PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Association between Normal Tension Glaucoma and the Risk of Alzheimer's Disease: A Nationwide Population-based Cohort study in Taiwan
AUTHORS	CHEN, YU-YEN; Lai, Yun-ju; Yen, Yong-Fong; Shen, Ying-Cheng; Wang, Chun-Yuan; Liang, Chiao-Ying; Lin, Keng-Hung ; Fan, Lir-Wan

VERSION 1 – REVIEW

REVIEWER	Michael Goldacre Department of Population Health, University of Oxford
REVIEW RETURNED	24-Apr-2018

GENERAL COMMENTS	This is a well conducted study using a well tested dataset. Two minor points: On page 7, line 40, the authors state that they 'used the whole population dataset and therefore had sufficient power…' Whilst the power statement is likely to be true, there are no power calculations; and it would be safer to say, more simply, that they '…therefore had large numbers of patients and a high level of statistical power' On page 7, line 42, the authors state that they used the 'International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes and 'thus, the diagnostic criteria used in our study are generally accepted worldwide'. This conflates the concepts of diagnostic criteria and of coding systems. The points are made better later, on page 17, lines 40-52. The authors might like to explain the earlier statement more clearly. Page 14, line 34: the title 'Risk factors for depression…' is wrong. The authors mean 'Risk factors for Alzheimer's disease…' Figure 1: The lines for the NTG group and the comparison group converge from the outset, i.e. they do not show any latent period between the onset of NTG and the emerging excess risk of AD. Does this offer any clues about possible mechanisms of effect? To me, it suggests that the same risk factors that influence NTG also influence AD, rather than that any excess risk of AD is a consequence (with a time delay) of NTG or its treatment. Would the authors like to comment?

REVIEWER	Catherine HELMER INSERM U1219, Bordeaux Population Health research center, Bordeaux, France
REVIEW RETURNED	02-May-2018

GENERAL COMMENTS	This paper investigates the risk of AD associated with normal tension glaucoma (NTG) within the Taiwan National Health Insurance Research Database. The authors found an increased risk of developing AD associated with NTG. This study has the advantages of a large Health Insurance Database, with a large number of participants, both with and without NTG and a long follow-up (up to 13 years here). Results presented are interested as very few previous studies have been published on this topic, with conflicting results until now. However, I have some concerns and I think that this paper could be improved and could bring more to this research field: - My first concern is about the diagnosis of AD in Health Insurance Database. It is well known that dementia and AD are largely underdiagnosed in the population, and thus under-reported in Health Insurance Database, with about 50% of undiagnosed cases. The authors do not tackle this issue in the paper and do not even considered this as a limitation. At least, the author must discuss this limit in the paper. Moreover, as having a pathology (such as NTG) implies more frequent contacts with health care professionals, and thus a higher chance of being diagnosed demented, it would be interesting to evaluate the frequency of contacts with health care professionals (excluding ophthalmologist contacts), comparatively in the 2 groups to investigate the potential bias and whether it could explain the higher risk of AD in the NTG. - Second, the diagnostic criteria used to define AD are very restrictive, in particular due to the fact that most of dementia cases among the elderly are mixed cases. Thus, in addition to AD, the analysis should be done also for other dementia cases, to evaluate if the increased risk is similar or not. This could bring additional clues regarding the potential physiopathological mechanisms between NTG and AD. Moreover, as the mean age of the population is relatively low to analyze AD risk, age at dementia onset should be presented in the results. - Third, beyond NTG, as diagnoses of other POAG are available in the database, the authors should also investigate other POAG and discuss whether the risk is specific of NTG or not. Again, it would enrich the discussion to better understand the potential mechanisms involves in the relationship between NTG and AD and provide suggestions for future researches. - Discussion limitations only address the problem of the severity of visual field defects whereas other limitations regarding Health Insurance Database analyses must be discussed; in particular the under-diagnosis of AD, the potential selection of diagnosed cases (those who recourse to care for their dementia), the potential differential bias for the diagnosis between NTG and non-NTG patients, ... - Discussion should be enriched regarding hypotheses on the relationship between NTG (and/or other POAG) and AD. - The absence of significant association between diabetes and AD is surprising. Please comment on this. Minor comments: p3, line 52: please replace yes by years. p4: the second point of strengths and limitations seems inappropriate. p7: Please modify the sentence "To eliminate the limitations of previous studies" as Health Insurance Database analyses have a lot of other limitations and do not overcome all the limitations of other studies. p 17, line 37: please delete "must".

