well as along the optic nerve, thus playing a role in NTG and AD.[51-54]

From our study, we cannot conclude that NTG or its treatment causes AD, because the association of the two diseases may result from their common pathogenesis. In Figure 1, the two lines converge from the outset without any latent period, suggesting the same risk factors that influence NTG also influence AD. Further studies are warranted to elucidate the explanations of relationship between NTG and AD.

A limitation of our study is that NHIRD does not provide information regarding the severity of visual field defects or visual acuity. Therefore, we could not evaluate whether the risk of AD was positively correlated with the severity of NTG or visual impairment. Future studies including chart review will be conducted to consider these factors. Another limitation is NTG or AD may be under-diagnosed in database studies. Thus, those with NTG may have more healthcare visits, leading to a higher chance of being diagnosed AD. Fortunately, in our healthcare system, NHI covers the fee of the comprehensive, regular health checkup of all beneficiaries. Individuals over 40 years are compelled to receive health checkup once per 3 years and those over 65 years should have once per year. The high accessibility of healthcare ensures the similar chance of diagnosis in NTGs and comparisons if they had AD. It is proved by the similar frequencies of healthcare professionals contacts (excluding ophthalmologists contacts) in NTG and control subjects (10.2±7.6 vs. 10.0±7.7 times per year; p=0.08). Even if NTG and AD are under-diagnosed, the misclassification is non-differential and causes toward-the-null bias.

The findings from our study have both clinical and public health implications.

Clinically, when treating NTG patients, ophthalmologists need to focus not only on the medical aspects of NTG but also on changes in cognitive function or memory. NTG patients at a higher risk for AD, such as older patients, female patients, and patients with stroke, should be referred to a neurologist or psychiatrist if early signs of AD become apparent. From a public health perspective, policy makers are encouraged to enforce screening for AD risk in patients with NTG and to provide more substantial and integrated care.

References

1. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Archives of ophthalmology* 2002;120(6):714-20.
2. Sommer A, Tielsch JM, Katz J, et al. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. The Baltimore Eye Survey. *Archives of ophthalmology* 1991;109(8):1090-5.
3. Weih LM, Nanjan M, McCarty CA, et al. Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. *Ophthalmology* 2001;108(11):1966-72.
4. Bonomi L, Marchini G, Marraffa M, et al. Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study. *Ophthalmology* 1998;105(2):209-15.
5. Cho HK, Kee C. Population-based glaucoma prevalence studies in Asians. *Survey of ophthalmology* 2014;59(4):434-47.
6. Klein BE, Klein R, Sponsel WE, et al. Prevalence of glaucoma. The Beaver Dam Eye Study. *Ophthalmology* 1992;99(10):1499-504.
7. Rotchford AP, Johnson GJ. Glaucoma in Zulus: a population-based cross-sectional survey in a rural district in South Africa. *Archives of ophthalmology* 2002;120(4):471-8.
8. Caprioli J, Coleman AL. Blood pressure, perfusion pressure, and glaucoma. *Am J Ophthalmol* 2010;149(5):704-12.
9. Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. *The EPMA journal* 2013;4(1):14.
10. Mozaffarieh M, Flammer J. New insights in the pathogenesis and treatment of normal tension glaucoma. *Current opinion in pharmacology* 2013;13(1):43-9.
11. Park HY, Park SH, Park CK. Central visual field progression in normal-tension glaucoma patients with autonomic dysfunction. *Investigative ophthalmology & visual science* 2014;55(4):2557-63.
12. Siaudvytyte L, Januleviciene I, Daveckaite A, et al. Literature review and meta-analysis of translaminar pressure difference in open-angle glaucoma. *Eye* 2015;29(10):1242-50.
13. Mroczkowska S, Benavente-Perez A, Negi A, et al. Primary open-angle glaucoma vs normal-tension glaucoma: the vascular perspective. *JAMA ophthalmology* 2013;131(1):36-43.
14. Burns A, Iliffe S. Alzheimer's disease. *BMJ* 2009;338:b158.
15. Tiraboschi P, Hansen LA, Thal LJ, et al. The importance of neuritic plaques and

tangles to the development and evolution of AD. *Neurology* 2004;62(11):1984-9.

