**Author's response to reviews**

**Title:** Hyperintense putaminal rim at 1.5T: prevalence in normal subjects and distinguishing features from multiple system atrophy

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**Author's response to reviews:** see over
To,

The Editors and Referees,

BMC Neurology

Date: April 14, 2012

Subject: Submission of the revised version of the article (Manuscript ID - 2032669564673251)

Dear Sirs,

We hereby submit the revised version of the research article entitled “Hyperintense putaminal rim at 1.5T: prevalence in normal subjects and distinguishing features from multiple system atrophy”. We have made corrections to the article, according to the advice and comments from the Referees. We hope the Referees would satisfy with the corrections. Along with submission of the article, we include a point-by-point response to the comments by the Referees. The revised version includes 5 Figures and 4 Tables. Please kindly see the revised version of the article.

Sincerely,

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Point-by-point response to the comments by the Referees

Referee 1

This MR imaging study aims to evaluate HPR conspicuity as a diagnostic marker for MSA using T2W and DTI at 1.5T. Two experienced neuroradiologists diligently performed detailed evaluation on many variables across a respectable sample size (N=130), and the results indicate that HPR is also prevalent (39%) among neurologically normal subjects. The conspicuity does not dependent on age, gender, and laterality, extending the implication of the study across a broad spectrum of HPR diagnosis. The authors suggest utility of HPR continuity, putaminal atrophy, and MD, as useful diagnostic markers in discriminating the three groups, though generalizability is limited by small sample sizes for some outcome variables. Overall these findings may be promising and suggest additional features that can enhance clinical diagnosis of MSA, pending replication and expansion of findings.

Major Compulsory Revisions

1. Generally, the results and discussion sections are too long and unfocused, spending too much space on detailed results that can better be listed in tables. The discussion contains lengthy explanations for a histological basis of HPR. I would suggest a tighter focus on diagnostic utility.

Response

The results were shortened, and compiled in tables (Table 2-4). The information obtainable from Table 4 and Figure 5 are almost the same. If considered redundant by the Editor or Referees, we will be willing to delete Figure 5. The paragraph explaining the
histology of HPR was shortened to two-thirds of the original manuscript. The “Discussion” as a whole was also shortened.

2. Blindness was arranged for the patients, but it is unclear what information the raters received. Were they aware that MSA was one of major groups and diagnostic concerns? Also, when the normals were reviewed, were the raters aware that these were all normals? These two points render the blindness very weak. Clarify this in the method section.

**Response**

Regarding rating of normal subjects, the raters were informed that these subjects were normal. Regarding rating of the patients, the raters were aware that the group belonged to a diseased state, but were not informed of the name of the disease or other clinical information — similar to previous studies [Ref: 4, 29]. It is, however, possible that the raters knew, from a mere guess, that MSA was included in the group as they were evaluating HPR and HPR has been considered as an MRI sign of MSA. To limit bias in rating of the patients’ data, 24 patients with systemic diseases other than MSA were included in rating of the patients.

3. Contrary to a previous study (Ref 9), this study finds high HPR prevalence among neurologically normal subjects, but a care should be taken in the interpretation of the actual number (39%). Absence of sensitivity and specificity figures at both 1.5T and 3.0T limits a fair comparison between the two field strengths and undermines the reliability of the number. Further, ambiguous blindness of the raters and absence of inter-rater reliability (see Ref 11), can distort the interpretation. This should be discussed in the discussion.

**Response**
We admit that variation in the scan parameters can lead to variation in the conspicuity of HPR. Ideally, identical scan parameters would be desired. In the revised version of the manuscript, we added this fact in the last paragraph of the “Discussion” section. In addition, we have suggested in the first paragraph of the “Discussion” section about inability to exactly pointing out the reason underlying the difference in the prevalence of HPR between the two studies. We also included that the difference of TE can also induce difference in conspicuity of HPR, as suggested by the Referee below.

4. As is the case with most of the published studies (Ref 9~12), relying on T2-weighted image instead of T2 (transverse relaxation time) probably contributes to inconsistency across studies. Contrast between putaminal hypo-intensity and HPR, for example, can depend on the choice of echo time (TE), affecting raters ability to score HPR conspicuity. Quantifying T2 in HPR, may mitigate a potential systematic bias across the studies. Consider adding this point in the discussion.