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:
Reviewer: 1
Please leave your comments for the authors below
This is a well conducted study using a well tested dataset. Two minor points:
1. On page 7, line 40, the authors state that they 'used the whole population dataset and therefore had sufficient power…' Whilst the power statement is likely to be true, there are no power calculations; and it would be safer to say, more simply, that they '…therefore had large numbers of patients and a high level of statistical power'
->Answer: Thank you. We have revised the sentences according to your comment. Please see page 6, line 37-39: "We utilized the whole population database, therefore, had large numbers of patients and a high level of statistical power."

2. On page 7, line 42, the authors state that they used the 'International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes and 'thus, the diagnostic criteria used in our study are generally accepted worldwide'. This conflates the concepts of diagnostic criteria and of coding systems. The points are made better later, on page 17, lines 40-52. The authors might like to explain the earlier statement more clearly.
->Answer: Thank you for your comment. We have revised the article according to your comment. Please see page 6, line 39-48: "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".
And, please see page 7, line 15-23: "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."

3. Page 14, line 34: the title 'Risk factors for depression…' is wrong. The authors mean 'Risk factors for Alzheimer's disease…'
->Answer: Thank you. We have revised the sentence according to your comment. Please see page 13, line 34: "Risk factors for AD among NTG patients".

4. Figure 1: The lines for the NTG group and the comparison group converge from the outset, i.e. they do not show any latent period between the onset of NTG and the emerging excess risk of AD. Does this offer any clues about possible mechanisms of effect? To me, it suggests that the same risk factors that influence NTG also influence AD, rather than that any excess risk of AD is a consequence (with a time delay) of NTG or its treatment. Would the authors like to comment?
->Answer: Thank you for your comment. We have described and explained this. Please see page 17, line 55 to page 18, line 5 :"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".

Reviewer: 2
Please leave your comments for the authors below
This paper investigates the risk of AD associated with normal tension glaucoma (NTG) within the Taiwan National Health Insurance Research Database. The authors found an increased risk of developing AD associated with NTG.

This study has the advantages of a large Health Insurance Database, with a large number of participants, both with and without NTG and a long follow-up (up to 13 years here). Results presented are interested as very few previous studies have been published on this topic, with conflicting results until now. However, I have some concerns and I think that this paper could be improved and could bring more to this research field:
1.- My first concern is about the diagnosis of AD in Health Insurance Database. It is well known that dementia and AD are largely underdiagnosed in the population, and thus under-reported in Health Insurance Database, with about 50% of undiagnosed cases. The authors do not tackle this issue in the paper and do not even considered this as a limitation. At least, the author must discuss this limit in the paper. Moreover, as having a pathology (such as NTG) implies more frequent contacts with health care professionals, and thus a higher chance of being diagnosed demented, it would be interesting to evaluate the frequency of contacts with health care professionals (excluding ophthalmologist contacts), comparatively in the 2 groups to investigate the potential bias and whether it could explain the higher risk of AD in the NTG.
->Answer: Thank you. We have revised the article according to your comment.
Please see page 18, line 16-40: “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”.

2.- Second, the diagnostic criteria used to define AD are very restrictive, in particular due to the fact that most of dementia cases among the elderly are mixed cases. Thus, in addition to AD, the analysis should be done also for other dementia cases, to evaluate if the increased risk is similar or not. This could bring additional clues regarding the potential physiopathological mechanisms between NTG and AD.
->Answer: Thank you. We have added some descriptions and explanations according to your comments.
Please see page 17, line 25-47: “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 β-amyloid, are linked to retinal ganglion cell death in glaucoma and contribute to neuronal apoptosis in AD. 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”.

3. Moreover, as the mean age of the population is relatively low to analyze AD risk, age at dementia onset should be presented in the results.
Answer: Thank you. Please see page 10, line 24: “Age of AD onset was 73.8±8.1 years”.

