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Investigation of quantitative susceptibility mapping (QSM) in diagnosis of tuberous sclerosis complex (TSC) and assessment of associated brain injuries at 1.5 Tesla

Lei Zhang, Hongqiang Xue, Tao Chen, Hongzhe Tian, Xiaohu Wang, Xiaocheng Wei, Huawen Zhang, Hui Ma, Zhuanqin Ren

Corresponding author:
Zhuanqin Ren
Department of Radiology, Baoji Center Hospital, Baoji, Shaanxi, P.R. China

Handeling editor:
Michal Heger
Department of Pharmaceutics, Utrecht University, the Netherlands
Department of Pharmaceutics, Jiaxing University Medical College, Zhejiang, China

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1st Editorial decision

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Ref.: Ms. No. JCTRes-D-19-00018
Investigation of quantitative susceptibility mapping (QSM) in diagnosis of tuberous sclerosis complex (TSC) and assessment of associated brain injuries at 1.5 Tesla
Journal of Clinical and Translational Research

Dear Dr. zhang,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Nov 15, 2019.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This manuscript investigates the use of quantitative susceptibility mapping (QSM) for the detection ofe subependymal nodules and the assessment of brain tissue injurie induced by cortical and subcortical tubers in tuberous sclerosis complex (TSC) patients. The study compares conventional MRI sequence, DTI, QSM and CT in twelve TSC patients and 15 gender and age matched healthy controls. They find good agreement in the detection of calcifications in TSC between QSM and TSC. They also find higher susceptibilities and lower fractional anisotropy (FA) in TSC, and show a significant negative correlation between the two.
Major concerns
1. The use of QSM for TSC seems to lie in the detection of calcifications, and may displace CT for that purpose. Although non-calcified tubers have higher susceptibility, the lesion is not as well delineated as in say T2 or T2 FLAIR, where the hyperintensity is clearly seen. Unfortunately, the authors do not measure the diagnostic performance of QSM for detection of calcification because they have no ground truth. They simply count the number of detected calcifications on QSM and CT, find that the number for QSM exceeds that for CT, and then concluded that QSM is at least as good as, if not better than, CT. Since no ground truth is available, this is not an appropriate conclusion: how do we know that the detected hypointensities on QSM (negative susceptibilities) actually are calcifications? The authors state that "QSM had similar performance in identifying calcified nodules, and detected more micro and immature calcification nodules than CT". How do we know for sure that this is the case? There is no independent characterization of these lesions. The proper study to perform is to consider CT as the ground truth (as the authors state in the introduction) and then perform a sensitivity/specificity analysis.
2. The manuscript is riddled with grammatical errors, especially in the abstract. The authors should perform a thorough editing of the entire manuscript.
3. What is a "susceptibility ringing artifact" ? (page 14, line 10)
Minor

4. Page 2, line 19, "Tuberous …" is not a grammatically correct sentence.
Please rephrase.
5. Page 2, line 23, "include"
6. Page 2, line 25, "Quantitative .."
7. Page 2, line 27, "measuring"
8. Page 2, line 35, "clinically"
9. Page 3, line 6, "… TSC compared to CT and DTI"
10. Page 3, line 6, "QSM may provide …"
11. Page 3, line 9, "… may simplity imaging of patients with TSC."
12. Page 3, line 13, " This study shows the feasibility of QSM to detect subependymal calcified nodules. It may provide quantitiative …"
13. Page 4, line 25 "closely"
14. Page 4, line 29, "even more"
15. Page 4, line 29, remove "on the other hand" and "also"
16. Page 4, line 54 "gold standard"
17. Page 4, line 56 For clarity, move the sentence starting with "Accurate localization .." to after the next sentence.
18. Page 7, line 4, "A multi-echo …"
19. Page 7, line 19, "An FA map…"
20. Page 9, line 1, please rephrase "QSM was advantageous…". However, this sentence will likely be removed, since a different study should be performed (see comment 1 above)
21. Page 14, line 48, "It has been shown that FA value…"
22. Page 14, line 52, "As myelin …"
23. Page 15, line 10, Reference 34 deals with SWI is not an appropriate reference here.
24. Page 15, line 11, the skull definitely does not have a low susceptibility (in absolute terms): it is very negative and creates a significant field surrounding it.
25. Page 15, Line 17, the sentence starting with "Therefore, the susceptibility …" does not obviously follow from the previous sentence. Please rephrase or remove.
26. Page 15, line 44, The section starting with "In conclusion" is similar to the conclusion in the abstract and suffers from the same grammatical deficiencies. Please correct accordingly.