16. Mallick J, Devi L, Malik PK, et al. Update on Normal Tension Glaucoma. *Journal of ophthalmic & vision research* 2016;11(2):204-8.
17. Berisha F, Feke GT, Trempe CL, et al. Retinal abnormalities in early Alzheimer's disease. *Investigative ophthalmology & visual science* 2007;48(5):2285-9.
18. Guo L, Duggan J, Cordeiro MF. Alzheimer's disease and retinal neurodegeneration. *Current Alzheimer research* 2010;7(1):3-14.
19. Kesler A, Vakhapova V, Korczyn AD, et al. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. *Clinical neurology and neurosurgery* 2011;113(7):523-6.
20. Sadun AA, Bassi CJ. Optic nerve damage in Alzheimer's disease. *Ophthalmology* 1990;97(1):9-17.
21. Vickers JC, Craig JE, Stankovich J, et al. The apolipoprotein epsilon4 gene is associated with elevated risk of normal tension glaucoma. *Molecular vision* 2002;8:389-93.
22. Liu YH, Tian T. Hypothesis of optineurin as a new common risk factor in normal-tension glaucoma and Alzheimer's disease. *Medical hypotheses* 2011;77(4):591-2.
23. Osawa T, Mizuno Y, Fujita Y, et al. Optineurin in neurodegenerative diseases. *Neuropathology : official journal of the Japanese Society of Neuropathology* 2011;31(6):569-74.
24. Tamura H, Kawakami H, Kanamoto T, et al. High frequency of open-angle glaucoma in Japanese patients with Alzheimer's disease. *Journal of the neurological sciences* 2006;246(1-2):79-83.
25. Bayer AU, Ferrari F, Erb C. High occurrence rate of glaucoma among patients with Alzheimer's disease. *European neurology* 2002;47(3):165-8.
26. Bayer AU, Keller ON, Ferrari F, et al. Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. *American Journal of Ophthalmology* 2002;133(1):135-37.
27. Helmer C, Malet F, Rougier MB, et al. Is there a link between open-angle glaucoma and dementia? The Three-City-Alienor cohort. *Annals of neurology* 2013;74(2):171-9.
28. Lin IC, Wang YH, Wang TJ, et al. Glaucoma, Alzheimer's disease, and Parkinson's disease: an 8-year population-based follow-up study. *PloS one* 2014;9(9):e108938.
29. Bach-Holm D, Kessing SV, Mogensen U, et al. Normal tension glaucoma and

Alzheimer disease: comorbidity? *Acta ophthalmologica* 2012;90(7):683-5.
30. Ekstrom C, Kilander L. Open-angle glaucoma and Alzheimer's disease: a population-based 30-year follow-up study. *Acta ophthalmologica* 2017;95(2):e157-e58.
31. Lee SH, Kim GA, Lee W, et al. Vascular and metabolic comorbidities in open-angle glaucoma with low- and high-teen intraocular pressure: a cross-sectional study from South Korea. 2017;95(7):e564-e74.
32. Kessing LV, Lopez AG, Andersen PK, et al. No increased risk of developing Alzheimer disease in patients with glaucoma. *Journal of glaucoma* 2007;16(1):47-51.
33. Ou Y, Grossman DS, Lee PP, et al. Glaucoma, Alzheimer disease and other dementia: a longitudinal analysis. *Ophthalmic epidemiology* 2012;19(5):285-92.
34. Keenan TD, Goldacre R, Goldacre MJ. Associations between primary open angle glaucoma, Alzheimer's disease and vascular dementia: record linkage study. *The British journal of ophthalmology* 2015;99(4):524-7.
35. Kim KE, Park KH. Update on the Prevalence, Etiology, Diagnosis, and Monitoring of Normal-Tension Glaucoma. *Asia-Pacific journal of ophthalmology (Philadelphia, Pa)* 2016;5(1):23-31.
36. Hersi M, Irvine B, Gupta P, et al. Risk factors associated with the onset and progression of Alzheimer's disease: A systematic review of the evidence. *Neurotoxicology* 2017;61:143-87.
37. Stefanova E, Pavlovic A, Jovanovic Z, et al. Vascular risk factors in Alzheimer's disease - preliminary report. *Journal of the neurological sciences* 2012;322(1-2):166-9.
38. Baptiste DC, Hartwick AT, Jollimore CA, et al. Comparison of the neuroprotective effects of adrenoceptor drugs in retinal cell culture and intact retina. *Investigative ophthalmology & visual science* 2002;43(8):2666-76.
39. Lee D, Kim KY, Noh YH, et al. Brimonidine blocks glutamate excitotoxicity-induced oxidative stress and preserves mitochondrial transcription factor a in ischemic retinal injury. *PloS one* 2012;7(10):e47098.
40. Osborne NN, DeSantis L, Bae JH, et al. Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the retina. *Experimental eye research* 1999;69(3):331-42.
41. Tatton W, Chen D, Chalmers-Redman R, et al. Hypothesis for a common basis for neuroprotection in glaucoma and Alzheimer's disease: anti-apoptosis by alpha-2-adrenergic receptor activation. *Survey of ophthalmology* 2003;48 Suppl 1:S25-37.

