Dear Professor Ya-Juan Ma,
Science editor, *World Journal of Gastroenterology*

Thank you very much for your response regarding our manuscript entitled "Clinically diagnosed late-onset fulminant Wilson disease without cirrhosis: A case report". We carefully examined the reviewers' comments, and point-by-point responses are attached for your convenience. We used red font to indicate changes to our manuscript.
We sincerely hope that the revised manuscript is acceptable for publication in ***World Journal of Gastroenterology***.

Name of journal: World Journal of Gastroenterology
Manuscript NO: 36581
Title: Clinically diagnosed late-onset fulminant Wilson disease without cirrhosis: A case report

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Sincerely,

Tokuhiro Matsubara, M.D., Ph.D.

Responses to Reviewer #1's comments (Reviewer's code: 00007470)

Thank you very much for your valuable and constructive comments regarding our manuscript. We believe that your comments significantly improved the manuscript. We used red font and underlined the modified text to indicate changes in our revised manuscript.
My responses to your comments are as follows:

Comments (Major revision):

1. This case report is very interesting because describes a patient with wilsonian hepatic failure in absence of histological evidence of cirrhosis. Unfortunately, there some diagnostic points that are not clear and need to be clarified. The absence of mutations at molecular analysis, the lack of information about copper liver concentration and ultrastructural liver aspects (typical mitochondrial lesions). The absence of this information weakens diagnosis of Wilson disease. On the other hand, the low serum levels of ceruloplasmin and the high levels of cupriuria may be observed in fulminant liver failure of other causes. Discussion should address this critical points.

   Response to comment 1
   Thank you for your helpful point. We also think your advice is very important. Unfortunately, we were unable to weigh precise copper levels in the liver, analyze mutations with molecular analysis or examine ultrastructural liver aspects due to inadequate specimen processing for analysis. Specifically, all liver tissues were formalin fixed.
   As indicated, low serum levels of ceruloplasmin and high levels of cupriuria may be observed in acute liver failure from other causes. First, we ruled out other acute liver injury, such as viral infection, autoimmune or drug-induced hepatic damage, even though there was a possibility of other unknown hepatitis. However, the following features were recently reported in ***Hepatology 2008*** as useful predictive markers for the diagnosis of WD-induced acute hepatic failure: reduced hemoglobin (<10 g/dL), elevated serum copper levels (>200 μg/dL), decreased ratios of alkaline phosphatase (ALP) to total bilirubin (T-Bil) (<4) and elevated ratios of

aspartate aminotransferase (AST) to alanine aminotransferase (ALT) (>2.2). Our case met all of these criteria. Therefore, we included the above underlined information in the revised text of the discussion section on Page 11, line 22 to Page 12, line 5.

Thank you very much. We believe that your comments significantly improved the manuscript. We hope that the revised manuscript is acceptable for publication in ***World Journal of Gastroenterology.***

Responses to Reviewer #2's comments (Reviewer's code: 03699990**)**

Thank you very much for your valuable and constructive comments regarding our manuscript. We believe that your comments significantly improved the manuscript.

My responses to your comments are as follows:

Comments (High priority for publication):

1. There are some Incorrect spelling of words such as "ithas" which should be" it has" and so on. Please check. Some expressions require professional polish. In the first sentence of ITRODUCTION "Wilson Disease (WD) was initially described by Kinnier Wilson in 1912 and…". The year is 1911 in some literature. Please check the literature.

Response to comment 1

Thank you for your advice. As per your comment, we checked and corrected spelling in our manuscript. Additionally, we asked a copy-editing company (**American Journal Experts; http://www.aje.com/jp/**) to correct syntax errors. Then, we re-reviewed the literature, but it seemed that Wilson disease was initially described by Kinnier Wilson in 1912 [1]. Therefore, we cited the reference below[1].

Reference

1 Compston A. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver, by S. A. Kinnier Wilson, (From the National Hospital, and the Laboratory of the National Hospital, Queen Square, London) Brain 1912: 34; 295-509. *Brain* 2009; **132**(Pt 8): 1997-2001 [PMID: 19634211]

Thank you very much. We believe that your comments significantly improved the manuscript. We hope that the revised manuscript is acceptable for publication in ***World Journal of Gastroenterology***.