4. - Third, beyond NTG, as diagnoses of other POAG are available in the database, the authors should also investigate other POAG and discuss whether the risk is specific of NTG or not. Again, it

would enrich the discussion to better understand the potential mechanisms involves in the relationship between NTG and AD and provide suggestions for future researches.
Answer: Thank you. Please see page 17, line 25-47: “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 β-amyloid, are linked to retinal ganglion cell death in glaucoma and contribute to neuronal apoptosis in AD. 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”.

5.- Discussion limitations only address the problem of the severity of visual field defects whereas other limitations regarding Health Insurance Database analyses must be discussed; in particular the under-diagnosis of AD, the potential selection of diagnosed cases (those who recourse to care for their dementia), the potential differential bias for the diagnosis between NTG and non-NTG patients, ...
->Answer: Thank you. Please see page 18, line 16-40: “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”.

6.- Discussion should be enriched regarding hypotheses on the relationship between NTG (and/or other POAG) and AD.
->Answer: Thank you. We have added some hypotheses on the relationship between NTG and AD. Please see page 17, line 33-47: “One of the possible explanation is the common pathogenesis of neurotoxic substances in NTG and AD. Abnormal hyperphosphorylated tau and β-amyloid, are linked to retinal ganglion cell death in glaucoma and contribute to neuronal apoptosis in AD. 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”.

7.- The absence of significant association between diabetes and AD is surprising. Please comment on this.
->Answer: Thank you for your comment. We have performed subgroup analyses, stratified by age, to explain this.
Please see page 12, line 20-33:” 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.”
Please see page 13, line 46-55: “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".
The results of subgroup analyses were presented in supplementary Table 1 and supplementary Table 2.
We further comment on this in the discussion section. Please see page 17, line 5-23: "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. 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".

Minor comments:
1. p3, line 52: please replace yes by years.
->Answer: Thank you. We have corrected it. Please see page 3, line 52: "The mean age of the cohort was 62.1±12.5 years."
2. p4: the second point of strengths and limitations seems inappropriate.
->Answer: Thank you. We have deleted the second point of strengths and limitations.
3. p7: Please modify the sentence "To eliminate the limitations of previous studies" as Health Insurance Database analyses have a lot of other limitations and do not overcome all the limitations of other studies.
-> Answer: Thank you. We have deleted the sentence according to your comment. Thank you.
4. p 17, line 37: please delete "must".
->Answer: Thank you. We have deleted "must". Please see page 18, line 43-46: "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".

VERSION 2 – REVIEW

REVIEWER	Michael Goldacre University of Oxford, UK
REVIEW RETURNED	05-Jun-2018

GENERAL COMMENTS	The authors have adequately addressed the reviewers' comments.

REVIEWER	Catherine HELMER Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, France
REVIEW RETURNED	10-Jul-2018

GENERAL COMMENTS	Thank you for revising the manuscript. There are still a few points which should be considered. I thank the authors for performing new analyses in response to my 2nd point regarding the relationship between HTG and AD as well as NTG and dementia. However, I'm a bit disappointed that these analyses were presented only in the discussion and not included in the core of the paper, as it is one of the most interesting points of the paper. I would recommend including them within the main

	analysis. Moreover, as it is currently presented, the wording for speaking about these analyses "In our next studies ... " does not totally fit. p19 line 1-2: I'm not sure to totally understand the meaning of this sentence "Abnormal hyperphosphorylated tau and β-amyloid, are linked to retinal ganglion cell death in glaucoma and contribute to neuronal apoptosis in AD." Could you please reformulate the sentence or explain more in depth? Supplementary tables 1 and 2. Thank you for the supplementary analyses. These supplementary analyses should however be announced and justified in the methods section (statistical analyses part). Moreover, the supplementary Table 1 may be enough, with no needs for supplementary Table 2. Numbers of participants (and dementia cases) in each subgroup should be added in supplementary tables. Even if diabetes is significantly associated with AD among those ≥ 65y, the HR is really very low, at 1.02. It would be interesting to see the HR of dementia (all types) associated with diabetes in this database. Whatever the result, it requires to adjust the comment in the discussion, to indicate whether this low HR is coherent or not with previous studies in Taiwan. Minor comments p18, line 15: to be a significant risk factors: please delete the "s" at the end of factor p19, line 17: Besides, those with NTG...: please replace by "Thus, those with NTG..." p19 line 6: we can not conclude: please replace by "we cannot conclude" p19 line 7: may be resulted: please replace by "may result" p20, line 1: Therefore, the positive association between NTG and AD is true and more prominent in real situation: this sentence is too affirmative and should be deleted.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 2

Reviewer Name: Catherine HELMER

Institution and Country: Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, France

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

Thank you for revising the manuscript. There are still a few points which should be considered.