Response

As mentioned above, we acknowledge the influence of TE on conspicuity of HPR, in the first paragraph of the “Discussion” section. We also acknowledge the possibility of influence of scan parameters on the appearance of HPR, in the 2nd paragraph of the “Discussion” section. In addition, a statement mentioning the possible application of T2 values in evaluation of HPR was added to the last paragraph of the “Discussion” section, in the revised version of the manuscript.
5. The study suggests continuity and length of HPR as useful diagnostic markers for discriminating the groups, but it’s not clear how the authors decided to use 2 mm as a threshold. First, in-plane resolution of the study is 1.02x0.47mm, hence detection of 2mm would translate to only ~4 voxels at most which is not easily discernible. Second, the continuity and length of HPR depend on the angulation of the image plane. The paper appears to indicate that only a single image slice (at the foramen of Monro level) was evaluated for HPR conspicuity. Unless all images were taken in a standardized plane as described in Ref 11, several adjacent slices need to be evaluated for HPR length and continuity as well. Clarify these points in the method and discussion sections.

Response

The raters evaluated the maximum transverse dimension of HPR after zooming the images. The raters measured the dimension, and answered in integer numbers. For assessment of continuity and length of HPR, the raters were also allowed to view adjacent slices. However, the raters were not allowed to view images of the infratentorial compartment (to avoid obtaining diagnosis of MSA-C from infratentorial findings). This information was added to the “Methods: Visual analysis” section of the revised version of the manuscript.

Minor Essential Revisions

1. In the abstract, MSA patients (which?) had significantly higher (not larger) MD values.

Response
Corrections were made to the revised version of the manuscript. The former sentence in the “Abstract” was replaced by “Patients with MSA of either type had significantly higher MD values of putamen than normal subjects.”

2. Background should be shorter.

**Response**

Background was shortened to 581 words (Word count of the “Background” section in the former manuscript = 626). The sentences “in subjects between 30 and 70 years of age, the prevalence ranged from 10% in subjects in their 70s to 70% in those in their 30s and 50s”, and “Thus, in these recent studies, HPR was considered pathological only if the signal arc had less than 50% continuity [11], or if it revealed dorsolateral predominance [12]”, and the phrase “between 13 and 85 years of age” were deleted.

3. In the methods, provide MRI magnet manufacture.

**Response**

The manufacturer was added to the “Methods: MRI and image processing” section of the revised version.

4. In the methods section first paragraph, it’s unclear why consent was waived for patients. Do the authors mean that consent was not required because the scans were clinically acquired and not research?

**Response**
According to the Institutional Regulations, written informed consent for this retrospective study is not necessary because the study is retrospective and anonymity is maintained. Regarding volunteers, written informed consent for MRI was obtained at the time they consented for participation in construction of the normal database. An announcement about this retrospective study protocol was made on the Hospital’s homepage; and the patients who were possibly included in the study were allowed to contact for confirmation and denial for inclusion in the study. In addition, all patients were informed in prior that the images or any clinical information may be used for future research purposes.

5. In the methods section, there is no mention of the number of subjects being scanned with DTI. Figure 5 clearly does not have 10 MSA-P patients.

**Response**

The number of subjects scanned with DTI is mentioned in the 1st paragraph of the “Methods: MRI and imaging processing” section, in the former as well as the revised version of the manuscript. DTI was performed in 3 MSA-P patients. Of whom, the number of putamen with which HPR was observed is 4.

6. In the visual analysis section, a lot of redundancy between Table1-2 and the text. Reduce it substantially.

**Response**

Redundant parts in the text were removed.
7. Comments on table 2. Express values in % and provide p-values. In normals, there are 13 males and 11 females which don’t add up to the total of N=39. In “hypo-intensity of nearby putamen (anterior half)”, the number of MSA-C does not add up to 18. It’s 17 in the table. In “hypo-intensity of nearby putamen (posterior half)”, the numbers of all 3 groups do not add up. Check these numbers.

Response

In the revised version of the Table 3 (formerly, Table 2), the values are given in %. The P-values are also given. The numbers, including P-values, were checked and corrected.

8. In the discussion, “Imaging at 3T allows acquisition of images with smaller pixel sizes” is an inaccurate statement. Higher field strength enhances overall signal to noise ratio (SNR) but the image resolution is dictated by a imaging gradient strength.

Response

High field strength improves signal-to-noise ratio (SNR). Improved SNR can be used to either increase spatial resolution (acquisition of images with smaller pixel sizes) or to reduce acquisition time (via reduced signal averaging). For clarity, a phrase “without additional time constraints” was added to the statement, in the revised version.

9. “An improved signal-to-noise-ratio” should be “An improved contrast-to-noise-ratio”

Response

In the revised version of the manuscript, the term “contrast-to-noise ratio” is used instead.
10. Whenever mentioning MSA, the authors should specify whether they mean C, P, or both.