Responses to Reviewer #3's comments (Reviewer's code: 00159633**)**

Thank you very much for your valuable and constructive comments regarding our manuscript. We believe that your comments significantly improved the manuscript.

My responses to your comments are as follows:

Comments (Accept):

1. In some parts English language is very poor and requires a thoroughly edition. Moreover, it is very long and written beyond a case report and seems a novel. Hence, it also should be shortened.

Response to comment 1

Thank you for your advice. As suggested, we asked a copy-editing company (**American Journal Experts; http://www.aje.com/jp/**) to correct syntax errors.

Thank you very much. We believe that your comments significantly improved the manuscript. We hope that the revised manuscript is acceptable for publication in ***World Journal of Gastroenterology***.

Responses to Reviewer #4's comments (Reviewer's code: 00003629**)**

Thank you very much for your valuable and constructive comments regarding our manuscript. We believe that your comments significantly improved the manuscript. We used red font and underlined the modified text to indicate changes to our manuscript.

My responses to your comments are as follows:

General comments (Major revision):

1. The article has major English language problems. In this respect, it needs thorough re-writing.

Response to comment 1

Thank you for your advice. As suggested, we asked a copy-editing company (**American Journal Experts; http://www.aje.com/jp/**) to correct syntax errors.

2. Since the whole liver is available, as stated, authors should consider hepatic copper concentration measurement in an appreciable part of the organ. In the absence of chronic cholestasis, as in this case, finding copper concentration ≥250mg/g of dry liver weight will increase the probability that the reported case had indeed WD (Am J Clin Pathol 1994;102:443, Clin Gastroenterol Hepatol 2005;3:811).

Response to comment 2

Thank you for your helpful point. We also think your advice is very important. As you know, dried liver tissue is necessary to measure hepatic copper levels. Unfortunately, dried tissue was not available because all liver tissues were formalin fixed. Therefore, we were unable to weigh the precise copper levels in the liver. Then, we evaluated copper deposition in the liver using rhodanine staining. However, previous reports cited in the Wilson disease practice guidelines in Japan said that immunohistochemistry staining is insufficient for examining copper deposition [2, 3]. Therefore, we think that rhodanine staining insufficiently examined copper deposition in the scattered residual hepatocytes because of massive necrosis and collapse

of the intervening parenchyma. We discussed the above information in the revised text. (Page 12, line 9 from the top).

References

2 Lindquist RR. Studies on the pathogenesis of hepatolenticular degeneration. II. Cytochemical methods for the localization of copper. *Archives of pathology* 1969; **87**(4): 370-379 [PMID: 5766764]

3 Polson J, Lee WM, American Association for the Study of Liver D. AASLD position paper: the management of acute liver failure. *Hepatology* 2005; **41**(5): 1179-1197 [PMID: 15841455 DOI: 10.1002/hep.20703]

Major comments (Major revision):

3. (Page 1, line 5 from top): What the authors mean by "was declined"? Do they mean that ceruloplasmin was low when first detected or that it was higher at the beginning and became lower latter?

Response to comment 3

We mean that ceruloplasmin levels were low on day 4 of hospitalization. We revised the text to avoid any confusion.

4. (Page 5, line 2 from top): Please give reference.

Response to comment 4

As suggested, we added a reference (Page 6, line 3 from the top).

5. (Table 1): Please give the MCV, MCH, MCHC and the RDW values of the admission RBC's.

Response to comment 5

As suggested, we added these values to Table 1 of the revised text.

6. (Table 1): Please mention the upper normal limits of AST, ALT, ALP and GGT measurements in your laboratory.

Response to comment 6

As suggested, we added the upper normal limits in the footnotes of Table 1.

7. (Figure 4): Please mention whether the AZAN staining was used to demonstrate absence of fibrous tissue in the preparation.

Response to comment 7

Thank you for your helpful comment. AZAN staining or Sirius red staining is mostly used to estimate fibrous tissue. We evaluated fibrous tissue using AZAN staining and added the results in Figure 4D.

