PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Grey and white matter abnormalities in chronic obstructive pulmonary disease: a case-control study
AUTHORS	Haiyan Zhang, Xiaochuan Wang, Jianzhong Lin, Yinchuan Sun, Yongxia Huang, Tianhe Yang, Shili Zheng, Ming Fan and Jiaxing Zhang

VERSION 1 - REVIEW

REVIEWER	Soo Borson MD Professor of Psychiatry and Behavioral Sciences University of Washinton School of Medicine Seattle WA 98195 USA No COI
REVIEW RETURNED	27/01/2012

THE STUDY	First: A disclaimed. I am not an expert in the imaging methods used in this study; I rather have acquaintance with them, so cannot adequately evaluate the technical aspects of image analysis. My critique is limited to the following as a result: 1. COPD patients need to be more fully characterized. a. Report GOLD stage. b. Report actual measures of lung function, PaO2 and SaO2, CO2, cognition, and ADLs, not merely statistics saying these values differ from controls. c. Report standards for use of oxygen supplementation in China, and what proportion of COPD subjects used 24-hour, nocturnal, or activity-based O2. d. Comment on comorbid conditions beyond the extremes indicated in the text. E.g. report known coronary artery disease, diabetes at a minimum. 2. The introduction and discussion go too far down speculative pathways. The authors find a large number of regional differences between cases and controlsm, as expected for a disease that can have global effects on the brain, and they then overinterpret the specificity of regional changes for unique symptoms of COPD (e.g. dyspnea) or associated disorders (e.g depression, which should have been measured in these patients). Their attribution of ADL deficits to cerebral effects of COPD reflects similar truncation of the known effects of severe respiratory insufficiency on other body tissues. 3. Missing reference: Borson S, Scanlan JM, Friedman S, Aylward E, Zuhr E, Fields J, Mahurin R, Richards T, Anzai Y, Yukawa M, and Yeh S. Modeling the impact of COPD on the brain. International Journal of Chronic Obstructive Pulmonary Disease 3(3):429-434, 2008.
RESULTS & CONCLUSIONS	Please see previous comments above. Specificity of interpretation

	seems excessive for the number of subjects and the level at which they are clinically characterized.
GENERAL COMMENTS	Despite its limitations (mostly of completeness and overinclusive interpretation), the authors make an important contribution to our understanding of COPD and the brain. The number of studies of the brain in COPD using modern imaging methods is very small. This study will help promote recognition and understanding of the cerebral effects of COPD and, as this body of work develops, dissemination of results to pulmonary disease specialists will assume greater importance in designing suitably comprehensive treatment plans.

REVIEWER	Chol Shin, MD, PhD. Division of Pulmonary, Sleep and Critical Care Medicine, Department of Internal Medicine,
REVIEW RETURNED	31/01/2012

THE STUDY	Authors aimed to explore the structural change in brain associated with COPD using the computational MRI analysis, and to document its correlation with COPD severity or neurocognitive performance. This study may have a potential to provide the information on the CNS impairment associated with COPD. However, in the present form, it is impossible to get exact information of information because of several major problems. 1. The sample size is small for multiple comparisons, although authors adopted statistical corrections and matched several clinical variables. 2. The subjects selection criteria were unclear. For example, how did authors exclude the presence of COPD and OSA? Authors seemed to perform the pulmonary function test in the control group, but did not describe in detail. OSA cannot be completely excluded by history or physical exam. OSA is common in the general population and more common and worse in the subjects with COPD. Besides, depression related to chronic illness, etc might result in the brain change. In this context, it is difficult to assume that the structrual and functional changes were solely related to COPD. 3. As for the statistical analysis, authors provided the details for the image analysis, but did not for the correlation analysis with disease severity or neurocognitive function. And the covariates for neurocog analysis were not adequate. They did not consider major variables such as gender and education.
RESULTS & CONCLUSIONS	4. It is better to provide the results of pulmonary and neurocognitive function test results in both groups, at least in the table. 5. I wonder if there was the change in gray matter or FA in the control group compared with the COPD 6. It is unclear how authors measured the absolute concentraion of gray matter in the specificed brain regions for the correlation analysis (Table 4). 7.The interpretation of the results were just enumerative and hypothetical. Furthermore, it is confusing the way that authors explained the possible background for the structural and functional change. Was it the top-down (brain-to-COPD) or reverse (COPD-to-brain) or both? 8. As the authors aimed to analyze the correlation between the specific anatomic region and cognitive function, it would be better to add the control group in the analysis and improve the statiscical

	analysis. Gender and education should be included.