Reviewer #2: The authors assess the value of quantitative susceptibility mapping (QSM) within the scope of diagnostic imaging of tuberous sclerosis complex (TSC). They compare conventional MR, CT and QSM, and find that QSM combined with conventional MRI can be an alternative to CT scans in the screening of TCS patients. This seems to be a very interesting application of QSM, however, several issues need to be worked on:
Major comments:
1. What is the ground truth? As the diagnostic value of QSM in TSC is assessed, there should be a ground truth it is compared to. The ground truth could be CT plus FLAIR. However, then, it needs to be explained why the two extra lesions found with QSM are not false positives/artifacts. Please consider.
Minor comments:
2. The manuscript requires extensive English language editing if possible by a native language speaker.
3. Introduction line 29/30: Remove 'On the other hand'.
4. The abstract on the title page and within the manuscript itself are different, please correct this.

5. Add to 2.1 that the patients also received a CT scan.
6. Was the gradient echo data for QSM acquired with a 2D or 3D sequence?
7. Section2.3 line 48 'this method has high…'should be moved to the discussion.
8. Section 2.3 line 48: '…are seen as iso-intense...' iso-intense compared to what?
9. The small number of patients should be added to the limitations sections of this study.
10. Abbreviations should be introduced when used first.
11. In the last section of the discussion, the authors claim '…susceptibility measurements have good reproducibility'; however, this was not shown in this study. It should be emphasized instead that QSM might have the potential to replace the CT scan for the diagnosis/ monitoring of TSC.
12. In Figure 1, Slice 2 QSM: What is the dark region in the left bottom corner? Is this an artifact?
13. In Figure 2, QSM, the frontal white matter appears hyperintense, the caudal white matter dark; please explain in the caption/results.
14. For the identifications of calcifications in the brain, the authors should add the citation: Quantitative susceptibility mapping differentiates between blood depositions and calcifications in patients with glioblastoma. Deistung A. et al.
15. In the discussion, last page, line 6/7 'Furthermore….' to 15 '…sub-cortex.' is irrelevant for this study.
16. Reference 32 is not used in the manuscript. Remove.

Reviewer #3: This manuscript investigated the use of QSM in detecting the subependymal nodules and assessing brain tissue injuries induced by cortical/subcortical tubers in TSC patients. The manuscript is overall well written.The manuscript can be improved by addressing the following concerns.

1. The authors should provide detailed information about QSM reconstruction.
2. QSM only determines susceptibility differences rather than absolute susceptibility values, so which tissue was used as the reference for susceptibility in this study?
3. Which software was used to draw ROIs? The authors should also provide detailed information how to draw ROIs.
4. I would recommend authors provide scale bar for the susceptibility maps and FA maps.

Reviewer #4: This manuscript reports a first QSM study of tuberous sclerosis complex (TSC), rare disease. Results are very interesting and impressive and worthy of publication.

Major comments:
Why would QSM detects more calcified lesions than CT? Some biophysics explanation is needed. Details of agreement/disagreement on lesion size and contrasts should be provided. Also the biophysical explanation for correlation among QSM vs FA and other MRI should be provided? QSM biometal imaging (https://www.ncbi.nlm.nih.gov/pubmed/28295954) has some biological explanations for things.
introduction:
2nd page - QSM was coined by de Rochefort (ref. 28)
Materials and methods:
1st page: five males: 7 females?

2nd page: ref 23 is commonly known as iLSQR?
Results:
Table 1 and Fig.1: it is interesting and impressive to see CT does not show lesion as well as QSM. why?
Figs.2&4: interesting - what's the biological interpretation - hemorrhages associated with microstructure destruction?