Minor comments (Major revision):

8. (Page 3, line 2 from top): "Hepatopathy" is a very vague expression. Please be more precise.

Response to comment 8

Thank you for your advice. We revised hepatopathy to jaundice of the bulbar conjunctiva and general fatigue in the revised text (Page 4, lines 2-3 from the top).

9. (Page 3, line 3 & 4 from top): Consider: "she developed hepatic encephalopathy and the diagnosis of fulminant liver failure was made".

Response to comment 9

Thank you for your useful comment. We added "based on the American Association for the Study of Liver Disease (AASLD) position paper" to the revised text (Page 4, line 4 from the top).

10. (Page 5, line 2 from top): Kinnier Wilson.

Response to comment 10

Thank you for your advice. We misspelled the word and revised "Kinnear" to "Kinnier" (Page 6, line 2 from the top).

11. (Page 6, line 5 from bottom): Hemofiltration.

Response to comment 11

We revised hemodiafiltration to hemofiltration. (Page 7, line 5 from the bottom)

Thank you very much. We believe that your comments significantly improved the manuscript. We hope that the revised manuscript is acceptable for publication in ***World Journal of Gastroenterology***.

Responses to Reviewer #5's comments (Reviewer's code: 02996674**)**
Thank you very much for your valuable and constructive comments regarding our manuscript. We believe that your comments significantly improved the manuscript. We used red font and underlined the modified text to indicate changes to our manuscript.
My responses to your comments are as follows:

Comments (Minor revision):

1. In fulminant Wilson disease, serum copper level may increase. In this case, the authors mentioned that 'serum copper level was greatly elevated up to 105μg/dl'. However, the normal serum copper level is 70～132μg/dl. Therefore, serum copper did not increase in this patient.

Response to comment 1

Thank you for your advice. As indicated, urinary copper levels decreased; however, serum copper levels did not increase. Therefore, we deleted "serum and" and added "serum copper levels did not increase". (Page 8, line 4 from the bottom)

2. In this case, rhodanine staining was unclear to examine copper deposition in the scattered residual hepatocytes. Alternatively copper content in the tissue can be relatively easily examined. Did not the authors examine copper content in her liver tissue?

Response to comment 2

Thank you for your comment. We also think your advice is very important. As you know, dried liver tissue is necessary to measure hepatic copper levels. Unfortunately, dried tissue was not available because all liver tissues were formalin fixed. Therefore, we were unable to weigh the precise copper levels in the liver. Previous reports cited in the Wilson disease practice guideline in Japan said that immunohistochemistry staining is insufficient for analyzing copper deposition [2, 3]. Therefore, we think that rhodanine staining did not clearly depict copper deposition in the scattered residual

hepatocytes because of massive necrosis and collapse of the intervening parenchyma. Then, we discussed this information in the text (Page 12, line 9 from the top).

References

2 Lindquist RR. Studies on the pathogenesis of hepatolenticular degeneration. II. Cytochemical methods for the localization of copper. *Archives of pathology* 1969; **87**(4): 370-379 [PMID: 5766764]

3 Polson J, Lee WM, American Association for the Study of Liver D. AASLD position paper: the management of acute liver failure. *Hepatology* 2005; **41**(5): 1179-1197 [PMID: 15841455 DOI: 10.1002/hep.20703]

3. Genetic test at ATP7B is mandatory to diagnose WD. DNA sample from the patient could be taken by autopsy. Why did not the authors extract her DNA from autopsy sample? The authors examined DNA of her son and DNA analysis was negative for mutation of ATP7B gene. But this information is quite incomplete.

Response to comment 3

Thank you for your helpful point. We also think your advice is very important. As you know, genetic testing for ATP7B is necessary for conserved serum or frozen hepatic tissue, but it is difficult to evaluate in formalin fixed liver. Unfortunately, liver or blood samples were not available because all liver tissues were formalin fixed. Therefore, we were unable to test for mutation of ATP7B.

Thank you very much. We believe that your comments significantly improved the manuscript. We hope that the revised manuscript is acceptable for publication in ***World Journal of Gastroenterology***.