**Response**

In the revised version, the detailed subtype of MSA (i.e. whether MSA-C or MSA-P) is given whenever possible. However, at certain points, it is not possible to differentiate between the two — either due to ambiguity of the subtype in the previous reports or the fact that the feature is shared between the two subtypes. At these points, the general term MSA is used.

11. MD values of the normal putamen should be quantitatively compared to previous literature.

**Response**

The MD values of the normal putamen were comparable with the values reported in previous literature (Ref. 25-27). This information is included in the 5th paragraph of the “Discussion” section, in the revised version.

12. Table 1 legend should specify that it refers to healthy controls

**Response**

The phrase “in the normal subjects” was added to the title of Table 1, in the revised version of the manuscript.

13. In Figure 5 high mean values in MSA-C and MSA-P are determined by outliers. These distributions raise questions about the nature of the patients and the reasons for this heterogeneity. Repeat the same statistical analysis without those outliers.

**Response**
Outliers were identified in the MSA-C group. One way ANOVA performed after removing the outliers also revealed significant difference in the MD values of putamen among the groups (F = 23.07, df = 33, P<0.0001). Post-hoc Bonferroni comparisons also revealed a significant increase in MD values of the putamen in the patients than normal subjects (p<0.0001 for MSA-P, and p=0.0085 for MSA-C). Between the two patient groups, MSA-P patients had significantly larger MD values than MSA-C patients (p<0.0001). In the “Results: Quantitative analysis” section of the revised version, a sentence describing that the differences remained statistically significant was added.

14. Report ANOVA result with F, df, and P-value

Response

In the revised version of the manuscript, ANOVA is reported with F, df, and P-value (F = 15.76, df = 37, P<0.0001).

15. In the discussion, “0.66 x 0.41 mm, whereas that in this study was 1.02 x 0.47 mm” provide slice thickness of both studies.

Response

In both studies, the slice thickness was 5 mm. To include this information, the sentence was rephrased as “The voxel size of T2-weighted images in the study by Fujii et al. was 0.66 x 0.41 x 5 mm, whereas that in this study was 1.02 x 0.47 x 5 mm”.
Referee 2

Tha et al in their study documented and characterized the appearance of HPR in normal individuals and in MSA patients. The novelty of the study is the fact that HPR is present in around third of normal individuals and therefore should not automatically be considered pathologic.

1. The question posed by the authors is well defined (at the end of the introduction section)

Response

The authors thank the Reviewer for the kind comment.

2. The methods section is generally clear but there is one point that is unclear with regard to the normal individuals. It is noted that the study was retrospective but later the authors note that they all had a normal neurological examination. The authors should be more specific noting the chronological relation between the examination and the scans.

Response

The phrase “performed on the day of MRI” was added to the sentence “The results of neurological examination were normal.”

3. The data are sound and the manuscript adheres to standards of reporting.

Response

The authors thank the Reviewer for the kind comment.
4. The discussion is well written and address most of the question raised (e.g. the difference between MSA-P and MSA-C).

**Response**

The authors thank the Reviewer for the kind comment.

5. The limitations of the study are sufficiently discussed in the discussion.

**Response**

The authors thank the Reviewer for the kind comment.

6. The writing is clear and the title and abstract accurately convey what has been found.

**Response**

The authors thank the Reviewer for the kind comment.

**Major revisions:**

**Abstract**

1. The authors claim that they "This study establish the MRI characteristics of HPR in 1.5T and the features which distinguish from HPR in MSA patients. (the end of the abstract). This claim is not supported by the data in table 2 and should be rephrased (maybe to summarize the differences in a table)"

**Response**

The last sentence of the abstract was rephrased. To keep the word limit, a few words were deleted from the “Methods” section of the “Abstract” (such as abbreviation for analysis of variance, age and gender distribution of the normal subjects).
Methods

2. As noted above for the time of neurological examination in normal individuals

Response

The phrase “performed on the day of MRI” was added to the sentence “The results of neurological examination were normal.”

3. It is not noted if MRI's of the normal individuals were also normal or revealed clinically silent pathologies.

Response

It is mentioned in the 2nd paragraph of the “Participants” section that “The MR images of the brain showed no obvious abnormalities.”

4. The logic of using dummy dataset is not clear to me

Response

As the evaluation of normal subjects and the patients was performed separately, there is likelihood of bias in interpretation of the patients’ data. To avoid this, the images of 24 patients with systemic diseases other than MSA were included in the interpretation session of the patients’ data.

Minor essential revisions

Results

1. The sentence "Thirty eight percent and 0.6 % of HPR had mild and moderate…..” is unclear
Response

In the revised version, the data are given in Table (Table 2).