REVIEWER	Emmanuel Stamatakis PhD University Of Cambridge, UK No competing Interests
REVIEW RETURNED	01/02/2012

THE STUDY	Written English, although of a reasonable standard, needs improvement, otherwise the manuscript is difficult to understand in places. Methods are not very well described. Techniques such as TBSS need more detailed description so that they can be fully understood by readers. Also, the physiological and psychological tests used need a more detailed description as to what it is they actually measure and some references to how they originated. Readers should have enough information to replicate this experiment if they so wish. What kind of statistical tests did the authors use to test for differences in these measures? Correlations between MRI measurements and disease severity: are these whole brain or ROI analyses? If ROI, how were the ROI data extracted? Were these analyses carried out only on grey matter data or on FA data too?
RESULTS & CONCLUSIONS	The result are presented in a sparse manner without much detail. This should be remedied. More detailed description will make the results credible. What do the colour bars in the figures represent?
GENERAL COMMENTS	Page 4 , line 52: TBSS does not measure FA, it is used to find differences in FA between groups or to relate behavioural or other measures to FA. Page 8, line 34: Why were the FA data upscaled to 1x1x1mm3? It seems like a huge step from the resolution the images were acquired. Page 8, line 46: p<.05 uncorrected is a very lenient threshold. Why not use an equivalent threshold to the one used for the grey matter analysis? Page 8, last line: The use of the eigen value data should be adequately motivated. Page 9: An idea of the values obtained for physiological and psychological data should be provided. Also, what tests did the authors use to analyse these data? Page 9, Did the authors find any grey matter (or indeed FA) increases in the patient group? If so please provide explanation

VERSION 1 – AUTHOR RESPONSE

Responses to Prof. Soo Borson

1. COPD patients need to be more fully characterized.
a. Report GOLD stage.
We have reported it.

b. Report actual measures of lung function, PaO2 and SaO2, CO2, cognition, and ADLs, not merely statistics saying these values differ from controls.
Response:
In fact, we used a table to report the results of physiological and cognitive tests in our initial manuscript. However, due to the word limitation we deleted this table. Now we have added this table as Table 2 in the text.

c. Report standards for use of oxygen supplementation in China, and what proportion of COPD subjects used 24-hour, nocturnal, or activity-based O2.
Response:
During study procedure, all patients were in stable condition. No oxygen supplementation was used.

d. Comment on co-morbid conditions beyond the extremes indicated in the text. E.g. report known coronary artery disease, diabetes at a minimum.
Response:
The patients and controls were carefully selected. They are all with no diseases such as coronary artery disease, diabetes, etc. We have added these comments in the text.

2. The introduction and discussion go too far down speculative pathways. The authors find a large number of regional differences between cases and controls, as expected for a disease that can have global effects on the brain, and they then over interpret the specificity of regional changes for unique symptoms of COPD (e.g. dyspnea) or associated disorders (e.g depression, which should have been measured in these patients). Their attribution of ADL deficits to cerebral effects of COPD reflects similar truncation of the known effects of severe respiratory insufficiency on other body tissues.
Response:
We have deleted the discussion about depression (Paragraph “Morphological impairments contribute to the depression”), and we added the discussion about ADL deficits.
In the paragraph in the Discussion “Morphological impairments play a role in respiratory and cardiovascular responses to dyspnea and hypoxia”, we tried to explain COPD symptoms, such as enhanced breathing movements (high respiratory rate and high heart rate) and cognitive deficits, using our findings in brain. According to your suggestion, we have simplified this section.

3. Missing reference:
Borson S, Scanlan JM, Friedman S, Aylward E, Zuhr E, Fields J, Mahurin R, Richards T, Anzai Y, Yukawa M, and Yeh S. Modeling the impact of COPD on the brain. International Journal of Chronic Obstructive Pulmonary Disease 3(3):429-434, 2008.
Response:
We have cited this research and discussed the results in paragraph two in Discussion.

Responses to Prof. Chol Shin

1. The sample size is small for multiple comparisons, although authors adopted statistical corrections and matched several clinical variables.
Response:
According to Tardif et al. (2010), the typical sample sizes in cross-sectional studies of VBM range

from 20 to 40 subjects per group. We used 3T field strength and MP-RAGE sequence, so the sample size which is more than 19 is enough.
Reference:
Tardif CL, Collins DL, Pike GB. Regional impact of field strength on voxel-based morphometry results. Hum Brain Mapp. 2010;31(7):943-57.

2. The subjects selection criteria were unclear. For example, how did authors exclude the presence of COPD and OSA? Authors seemed to perform the pulmonary function test in the control group, but did not describe in detail. OSA cannot be completely excluded by history or physical exam.
OSA is common in the general population and more common and worse in the subjects with COPD. Besides, depression related to chronic illness, etc might result in the brain change.
In this context, it is difficult to assume that the structrual and functional changes were solely related to COPD.
Response:
We assessed factors asso¬ciated with OSA, namely daytime sleepiness with the Epworth Sleepiness Scale (ESS) and sleep quality with the Pittsburg Sleep Quality Index (PSQI). These self-administered question¬naires were completed by subjects.

3. As for the statistical analysis, authors provided the details for the image analysis, but did not for the correlation analysis with disease severity or neurocognitive function. And the covariates for neurocog analysis were not adequate. They did not consider major variables such as gender and education.
Response:
(1) We provided the details about the correlation analysis of image values with disease severity or cognitive function in the paragraph “VBM analysis of 3D T1 images” in METHODS section.
(2) We have reanalyzed the correlations of image values with disease severity, controlling for disease duration, Po2, FEV1/FVC, age, education, and gender.

4. It is better to provide the results of pulmonary and neurocognitive function test results in both groups, at least in the table.
Response:
We have added a Table 2 in the text.

5. I wonder if there was the change in gray matter or FA in the control group compared with the COPD
Response:
We did not find any increased GM density or increased FA in the control group compared with the COPD.

6. It is unclear how authors measured the absolute concentraion of gray matter in the specificed brain regions for the correlation analysis (Table 4).
Response:
We have provided the details about the measure of the absolute concentraion in the paragraph “VBM analysis of 3D T1 images” in METHODS section.

7. The interpretation of the results were just enumerative and hypothetical. Furthermore, it is confusing the way that authors explained the possible background for the structural and functional change. Was it the top-down (brain-to-COPD) or reverse (COPD-to-brain) or both?
Response:
In the third paragraph in Discussion, we tried to explain the mechanism for the brain structural change (COPD-to-brain).
Then during the next three sections we try to explain the contribution of our findings in brain to COPD symptoms that found in our study, including respiratory and cardiovascular symptoms, such as

enhanced breathing movements (high respiratory rate and high heart rate) and cognitive deficits (brain-to-COPD).

8. As the authors aimed to analyze the correlation between the specific anatomic region and cognitive function, it would be better to add the control group in the analysis and improve the statiscical analysis. Gender and education should be included.
Response:
We have added the control group in the analysis of correlation between regional concentration and cognitive function (Table 5).

Responses to Prof. Emmanuel Stamatakis

Written English, although of a reasonable standard, needs improvement, otherwise the manuscript is difficult to understand in places.
Response:
We have carefully corrected the error sentences and a lot of sentences were revised.

Methods are not very well described. Techniques such as TBSS need more detailed description so that they can be fully understood by readers.
Response:
We have described the techniques more detailed.

Also, the physiological and psychological tests used need a more detailed description as to what it is they actually measure and some references to how they originated. Readers should have enough information to replicate this experiment if they so wish. What kind of statistical tests did the authors use to test for differences in these measures?
Response:
We have described the physiological and psychological tests more detailed than previous version.
We have added two references related to psychological tests.
All data were analyzed using SPSS 19.0 (Chicago, IL, USA). Independent t test measures between-group differences. Statistical significance was set at $p < 0.05$.

Correlations between MRI measurements and disease severity: are these whole brain or ROI analyses? If ROI, how were the ROI data extracted? Were these analyses carried out only on grey matter data or on FA data too?
Response:
We have provided the details about the measure of the absolute concentraion in the paragraph “VBM analysis of 3D T1 images” in METHODS section.
We analyzed the correlation between regional FA and disease severity, but the statistic was not significant.

The result is presented in a sparse manner without much detail. This should be remedied. More detailed description will make the results credible.
Response:
We used a table (Table 2) to report the results of physiological and cognitive tests.

What do the colour bars in the figures represent?
Response:
The colour bars show t values corresponding to colours in the figure. We have indicated it in the figure legend.

Page 4 , line 52:

TBSS does not measure FA, it is used to find differences in FA between groups or to relate behavioural or other measures to FA.
Response:
We are sorry for this mistake. We have corrected it.

Page 8, line 34:
Why were the FA data upscaled to 1x1x1mm3? It seems like a huge step from the resolution the images were acquired.
Response:
This resolution is chosen as the later skeletonisation and projection steps work well at this resolution, and the choice of working in MNI152 space is chosen for convenience of display and coordinate reporting later.

Page 8, line 46:
$p<.05$ uncorrected is a very lenient threshold. Why not use an equivalent threshold to the one used for the grey matter analysis?
Response:
The limitation of our study is the weak statistical power of FA value analysis, because the results obtained in the TBSS analysis could not survive multiple comparison correction. We indicated this limitation in the last paragraph in Discussion.

Page 8, last line:
The use of the eigen value data should be adequately motivated.
Response:
We have revised the extraction of mean λ1 and λ23 values. The meaning of use the eigen value data were explain in the second paragraph in Introduction section.

Page 9:
An idea of the values obtained for physiological and psychological data should be provided. Also, what tests did the authors use to analyse these data?
Response:
We have added a Table 2 to report the physiological and psychological data.
The analysis method has been mentioned in the Method section (Page 6). Here we added "Independent t test showed that".

Page 9,
Did the authors find any grey matter (or indeed FA) increases in the patient group? If so please provide explanation.
Response:
We did not find any increases in grey matter density or FA in COPD patients compared with the control group.

VERSION 2 – REVIEW

REVIEWER	Emmanuel Stamatakis PhD University Of Cambridge, UK No competing Interests
REVIEW RETURNED	27/02/2012

THE STUDY	I'm afraid the use of English must improve further
RESULTS & CONCLUSIONS	Some speculation still remains in the discussion.

GENERAL COMMENTS	Page 5, lines: 19-26: Move the addition from the first revision to the methods section. Page 6, line 19: Have the authors accounted for the fact that patients fall under different disease staging categories in their analyses? Do they need to? Where thresholds of whole brain voxel based analyses are provided the authors need to tell us whether they used voxel or cluster statistics. Page 8, lines 37-47 and Page 9, lines 51-57: Did the authors account for variables such as age, gender etc. in their SPSS ROI analyses? Page 9, lines31-32: A reference is needed for using an FA threshold of 0.20

VERSION 2 – AUTHOR RESPONSE

Responses to Prof. Emmanuel Stamatakis

I'm afraid the use of English must improve further
Response:
The grammar errors have been carefully corrected by two native English speakers.

Some speculation still remains in the discussion.
Response:
We have rewritten the fourth paragraph in the Discussion section and some speculated sentences were removed.

Page 5, lines: 19-26:
Move the addition from the first revision to the methods section.
Response:
We have moved “(longitudinal diffusivity [the magnitude of diffusion along the principal diffusion direction, λ1] and radial diffusivity [the magnitude of diffusion in the two orthogonal directions perpendicular to the principal diffusion direction, λ23])” in the Introduction section to the paragraph “TBSS analysis of DTI” in the Methods section.

Page 6, line 19:
Have the authors accounted for the fact that patients fall under different disease staging categories in their analyses? Do they need to?
Response:
We deleted the sentence “at stage I (4%), stage II (32%), stage III (28%) and stage IV (36%)” in the Methods section. We accounted for the fact that patients fall under different disease staging categories in the paragraph “Physiological and behavioral findings (Table 2)” in the Results section.

Where thresholds of whole brain voxel based analyses are provided the authors need to tell us whether they used voxel or cluster statistics.
Response:
Thresholds of whole brain voxel based analyses are provided in the paragraph “VBM analysis of 3D T1 images” as “The statistical parametric map was generated with the voxel level threshold at $t > 3.7734$, $p < 0.01$”.

Voxel-wise statistical analysis of the GM data was carried out using VBM.

Page 8, lines 37-47 and Page 9, lines 51-57:
Did the authors account for variables such as age, gender etc. in their SPSS ROI analyses?
Response:
In fact, during data analysis, we analysed the correlation of GM image values with cognitive or physiological measurement, with gender, age, and education as covariates. We have added this statement in the paragraph “VBM analysis of 3D T1 images” in the Methods section.

Page 9, lines31-32:
A reference is needed for using an FA threshold of 0.20
Response:
“FA threshold of 0.20” was cited from the eighth reference in the reference list.
We have marked it in the paragraph “TBSS analysis of DTI” in Methods section.

Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006; 31:1487-505.