Author's rebuttal

Reviewer #1:

This manuscript investigates the use of quantitative susceptibility mapping (QSM) for the detection ofe subependymal nodules and the assessment of brain tissue injurie induced by cortical and subcortical tubers in tuberous sclerosis complex (TSC) patients. The study compares conventional MRI sequence, DTI, QSM and CT in twelve TSC patients and 15 gender and age matched healthy controls. They find good agreement in the detection of calcifications in TSC between QSM and TSC. They also find higher susceptibilities and lower fractional anisotropy (FA) in TSC, and show a significant negative correlation between the two.

Major concerns

1. The use of QSM for TSC seems to lie in the detection of calcifications, and may displace CT for that purpose. Although non-calcified tubers have higher susceptibility, the lesion is not as well delineated as in say T2 or T2 FLAIR, where the hyperintensity is clearly seen. Unfortunately, the authors do not measure the diagnostic performance of QSM for detection of calcification because they have no ground truth. They simply count the number of detected calcifications on QSM and CT, find that the number for QSM exceeds that for CT, and then concluded that QSM is at least as good as, if not better than, CT. Since no ground truth is available, this is not an appropriate conclusion: how do we know that the detected hypointensities on QSM (negative susceptibilities) actually are calcifications? The authors state that "QSM had similar performance in identifying calcified nodules, and detected more micro and immature calcification nodules than CT". How do we know for sure that this is the case? There is no independent characterization of these lesions. The proper study to perform is to consider CT as the ground truth (as the authors state in the introduction) and then perform a sensitivity/specificity analysis.

[Response]: Thanks for the comment. In this study, the observation of calcification on QSM was to consider CT as the ground truth, and the data of this study was reanalyzed.(marked as

R1.1)

2. The manuscript is riddled with grammatical errors, especially in the abstract. The authors should perform a thorough editing of the entire manuscript.

[Response]: Thank you for the suggestion, the revised manuscript has undergone a thorough grammatical editing.

3. What is a "susceptibility ringing artifact" ? (page 14, line 10)

It said that susceptibility ringing artifact was the susceptibility artifacts of hypointensity surrounding calcification. Now, the sentence was deleted. (marked as R1.2)

Minor

4. Page 2, line 19, "Tuberous …" is not a grammatically correct sentence. Please rephrase.

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.3)

5. Page 2, line 23, "include"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.4)

6. Page 2, line 25, "Quantitative .."

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.5)

7. Page 2, line 27, "measuring"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.6)

8. Page 2, line 35, "clinically"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.7)

9. Page 3, line 6, "… TSC compared to CT and DTI"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.8)

10. Page 3, line 6, "QSM may provide …"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.9)

11. Page 3, line 9, "… may simplity imaging of patients with TSC."

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.10)

12. Page 3, line 13, " This study shows the feasibility of QSM to detect subependymal calcified nodules. It may provide quantitiative …"

[Response]: Thanks for the comment. (marked as R1.11)

13. Page 4, line 25 "closely"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.12)

14. Page 4, line 29, "even more"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.13)

15. Page 4, line 29, remove "on the other hand" and "also"

[Response]: Thanks for the comment. (marked as R1.14)

16. Page 4, line 54 "gold standard"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.15)

17. Page 4, line 56 For clarity, move the sentence starting with "Accurate localization .." to after the next sentence.

[Response]: Thanks for the comment. (marked as R1.16)

18. Page 7, line 4, "A multi-echo …"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.17)

19. Page 7, line 19, "An FA map…"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.18)

20. Page 9, line 1, please rephrase "QSM was advantageous…". However, this sentence will likely be removed, since a different study should be performed (see comment 1 above)

[Response]: Thanks for the comment. (marked as R1.19)

21. Page 14, line 48, "It has been shown that FA value…"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.20)

22. Page 14, line 52, "As myelin …"

[Response]: Sorry for this typo, it has been corrected in revised abstract. (marked as R1.21)

23. Page 15, line 10, Reference 34 deals with SWI is not an appropriate reference here.

[Response]: Thanks for the comment. This reference has been deleted. (marked as R1.22)

24. Page 15, line 11, the skull definitely does not have a low susceptibility (in absolute terms): it is very negative and creates a significant field surrounding it.

[Response]: Thanks for the comment. This sentence was irrelevant for this study, and it has been deleted. (marked as R1.23)

25. Page 15, Line 17, the sentence starting with "Therefore, the susceptibility …" does not

obviously follow from the previous sentence. Please rephrase or remove.

