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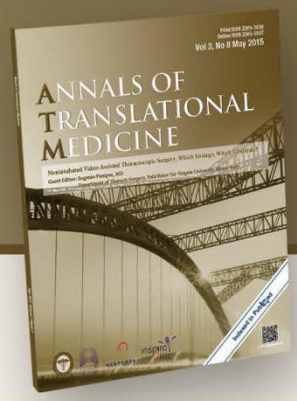

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Peer Review File

Article information: http://dx.doi.org/10.21037/atm-21-217

Reviewer Comments

Thanks to advances in molecular genetics, the complexity of hereditary leukoencephalopathies has been gradually unveiled in recent years. In this study (ATM-21-217-R1), Chu and colleagues identified potential new CSF1R mutations in Chinese patients with CSF1R-related leukoencephalopathy. Although the objective of the study is of interest, significant improvement of the current version is needed before publishing in Annals of Translational Medicine.

1. Hereditary diffuse leukoencephalopathy with axonal spheroids (HDLS) was first reported in a Swedish family as early as 1984 based on the pathological hallmarks (ref 1 of this manuscript). However, the alanyl tRNA synthetase (AARS) gene mutation has recently been identified in this original Swedish HDLS family, who have also been tested for CSF1R gene mutation status with negative results (An AARS variant as the likely cause of Swedish type hereditary diffuse leukoencephalopathy with spheroids, Acta Neuropathologica Communications, 2019). In addition, all pigmentary orthochromatic leukodystrophy (POLD) families tested for CSF1R gene mutations have been positive for the mutation. HDLS was named based on its pathologic characteristics: loss of myelin sheaths and axons, widespread white matter degeneration, and numerous neuroaxonal spheroids. In this manuscript, the authors did not provide any neuropathological evidence. To avoid further nomenclature confusion and adopting current nomenclature trends, the term CSF1R-related leukoencephalopathy should be used (Microglial replacement therapy: a potential therapeutic strategy for incurable CSF1R-related leukoencephalopathy, Acta Neuropathologica Communications, 2020). This was also suggested in the review by Konno and colleagues (ref 2).

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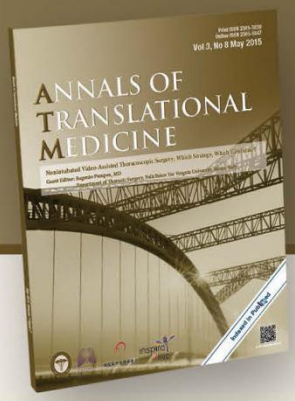

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Reply 1: Thanks for your valuable advice, we agree it will be more appropriate and understandable to use the term "CSF1R-related leukoencephalopathy". We have corrected the term "CSF1R-related leukoencephalopathy" as you suggested.
Changes in the text: We have included the term "CSF1R-related leukoencephalopathy" in the title (page 1, lines 1–2), and elsewhere in the manuscript (page 3, line 35; page 5, line 64 and lines 70-71; page 11, line 220).

2. In the main text, the authors said that the colony-stimulating factor 1 receptor (CSF1R) gene on chromosome 5q32, which was identified as a causative gene in HDLS in 2012. However, this original study was published in the year of 2011 (ref 10). Please double check.
Reply 2: Many thanks for your suggestion. We have corrected the published year as suggested.
Changes in the text: Please see the changes from Page 4, line 73.

3. In the introduction, the authors said that 164 patients in 116 families worldwide and 39 patients in 23 families in China have been reported (refs 4-9). And 90 CSF1R mutations have been reported. However, it is not clear for readers how these numbers were counted since these are not consistent with available literature. Did you include non-English literature? In addition, a recent paper was not cited and thus might not be counted (A Novel Missense Mutation of the CSF1R Gene Causes Incurable CSF1R-Related Leukoencephalopathy: Case Report and Review of Literature, International Journal of General Medicine, 2020). In this very recent paper, Chen and colleagues found that 93 missense mutations, 13 splicing mutations, 6 deletion/insertion mutations, 1 code shift mutation and 1 nonsense mutation of the CSF1R gene have been reported in patients with CSF1R-related leukoencephalopathy (A Novel Missense Mutation of the CSF1R Gene Causes Incurable CSF1R-Related Leukoencephalopathy: Case Report and Review of Literature, International Journal of General Medicine, 2020).
Reply 3: Thank you for your suggestions. We have read the newly published literature you mentioned and checked and corrected the numbers detailed in our manuscript.
Changes in the text: Please see the changes from page 4-5, lines 77-79.

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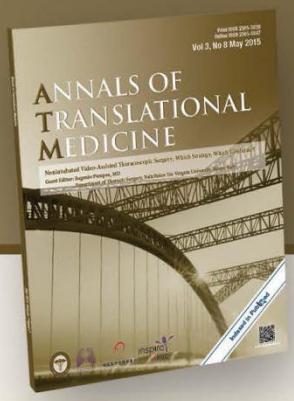

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4. Please provide the approval number given by the ethical board in the part of method.
Reply 4: The study was approved by institute Ethics Committee of Xuanwu Hospital, Capital Medical University (approval number 2014019). We have added the approval number in the part of method.
Changes in the text: Please see the changes from page 5, lines 81-82.