42. Wood JP, DeSantis L, Chao HM, et al. Topically applied betaxolol attenuates ischaemia-induced effects to the rat retina and stimulates BDNF mRNA. *Experimental eye research* 2001;72(1):79-86.
43. Lin JC, Hsu WC, Hsu HP, et al. Risk factors for vascular dementia: a hospital-based study in Taiwan. *Acta Neurol Taiwan* 2007;16(1):22-6.
44. Ahtiluoto S, Polvikoski T, Peltonen M, et al. Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. *Neurology* 2010;75(13):1195-202. doi: 10.1212/WNL.0b013e3181f4d7f8 [published Online First: 2010/08/27]
45. Ohara T, Doi Y, Ninomiya T, et al. Glucose tolerance status and risk of dementia in the community: the Hisayama study. *Neurology* 2011;77(12):1126-34. doi: 10.1212/WNL.0b013e31822f0435 [published Online First: 2011/09/21]
46. Qiu C, Xu W, Winblad B, et al. Vascular risk profiles for dementia and Alzheimer's disease in very old people: a population-based longitudinal study. *Journal of Alzheimer's disease : JAD* 2010;20(1):293-300. doi: 10.3233/jad-2010-1361.
47. Huang CC, Chung CM, Leu HB, et al. Diabetes mellitus and the risk of Alzheimer's disease: a nationwide population-based study. *PLoS One* 2014;9(1):e87095.
48. Chang TJ, Jiang YD, Chang CH, et al. Accountability, utilization and providers for diabetes management in Taiwan, 2000-2009: an analysis of the National Health Insurance database. *J Formos Med Assoc* 2012;111(11):605-16.
49. Li TC, Yang CP, Tseng ST, et al. Visit-to-Visit Variations in Fasting Plasma Glucose and HbA1c Associated With an Increased Risk of Alzheimer Disease: Taiwan Diabetes Study. *Diabetes Care* 2017;40(9):1210-17.
50. Jain S, Aref AA. Senile Dementia and Glaucoma: Evidence for a Common Link. *Journal of ophthalmic & vision research* 2015;10(2):178-83. doi: 10.4103/2008-322x.163766 [published Online First: 2015/10/02]
51. Wostyn P, De Groot V, Van Dam D, et al. Senescent changes in cerebrospinal fluid circulatory physiology and their role in the pathogenesis of normal-tension glaucoma. *Am J Ophthalmol* 2013;156(1):5-14.e2. doi: 10.1016/j.ajo.2013.03.003 [published Online First: 2013/04/24]
52. Serot JM, Zmudka J, Jouanny P. A possible role for CSF turnover and choroid plexus in the pathogenesis of late onset Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2012;30(1):17-26. doi: 10.3233/jad-2012-111964 [published Online First: 2012/02/22]
53. Preston JE. Ageing choroid plexus-cerebrospinal fluid system. *Microscopy research and technique* 2001;52(1):31-7. doi: 10.1002/1097-0029(20010101)52:1<31::aid-jemt5>3.0.co;2-t [published

Online First: 2001/01/03]

54. Silverberg GD, Mayo M, Saul T, et al. Alzheimer's disease, normal-pressure hydrocephalus, and senescent changes in CSF circulatory physiology: a hypothesis. *The Lancet Neurology* 2003;2(8):506-11. [published Online First: 2003/07/25]

Figure Legends

Fig. 1. Kaplan-Meier curves for Alzheimer's disease among NTG patients and the comparison group. The black line represents the NTG group and the gray line represents the comparison group.