[Response]: Thanks for the comment. The previous sentence has deleted. (marked as R1.24)

26. Page 15, line 44, The section starting with "In conclusion" is similar to the conclusion in the abstract and suffers from the same grammatical deficiencies. Please correct accordingly.

[Response]: Thanks for the comment. The sentence has been revised. (marked as R1.25)

Reviewer #2:

The authors assess the value of quantitative susceptibility mapping (QSM) within the scope of diagnostic imaging of tuberous sclerosis complex (TSC). They compare conventional MR, CT and QSM, and find that QSM combined with conventional MRI can be an alternative to CT scans in the screening of TCS patients. This seems to be a very interesting application of QSM, however, several issues need to be worked on:

Major comments:

1. What is the ground truth? As the diagnostic value of QSM in TSC is assessed, there should be a ground truth it is compared to. The ground truth could be CT plus FLAIR. However, then, it needs to be explained why the two extra lesions found with QSM are not false positives/artifacts. Please consider.

[Response]: Thank you for the suggestion. In this stugy, the observation of calcification on QSM was to consider CT as the ground truth, and the observation of subependymal nodules on QSM was to consider T2 FLAIR as the ground truth. The data of this study was reanalyzed.

Minor comments:

2. The manuscript requires extensive English language editing if possible by a native language speaker.

[Response]: Thank you for the suggestion, the revised manuscript has undergone a thorough grammatical editing.

3. Introduction line 29/30: Remove 'On the other hand'.

[Response]: Thanks for the comment. (marked as R2.2)

4. The abstract on the title page and within the manuscript itself are different, please correct this.

[Response]: Thanks for the comment. (marked as R2.3)

5. Add to 2.1 that the patients also received a CT scan.

[Response]: Thanks for the comment. (marked as R2.4)

6. Was the gradient echo data for QSM acquired with a 2D or 3D sequence?

[Response]: That was a 2D sequence.

7. Section2.3 line 48 'this method has high…'should be moved to the discussion.

[Response]: Thanks for the comment. (marked as R2.5)

8. Section 2.3 line 48: '…are seen as iso-intense...' iso-intense compared to what?

[Response]: Thanks for the comment, that were compared with white matter. (marked as R2.6)

9. The small number of patients should be added to the limitations sections of this study.

[Response]: Thanks for the comment. (marked as R2.7)

10. Abbreviations should be introduced when used first.

[Response]: Thanks for the comment. (marked as R2.8)

11. In the last section of the discussion, the authors claim '…susceptibility measurements have good reproducibility'; however, this was not shown in this study. It should be emphasized instead that QSM might have the potential to replace the CT scan for the diagnosis/ monitoring of TSC.

[Response]: In the Result 3.2, We verified this with Bland-Altman test. (marked as R2.9)

12. In Figure 1, Slice 2 QSM: What is the dark region in the left bottom corner? Is this an artifact?

[Response]: Yes, that was a partial volume effect from next slice.

13. In Figure 2, QSM, the frontal white matter appears hyperintense, the caudal white matter dark; please explain in the caption/results.

[Response]: Thanks for the comment. The QSM image was reconstructed again, and that appearance appears to be weak. (marked as R2.10)

14. For the identifications of calcifications in the brain, the authors should add the citation: Quantitative susceptibility mapping differentiates between blood depositions and calcifications in patients with glioblastoma. Deistung A. et al.

[Response]: Yes, that was a good paper. We have added it. (marked as R2.11)

15. In the discussion, last page, line 6/7 'Furthermore….' to 15 '…sub-cortex.' is irrelevant for this study.

[Response]: Yes, we have removed it. (marked as R2.12)

16. Reference 32 is not used in the manuscript. Remove.

[Response]: Yes, we have removed it. (marked as R2.13)

Reviewer #3:

This manuscript investigated the use of QSM in detecting the subependymal nodules and assessing brain tissue injuries induced by cortical/subcortical tubers in TSC patients. The manuscript is overall well written.The manuscript can be improved by addressing the following concerns.

1. The authors should provide detailed information about QSM reconstruction.

[Response]: Thanks for the comment. This is a clinical application study of QSM, so detailed information about QSM reconstruction can be obtained from reference No. 23 for the readers.

2. QSM only determines susceptibility differences rather than absolute susceptibility values, so which tissue was used as the reference for susceptibility in this study?