1. I thank the authors for performing new analyses in response to my 2nd point regarding the relationship between HTG and AD as well as NTG and dementia. However, I'm a bit disappointed that these analyses were presented only in the discussion and not included in the core of the paper, as it is one of the most interesting points of the paper. I would recommend including them within the main analysis. Moreover, as it is currently presented, the wording for speaking about these analyses "In our next studies ... " does not totally fit.

Answer: Thank you for your comments. We have included the analyses regarding the relationship between HTG and AD as well as NTG and dementia in the result Section of the paper, and add a new Table 3 to describe the findings. (Thus the previous Table 3 has become Table 4). As it is currently presented, the wording for speaking about these analyses "In our next studies ... " has been deleted, according to your comment. Thank you.

Please see the Method section (page 8, line 48-56):

“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.”

And, please see the Result section (page 13, line 32-41):

“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).”

The findings are also presented in the new Table 3. Please also see the Table 3 (page 14, line 7-24).

2. p19 line1-2: I'm not sure to totally understand the meaning of this sentence "Abnormal hyperphosphorylated tau and β-amyloid, are linked to retinal ganglion cell death in glaucoma and contribute to neuronal apoptosis in AD." Could you please reformulate the sentence or explain more in depth?

Answer: Thank you for your comment. We have re-written the sentence. Please see the Discussion section (page 18, line 53-55):

“AD is characterized by abnormal hyperphosphorylated tau and β-amyloid in the brain. These abnormal protein are also related to retinal ganglion cell death in glaucoma.”

3. Supplementary tables 1 and 2.

Thank you for the supplementary analyses. These supplementary analyses should however be announced and justified in the methods section (statistical analyses part). Moreover, the supplementary Table 1 may be enough, with no needs for supplementary Table 2. Numbers of participants (and dementia cases) in each subgroup should be added in supplementary tables.

Even if diabetes is significantly associated with AD among those ≥ 65y, the HR is really very low, at 1.02. It would be interesting to see the HR of dementia (all types) associated with diabetes in this database. Whatever the result, it requires to adjust the comment in the discussion, to indicate whether this low HR is coherent or not with previous studies in Taiwan.

Answer: Thank you for your comments. According to your comments, we have deleted the supplementary Table 2. We also added some announcements in the method section. Numbers of participants and dementia cases were also added in supplementary Table 1.

Please see the Method section (page 8, line 48-50):

“We additionally performed stratified analyses according to age, in order to evaluate the risk factors for AD among different age subgroups.”

Please also see the supplementary Table 1 (page 28).

And, according to your comments, we have added the comparison between our results and findings of previous studies in Taiwan. Please also see the Discussion section (page 18, line 3-40) and Table 3 (page 14):

“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. 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. 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. 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). The weaker association might result from the better diabetes care in recent years. Since poor-controlled fasting plasma glucose and HbA1c are significant predictors of AD, 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.”

Minor comments

p18, line 15: to be a significant risk factors: please delete the "s" at the end of factor

Answer: Thank you. We have revised the sentence according to your comment.

p19, line 17: Besides, those with NTG...: please replace by "Thus, those with NTG..."

Answer: Thank you. We have revised the sentence according to your comment.

p19 line 6: we can not conclude: please replace by "we cannot conclude"

Answer: Thank you. We have revised the sentence according to your comment.

p19 line 7: may be resulted: please replace by "may result"

Answer: Thank you. We have revised the sentence according to your comment.

p20, line 1: Therefore, the positive association between NTG and AD is true and more prominent in real situation: this sentence is too affirmative and should be deleted.

Answer: Thank you. We have deleted the sentence according to your comment.