5. In figure 1 (an autosomal dominant pattern of inheritance), does IV-6 (a man) also carry the same CSF1R mutation? How did the authors deal with this individual?
Reply 5: We conducted genetic screen analysis for this individual and he does not carry the same *CSF1R* mutation.
Changes in the text: No changes were made in the text.

6. In authors' single center, the mean disease duration from onset until death was 4.3 years (the average age of onset was 39.2 years), which is shorter than worldwide average disease duration of 6.8 years (the mean age at death was 53 years). This point should be discussed in the revised manuscript.
Ref: Clinical and genetic characterization of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia associated with CSF1R mutation, Eur J Neurol, 2017 Jan;24(1):37-45.
Reply 6: Thank you for your valuable advice. It is the case that mean disease duration of patients in our study was shorter than that previously reported. There are some potential explanations for this difference. First, neurodegenerative genetic diseases may progress rapidly if there is an early age of onset. Second, mean disease duration was calculated based on a very small number of patients (n = 3) who died during our study. Finally, the sample in the article cited by the reviewer comprised 26 Japanese patients with ALSP and 96 symptomatic carriers, based on literature research, whereas we focused only on the Chinese patients in our institution. Hence, regional, medical care condition, and ethnic variations may also help explain this observed difference.

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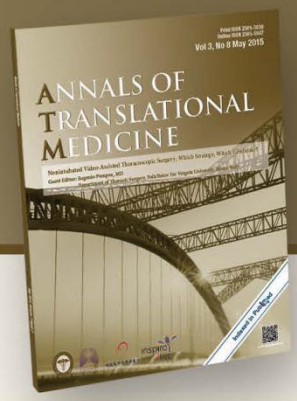

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Changes in the text: A discussion of our findings of disease duration compared with previous publications has been added to the "Discussion" section (from page 10-11, lines 208-218).

7. Experimental studies have convincingly demonstrated sex-dependent structural and functional differences in microglia (Transcriptional and translational differences of microglia from male and female brains. Cell Rep, 2018; Sex-Specific Effects of Microglia-Like Cell Engraftment during Experimental Autoimmune Encephalomyelitis, IJMS, 2020). Consistently, female patients with CSF1R-related leukoencephalopathy may develop clinical symptoms significantly earlier than do men (ref.7). Did the authors also observe sex (gender) clinical differences in your center? This point should be discussed.
Reply 7: Thank you for your valuable suggestion. We have compared the age at onset between male and female groups in our study and did not find a significant difference (male vs. female, 33.50 ± 8.06 vs. 43.80 ± 8.82, $p = 0.114$), which may because of the small sample size.
Changes in the text: We have included this data in the "Results" and "Discussion" sections (page 8, line 155–156; page 11, line 218–223).

8. Seven of the nine patients presented with cognitive decline, seven with pyramidal signs, and four with parkinsonism; two had personality changes, and one had epilepsy. However, it is not clear for readers how clinical severity (rapidly progressive?) they were. Did you perform any clinical rating scales?
Reply 8: We performed cognitive scales including MMSE or MoCA in four patients; however, the other patients were in a critical condition and could not cooperate to complete the cognitive scales. Other rating scales, such as UPDRS, were not performed and we collected the information on the rapidly progressive symptoms from the patients and their families through a comprehensive and standard medical history collection.
Changes in the text: No changes have been made to the text.

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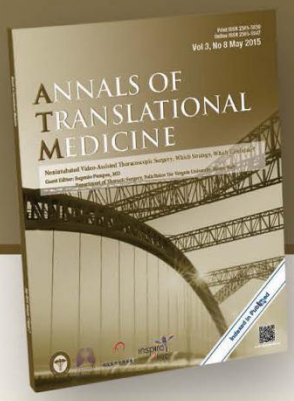

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9. In table 1, what about the first symptoms of these patients? How did you explain epilepsy in one of these patients?

Reply 9: The first symptoms of the patients were: cognitive deficit (patient 1), cognitive deficit (patient 2), parkinsonism and cognitive decline (patient 3), walking difficulties (patient 4), cognitive deficit (patient 5), personality changes and walking difficulties (patient 6), cognitive deficit (patient 7), cognitive decline (patient 8), and walking difficulties (patient 9). In consideration of your helpful questions, we have added this data to Table 1.