[Response]: Thanks for the comment. Normal white matter was used as the reference for susceptibility in this study.

3. Which software was used to draw ROIs? The authors should also provide detailed information how to draw ROIs.

[Response]: Thanks for the comment. The ROI was drawn on the Mango software.

4. I would recommend authors provide scale bar for the susceptibility maps and FA maps.

[Response]: Thanks for the comment. The scale bar for the susceptibility maps and FA maps have been provided. (marked as R2.10)

Reviewer #4:

This manuscript reports a first QSM study of tuberous sclerosis complex (TSC), rare

disease. Results are very interesting and impressive and worthy of publication.

Major comments:

Why would QSM detects more calcified lesions than CT? Some biophysics explanation is needed. Details of agreement/disagreement on lesion size and contrasts should be provided.

[Response]: Thanks for the comment. In this study, the observation of calcification on QSM was to consider CT as the ground truth, and the data of this study was reanalyzed. (marked as R4.1)

Also the biophysical explanation for correlation among QSM vs FA and other MRI should be provided? QSM biometal imaging (https://www.ncbi.nlm.nih.gov/pubmed/28295954) has some biological explanations for things.

[Response]: Thanks for the advice. This paper was cited as reference. (marked as R4.2)

introduction:

2nd page - QSM was coined by de Rochefort (ref. 28)

[Response]: Thanks for the comment. (marked as R4.3)

Materials and methods:

1st page: five males: 7 females?

[Response]: Thanks for the comment. This has been added. (marked as R4.4)

2nd page: ref 23 is commonly known as iLSQR?

[Response]: Thanks for the comment. That was named as iLSQR. This has been added. (marked as R4.5)

Results:

Table 1 and Fig.1: it is interesting and impressive to see CT does not show lesion as well as QSM. why?

[Response]: Thanks for the comment. In this study, the observation of calcification on QSM was to consider CT as the ground truth, and the data of this study was reanalyzed.

Figs.2&4: interesting - what's the biological interpretation - hemorrhages associated with microstructure destruction?

[Response]: Thanks for the comment. Figs.2&4 indicated that the cortical/subcortical lesions in the TSC patients, which contain varies concentration of abnormal giant cells, which have

both glial and neuronal characteristics, can induce gliosis, hypomyelination, neurons arrangement disorder. The above factors associated with microstructure destruction.

2d Editorial response

2-January-2020

Ref.: Ms. No. JCTRes-D-19-00018R1

Investigation of quantitative susceptibility mapping (QSM) in diagnosis of tuberous sclerosis complex (TSC) and assessment of associated brain injuries at 1.5 Tesla

Journal of Clinical and Translational Research

Dear Dr. zhang,

Reviewers have commented on the revised version of your paper. You will see that they are still advising that you re-revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made. Additionally, please ensure that points addressed in the rebuttal are also accounted for in the manuscript where warranted.

Your revision is due by Feb 01, 2020.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger

Editor-in-Chief

Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #2: The authors tried to address the reviewers' comments, however, in its current state the article is not suitable for publication:

Comments:

1. In this revision, as recommended by the reviewers, the authors selected a ground truth. In 2.3., the use of FLAIR for the identification of TSC nodules is described, in 2.4., CT is state to be the ground truth. It would be more natural to consider the modality that was used to detect the lesions as ground truth. However, it seems that non-calcified lesions that were detected by conventional MRI (including FLAIR)/QSM and not by CT are counted as false positives. Consequently, the specificity for QSM is only 50%. Use CT and FLAIR as ground truth instead. The discussion was not adjusted to that change.

2. Table 1 is very unclear, and the numbers of detected lesions changed compared to the previous manuscript. Why?

3. The article is still hard to read because of the large amount of typos and grammatical errors/wrong expressions used.

4. The authors answered questions in the answer letter, but did partly not include the answers in the manuscript to clarify unclear sections. E.g. R2.6, R3.3.

5. How are patients and controls age-matched? The age range for the patients reaches up to 38 years, for the controls up to 27 years?

6. For Figure 1, the darker regions in QSM in the dorsal white matter regions are still visible (R2.13), only the contrast has changed (as the histogram was adjusted differently in the revision than in the original manuscript).