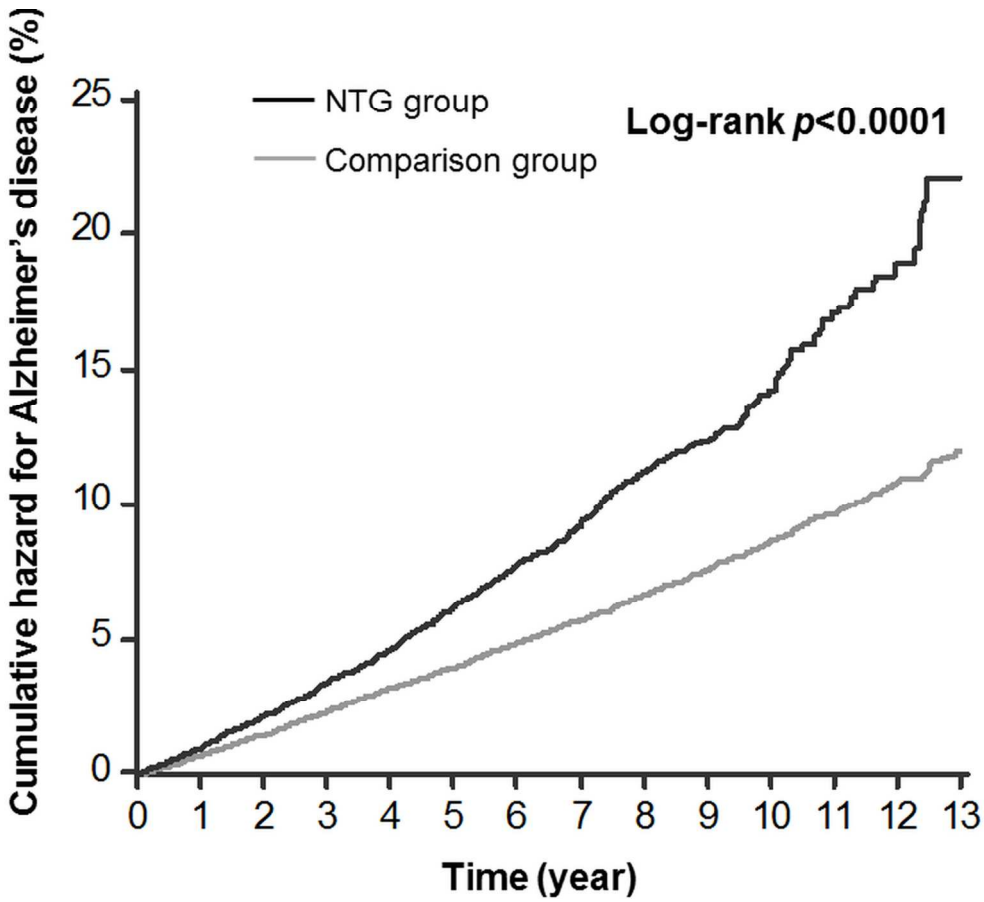

Fig. 1.Kaplan-Meier curves for Alzheimer's disease among NTG patients and the comparison group. The black line represents the NTG group and the gray line represents the comparison group.

135x120mm (300 x 300 DPI)

Supplementary Table 1. Subgroup analysis of risk factors for Alzheimer's disease in patients with and without normal tension glaucoma, stratified by age

Predictive variables	Age<65 (Total n=44165; AD n=447)		Age≥65 (Total n=32420; AD n=3150)	
	Adjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
NTG (Yes vs. No)	1.98 (1.63–2.42)	<0.0001	1.50 (1.39–1.63)	<0.0001
Gender (Male vs. Female)	0.68 (0.56–0.82)	<0.0001	0.93 (0.86–0.99)	0.03
Comorbidities				
Diabetes	1.19 (0.96-1.47)	0.12	1.02 (1.01–1.12)	0.03
Hypertension	1.05 (0.84–1.31)	0.69	1.09 (0.98-1.21)	0.10
Hyperlipidemia	1.22 (0.93-1.59)	0.15	1.01 (0.99-1.18)	0.08
Coronary artery disease	1.26 (0.94-1.65)	0.12	1.07 (0.99-1.13)	0.08
Stroke	4.19 (3.30–5.30)	<0.0001	1.73 (1.60–1.87)	<0.0001

NTG indicates normal tension glaucoma; n indicates numbers; AD indicates Alzheimer disease; HR indicates hazard ratio; CI indicates confidence interval; all the other variables listed in the table were included for adjustment.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any pre-specified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8-9
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8*
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	8

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	11
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	13
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.