Epilepsy is a major clinical feature of patients with hereditary diffuse leukoencephalopathy with spheroid (HDLS), occurring in 28% (38/137) of patients, as previously reported (Zhuang L, Liu C, Li Y, et al. Clinical features and genetic characteristics of hereditary diffuse leukoencephalopathy with spheroids due to CSF1R mutation: a case report and literature review. Ann Transl Med 2020; 8:11). We consider that the epilepsy in one patient may have been be caused by the CSF-1R leukoencephalopathy lesions. Cortex irritation syndromes can occur if brain lesions are located adjacent to the cortical area and cause abnormal brain discharge. MRI examination of patient 4 showed hyperintensities in the corpus callosum and right subfrontal region, which may be the lesions that explain the simple partial motor seizures observed in this patient.

Changes in the text: We have added the relevant data to Table 1.

10. In table 1, the scores of MMSE and MoCA from most patients were lacking?

Reply 10: This is correct. We have double checked our original cognitive scales and found some data were lacking, because the patients were in a critical condition and were unable to cooperate with cognitive examination.

Changes in the text: No changes were made to the text.

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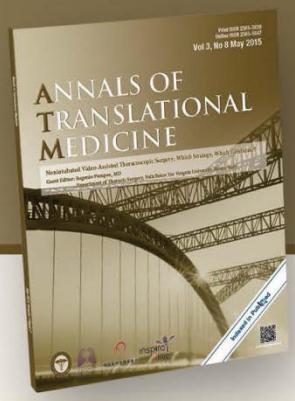

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11. Regarding MRI findings in table 1, please include persistent limited diffusion on diffusion-weighted imaging (DWI). In figure 2, were repeated brain MRI performed during follow-up? In patient 4, periventricular white matter hyperintensities are not obvious.
Reply 11: Many thanks for your valuable advice, we have added the suggested data to Table 1. Repeated brain MRI examinations were not performed during follow-up. It is correct that images C1–C7 (Patient 4, 6 months after disease onset) in Figure 2 do not show any obvious periventricular white matter hyperintensities; however, prominent hyperintensities were detected in the corpus callosum, as well as brain atrophy, which support our diagnosis.
Changes in the text: We have added the relevant data to Table 1.

12. I wonder how many patients had the involvement of subcortical U fibers? Any enhancement lesions in these patients?
Reply 12: No patient in our study had involvement of subcortical U fibers in the frontal white matter according to MRI. MRI enhancement examination was performed in five of the nine patients and no enhancement lesions were detected.
Changes in the text: No changes were made to the text.

13. A diagnostic value of unique calcifications distributed in selective brain regions on CT scans has been proposed (Diagnostic value of brain calcifications in adult-onset leukoencephalopathy with axonal spheroids and pigmented glia. AJNR Am J Neuroradiol, 2017). Please provide brain CT scans as well. The authors said that four of the five patients who underwent CT scanning had subcortical calcification. However, these calcifications are usually located at the frontal horns of the lateral ventricles.
Reply 13: Four of five patients had calcifications on CT in our study; however, two only had CT reports in their medical records, another two patients had CT images. It is correct that the

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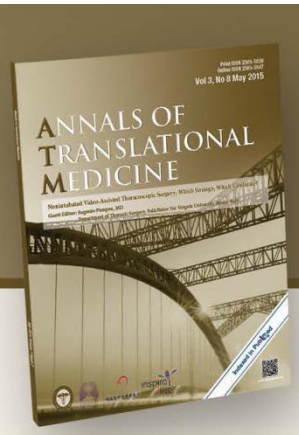

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calcifications are usually located at the frontal horns of the lateral ventricles, and we can also see extensive calcifications at subcortical area in our patients. The CT images are provided as a supplementary material.
Changes in the text: Please see Page 9 line 174

14. Were any neuropathological images from these patients available? If so, please provide.
Reply 14: Biopsy was not performed in our patients after definite diagnosis by genetic test and neither were autopsies conducted after the three patients died, because they were at home and refused. The older sister of one patient underwent biopsy in 2006, revealing widespread white matter degeneration with a loss of myelin and axons, as well as neuroaxonal spheroids; however, as it was performed at another hospital, images cannot be provided in our article.
Changes in the text: No changes have been made to the text.

15. In this study, the authors identified some potential novel mutations of CSF1R gene in Chinese HDLS patients, including p.R579Q. When I searched the literature, however, I found another Chinese HDLS patient (also in Beijing) carrying CSF1R missense mutation c.1736G>A (p.R579Q) (https://www.researchsquare.com/article/rs-611/v1).
Reply 15: Thank you for your kind reminder. You are correct that the p.R579Q mutation had been reported in that case report, which we had missed; however, the previously reported patient was a 30-year-old woman admitted to Beijing Chaoyang hospital who presented with a 30-month history and anti-GABAbR antibody positivity, and was not the same patient as we report here. Nevertheless, as the two patients had the same mutations, we have taken your advice and not noted that it is not a novel mutation in our manuscript.
Changes in the text: We have reported p.R579Q as a previously described mutation (page 3, line 50; page 9, line 184; page 10, line 200).