Reviewer #3: For my 1st concern, there may be some differences in QSM reconstruction pipelines between this manuscript and Ref. 24. For example, QSM data were obtained with a multi-echo GRE sequence in this manuscript while a single echo GRE was used in Ref. 24.

For my 2nd and 3rd concerns, the related information should be added in the manuscript.

Reviewer #4: am satisfied with the revision.

Author's response

Reviewer #2: The authors tried to address the reviewers' comments, however, in its current state the article is not suitable for publication:
Comments:
1. In this revision, as recommended by the reviewers, the authors selected a ground truth. In 2.3., the use of FLAIR for the identification of TSC nodules is described, in 2.4., CT is state to be the ground truth. It would be more natural to consider the modality that was used to detect the lesions as ground truth. However, it seems that non-calcified lesions that were detected by conventional MRI (including FLAIR)/QSM and not by CT are counted as false positives. Consequently, the specificity for QSM is only 50%. Use CT and FLAIR as ground truth instead. The discussion was not adjusted to that change.

[Response]: Thanks for the comment. There are some mistakes in expression. Firstly, for the calcified subependymal nodules, CT was considered as the ground truth in this study.

Secondly, for the cortical/subcortical nodules of TSC, the identification and localization of them were based on hyperintense region in T2 FLAIR images. (marked as R2.1)

2. Table 1 is very unclear, and the numbers of detected lesions changed compared to the previous manuscript. Why?

[Response]: Thanks for the comment. The format of table 1 has been changed. Since no ground truth is available in the previous manuscript. And now, to consider CT as the ground truth for the calcified subependymal nodules. So the number of detected lesions has been changed according to the result of CT, and the total of lesions in CT imaging didn't change. (marked as R2.2)

3. The article is still hard to read because of the large amount of typos and grammatical errors/wrong expressions used.

[Response]: Thanks for the comment. The typos and grammatical errors/wrong expressions had been corrected in this paper.

4. The authors answered questions in the answer letter, but did partly not include the answers in the manuscript to clarify unclear sections. E.g. R2.6, R3.3.

[Response]: Thanks for the comment. "Section 2.3 line 48: '…are seen as iso-intense...' iso-intense compared to what?" of **R2.6**, this section has been added to the appropriate location. (marked as R2.3)

[Response]: Thanks for the comment. "The ROI was drawn on the Mango software" of **R3.3**, this section has been added to the appropriate location. (marked as R2.4)

5. How are patients and controls age-matched? The age range for the patients reaches up to 38 years, for the controls up to 27 years?

[Response]: Thanks for the comment. Two volunteers in the control group have been replaced by volunteers aged 34 and 37. The susceptibility values and FA values of the control group (Table 2) have been updated, and the Bland-Altman test (Figure 3) and correlation line (Figure 4) have been recreated. (marked as R2.5)

6. For Figure 1, the darker regions in QSM in the dorsal white matter regions are still visible (R2.13), only the contrast has changed (as the histogram was adjusted differently in the revision than in the original manuscript).

[Response]: Thanks for the comment. For Figure 1, the darker regions in QSM located in the

dorsal white matter regions are the volume effect of the next image, which has been replaced by other layer image of the patient. (marked as R2.6)

Reviewer #3: For my 1st concern, there may be some differences in QSM reconstruction pipelines between this manuscript and Ref. 24. For example, QSM data were obtained with a multi-echo GRE sequence in this manuscript while a single echo GRE was used in Ref. 24.
[Response]: Thanks for the comment. The Ref. 24 has been replaced. (marked as R3.1)
For my 2nd and 3rd concerns, the related information should be added in the manuscript.

[Response]: Thanks for the comment. "which tissue was used as the reference for susceptibility in this study?" of **2nd concerns**. About this question, I asked Dr. Wei xiaocheng, a technical scientist, again. Referred to the literature "Region-specific disturbed iron distribution in early idiopathic Parkinson's disease measured by quantitative susceptibility mapping", the phase value was calculated based on healthy white matter, while the susceptibility value was an absolute value, in parts per million.

[Response]: Thanks for the comment. "The ROI was drawn on the Mango software" of **3rd concerns**, this section has been added to the appropriate location. (marked as R2.4)

3rd Editorial response

25-Feb-2020

Ref.: Ms. No. JCTRes-D-19-00018R2
Investigation of quantitative susceptibility mapping (QSM) in diagnosis of tuberous sclerosis complex (TSC) and assessment of associated brain injuries at 1.5 Tesla
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Mar 26, 2020.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #2: Major comments:
1. Please define your ground truth (for the various lesion types) more clearly in the data analysis section (page 6, line 23-30), and then stay consistent in the results (page 7, line 25-30).
2. Please clarify Table 1: using columns (CT, convent. MRI, QSM), and rows (calcified, true/false positive calcified, false negative calcified, non-calcified, true/false positive non-calcified, false negative non-calcified), or similar.
3. Please discuss, the sensitivity and specificity, you would achieve with the proposed methods more thoroughly with respect to the sensitivity and specificity achieved with the methods currently used. (The discussion has not been adapted).

Minor comments (expressions, typos, grammar):
3. calcified (subependymal) nodules instead of calcification (subependymal) nodules.
4. On page 4, four decimal places are used for QSM. In Table 2, three decimal places are used for QSM. Please be consistent (usually three is used, when using ppm).
5. Page 14, line 3: combination or combined use instead of combined.
6. When stating "compared to white matter", it might be more appropriate to say "compared to normal appearing white matter", in many cases.
7. Page 5, line 19: Delete "was" in "was consisted"
8. Page 3, line 10: reduced instead of improved
9. Page 3, line 6: of any age
10. Page 1, line 18: underwent measurement with...
11. Page 1, line 19: a computed tomography (CT) scan
12. Page 1, line 20: Considering instead of "To consider"

Reviewer #3: I have no concern.

Author’s rebuttal

Reviewer #2: Major comments:
1. Please define your ground truth (for the various lesion types) more clearly in the data

analysis section (page 6, line 23-30), and then stay consistent in the results (page 7, line 25-30).

[Response]: Thanks for the comment. The ground truths of subependymal nodules and cortical/subcortical nodules were defined clearly. (marked as R2.1)

2. Please clarify Table 1: using columns (CT, convent. MRI, QSM), and rows (calcified, true/false positive calcified, false negative calcified, non-calcified, true/false positive non-calcified, false negative non-calcified), or similar.

[Response]: Thanks for the comment. The columns and rows of Table 1 has been modified. (marked as R2.2)

3. Please discuss, the sensitivity and specificity, you would achieve with the proposed methods more thoroughly with respect to the sensitivity and specificity achieved with the methods currently used. (The discussion has not been adapted).

[Response]: Thanks for the comment. The discussion of sensitivity and specificity for QSM has added. (marked as R2.3)

Minor comments (expressions, typos, grammar):

3. calcified (subependymal) nodules instead of calcification (subependymal) nodules.

[Response]: Thanks for the comment. (marked as R2.4)

4. On page 4, four decimal places are used for QSM. In Table 2, three decimal places are used for QSM. Please be consistent (usually three is used, when using ppm).

[Response]: Thanks for the comment. (marked as R2.5)

5. Page 14, line 3: combination or combined use instead of combined.

[Response]: Thanks for the comment. (marked as R2.6)

6. When stating "compared to white matter", it might be more appropriate to say "compared to normal appearing white matter", in many cases.

[Response]: Thanks for the comment. (marked as R2.7)

7. Page 5, line 19: Delete "was" in "was consisted"

[Response]: Thanks for the comment. (marked as R2.8)

8. Page 3, line 10: reduced instead of improved

[Response]: Thanks for the comment. (marked as R2.9)

9. Page 3, line 6: of any age

[Response]: Thanks for the comment. (marked as R2.10)

10. Page 1, line 18: underwent measurement with...

[Response]: Thanks for the comment. (marked as R2.11)

11. Page 1, line 19: a computed tomography (CT) scan

[Response]: Thanks for the comment. (marked as R2.12)

12. Page 1, line 20: Considering instead of "To consider"

[Response]: Thanks for the comment. (marked as R2.13)

4th Editorial decision

9-Mar-2020

Ref.: Ms. No. JCTRes-D-19-00018R3

Investigation of quantitative susceptibility mapping (QSM) in diagnosis of tuberous sclerosis complex (TSC) and assessment of associated brain injuries at 1.5 Tesla
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: