PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Epidemiological evidence for a hereditary contribution to myasthenia gravis: a retrospective cohort study of patients from North America
AUTHORS	Green, Joshua; Barohn, Richard; Bartoccion, Emanuela; Benetar, Michael; Blackmore, Derrick; Chaudhry, Vinay; Chopra, Manisha; Corse, Andrea; Dimachkie, Mazen M.; Evoli, Amelia; Florence, Julaine; Freimer, Miriam; Howard, James; Jiwa, Theresa; Kaminski, Henry; Kissel, JT; Koopman, Wilma; Lipscomb, Bernadette; Maestri, Michelanglo; Marino, Mariapaola; Massey, Janice; McVey, April; Mezei, Michelle; Muppidi, Srikanth; Nicolle, Michael; Oger, Joel; Pascuzzi, Robert; Pasnoor, Mamatha; Pestronk, Alan; Provenzano, Carlo; Ricciardi, Roberta; Richman, DP; Rowin, Julie; Sanders, Donald; Siddiqi, Zaeem; Soloway, Aimee; Wolfe, Gil; Wulf, Charlie; Drachman, Daniel; Traynor, Bryan

VERSION 1 – REVIEW

REVIEWER	Jan Damoiseaux Maastricht University Medical Center The Netherlands
REVIEW RETURNED	20-Apr-2020

GENERAL COMMENTS	The manuscript of Green et al investigated in a relatively large cohort of MG patients the family history for MG and other autoimmune diseases. Also the presence of other autoimmune diseases in the MG patients themselves was investigated. Importantly, the study cohort involved a rather homogeneous population (anti-AChR positive, non-Hispanic/white patients) Results reveal that about 5-6% of the patients have a family history of MG; about 25-30% of the patients reported another autoimmune disease and a similar percentage reported to have another autoimmune disease in the family. Data are combined with a literature search. Major comments: 1. The first comment is about the novelty of this study. In the introduction it is mentioned that there are other studies (references are lacking in the introduction) and indeed, the literature review reveals 5 other studies (one of similar size from the same continent). The title starting with "new evidence" therefore is inappropriate. The question is: what does this study add to the already existing studies? 2. It is mentioned in the abstract, in the results and in the discussion that the only difference between familial and sporadic cases is the age of onset. This "difference" does not even reach a significance level close to a trend (p=0.23 with inappropriate

	statistics). This means that there is no difference. In addition, it is mentioned in the discussion that 3 other studies reported an association with age of onset in MG (this implies that 2 other studies did not): whatever the outcome of the current study, the claim that "this is a finding that is consistent with previous reports" is rather redundant. 3. It is not specified how many diagnoses of MG were made in the respective study period in the 14 participating centers. If not all patients were included, what was the reason? Could there have been any kind of selection bias? 4. The age of disease onset is given as mean (SD), but from figure 1 it is evident that data are not normally distributed (two age peaks). Therefore, such data should be presented as median (range) and require different statistical methods to determine a potential difference. 5. The data-set presented in the results is rather limited. Surprisingly, in the discussion (third paragraph) several new data are being introduced, while lacking in the results section. Either present these data in the results, or do not discuss. 6. According to figure 2C there is a personal as well as family history for thyroid disease of about 10%. It is important to unravel if those MG patients with thyroid disease also have a positive family history of thyroid disease. If so, the relation may be completely independent of MG (similarly for the other autoimmune diseases). 7. A discussion on the HLA A1-B8-DR3-DQ2 haplotype and common non-HLA genes related to the respective autoimmune diseases is warranted. Minor comments: 1. One of the problems with the study design (cross-sectional) is the rather late development of autoimmune diseases during life time. This implies that, in time, a sporadic case may become a familial case. This should be discussed. 2. In the paragraph on patient ascertainment it is mentioned that first degree relatives have 50% of DNA in common, which is more or less correct. However, the values given for second- and third-degree relatives are average values. For single individuals this may have a very large range because segregation of maternal and paternal chromosomes is random. 3. In the methods section it is mentioned that DNA samples were collected, but this study does not include any genetic data obtained from these samples. 4. It is recognized that self-reporting of autoimmune diseases in family members is a serious limitation, but this is in particular true for thyroid and rheumatic diseases. This should be discussed in relation to existing literature on the overestimation of these diseases due to self-reporting.

REVIEWER	Jung-Joon Sung Department of Neurology, Seoul National University Hospital, Seoul 03080, Korea.
REVIEW RETURNED	29-Apr-2020

GENERAL COMMENTS	The main purpose of this article is to provide new evidence for a genetic contribution to MG with the evidence of the high rate of familial MG and the coexistence of other autoimmune disease in the patients with MG. So, the certainty and the objectivity of familial history of MG is important.

	Major comment 1. The major strength of this article is inclusion of a large cohort of myasthenia gravis patients who were confirmed with anti-AchR antibodies. 2. The major weakness of this article is the lack of ascertainment of MG and other autoimmune diseases of the familial members of the patients. Considering the limitation to obtain the confirmed diagnosis of MG of familial members of the patients, I recommend the author to more specifically describe how to obtain the information of familial history of MG and other autoimmune disease from the patients and it would be better to provide the composition of a simple structured questionnaire the author mentioned. 3. Of fifty-eight patients who had family history of MG, the author reported parent-child (32.8%) type was the most common. The author needs to mention the presence of neonatal MG cases in the parent-child type, because neonatal MG is not the evidence of genetic contributive factor for MG. 4. The author compared the rate of other autoimmune disease in this cohort patients (26.6%) with the prevalence of autoimmunity in the general population (~3~9%). Because this result is meaningful and significant in this article, it's better to show it with p-value like other authoritative journals. 5. The cohort used in this study was the same as GWAS study of MG published on JAMA neurology in 2015. So, the genotype and imputed variant data might be already established. I recommend to analyze whether the patients with familial history of MG have tendency to show polymorphism in CTLA4 or other variants. If there is relevance between familial history of MG and the presence of genetic variants, it would be strong evidence for genetic contribution to MG. 6. The author suggested the fact that males had a slightly higher prevalence of familial MG compared to the females (1:1.32) reflected the different age distribution of MG cases between two gender (mean onset age for females =45.6; mean onset age for males=59.8). I need logical explanation about the relationship between higher prevalence of familial history of MG in male patients and the older age of symptom onset in male patients, because some reports including this article showed that age at symptom onset was younger among patients with familial history of MG than patients without familial history of MG. 7. According to this article, thyroid disease is the most common autoimmune disease concomitant in MG patients and in the families of MG patients. There is no in-depth discussion about this result. I recommend to describe further discussion about the pathologic relationship between thyroid disease and MG. 8. The author mentioned the familial MG patients were more likely to have a personal history of autoimmune disease than the sporadic cohort, but there is no numerical data about this. Considering the importance of this result, I recommend the author to show the number of patients with concomitant autoimmune

	disease in patients with familial history of MG and without familial history of MG, respectively. Minor comment 1. In table 1, the term of familial or sporadic MG seems inappropriate. The term of 'patients with or without familial history of MG' is more appropriate, because the presence of self-reported familial history of MG is not equivalent to 'familial MG'.

VERSION 1 – AUTHOR RESPONSE

RESPONSE TO REVIEWER ONE

The manuscript of Green et al investigated in a relatively large cohort of MG patients the family history for MG and other autoimmune diseases. Also the presence of other autoimmune diseases in the MG patients themselves was investigated. Importantly, the study cohort involved a rather homogeneous population (anti-AChR positive, non-Hispanic/white patients) Results reveal that about 5-6% of the patients have a family history of MG; about 25-30% of the patients reported another autoimmune disease and a similar percentage reported to have another autoimmune disease in the family. Data are combined with a literature search.

Major comments:

The first comment is about the novelty of this study. In the introduction it is mentioned that there are other studies (references are lacking in the introduction) and indeed, the literature review reveals 5 other studies (one of similar size from the same continent). The title starting with "new evidence" therefore is inappropriate. The question is: what does this study add to the already existing studies?

We believe that our manuscript contributes valuable epidemiological data supporting a hereditary contribution to myasthenia gravis. This work serves both to confirm and to strengthen previous work of others, thereby providing important additional information about the etiology of myasthenia gravis.

To address the reviewer's concern about our title, we have changed the title to read as follows:

"Epidemiological evidence for a hereditary contribution to myasthenia gravis: a retrospective cohort study of patients from North America."

In addition, we have added references for the relevant other studies in the last paragraph of the Introduction as follows:

"In fact, studies have described myasthenic patients with a family history of myasthenia gravis and/or a family history of autoimmune diseases[12-16].

2. It is mentioned in the abstract, in the results and in the discussion that the only difference between familial and sporadic cases is the age of onset. This "difference" does not even reach a significance level close to a trend (p=0.23 with inappropriate statistics). This means that there is no difference. In addition, it is mentioned in the discussion that 3 other studies reported an association with age of onset in MG (this implies that 2 other studies did not): whatever the outcome of the current study, the claim that "this is a finding that is consistent with previous reports" is rather redundant.

The reviewer raises a valid point. We have removed a sentence from the Abstract and paragraph 2 of the Discussion. We have changed the wording of paragraph 2 of the Results section to reflect this point. In addition, we changed the statistical test that was used. See the specific changes below:

"Age at symptom onset was similar among patients with a family history compared to patients without a family history (57.5 years of age, range = 8-80

years versus 58.5 years, range = 4-91 years, p-value = 0.183, Wilcoxon rank sum test, figure 1, table 1)."

3. It is not specified how many diagnoses of MG were made in the respective study period in the 14 participating centers. If not all patients were included, what was the reason? Could there have been any kind of selection bias?

The centers participating in this study did not provide that information during the development of this cohort, so we are not able to report on the number of diagnoses made during the study period. Despite this, we do not think there is any major selection bias that would have a significant influence on the major findings reported in our study. The following has been added to paragraph 1 of the Methods section to reflect this:

> "The numbers of myasthenia gravis patients attending each of these clinics was not available for this study."

4. The age of disease onset is given as mean (SD), but from figure 1 it is evident that data are not normally distributed (two age peaks). Therefore, such data should be presented as median (range) and require different statistical methods to determine a potential difference.

We have modified the manuscript to present the median and range when reporting the age of onset of myasthenia gravis among the various groups in our cohort.

The following changes were made:

1) Paragraph 1 of the Results: "The median age of symptom onset in this clinic-based cohort was 58 years of age (range = 4-91; median onset age for females = 46; median onset age for males = 62).

2) Paragraph 2 of the Results: "Age at symptom onset was similar among patients with a family history compared to patients without a family history (57.5 years of age, range = 8-80 years versus 58.5 years, range = 4-91 years, p-value = 0.183, Wilcoxon rank sum test, figure 1, table 1). "

3) Row 2 of Table 1: "Median age of disease onset (years) (range)

5. The data-set presented in the results is rather limited. Surprisingly, in the discussion (third paragraph) several new data are being introduced, while lacking in the results section. Either

present these data in the results, or do not discuss. We have added the following highlighted text to Paragraph 3 of the Results section:

> "The median age of symptom onset in this clinic-based cohort was 58 years of age (range = 4-91; median onset age for females = 46; median onset age for males = 62)."

6. According to figure 2C there is a personal as well as family history for thyroid disease of about 10%. It is important to unravel if those MG patients with thyroid disease also have a positive family history of thyroid disease. If so, the relation may be completely independent of MG (similarly for the other autoimmune diseases).

Our data indicate that thyroiditis is more common in patients with myasthenia gravis and among their family members. This is consistent with previous literature in this area. From a clinical perspective, this is important information, as it alerts physicians to test for the presence of thyroiditis among patients with myasthenia gravis, and among patients with a family history of myasthenia gravis. Determining the underlying genetic reason for this is beyond the scope of this article.

7. A discussion on the HLA A1-B8-DR3-DQ2 haplotype and common non-HLA genes related to the respective autoimmune diseases is warranted.

While we agree with the Reviewer that this is an interesting topic, this detailed topic is beyond the scope of our paper. Our study emphasizes the supporting epidemiological data for hereditary contributions to myasthenia. We have chosen not to discuss these kinds of genetic topics in the paper, so as not to distract from the main findings and conclusions.

Minor comments:

1. One of the problems with the study design (cross-sectional) is the rather late development of autoimmune diseases during lifetime. This implies that, in time, a sporadic case may become a familial case. This should be discussed.

The Reviewer correctly points out that when conducting studies on the family history of a disease, it is oftentimes difficult to validate if sporadic cases are truly sporadic cases or are familial cases that have not been detected. This is a problem across late-onset diseases and is not unique to myasthenia gravis. This caveat is now raised in the manuscript within a new paragraph near the end of the Discussion (note that this new paragraph also addresses this Reviewer's comment #4):

> "While our study has some notable strengths, it also has an inherent limitation related to its reliance on self-reported family histories, which could have over- or underestimated the prevalence of the diseases studied. For example, it is plausible that a patient could self-report as not having a family history of myasthenia gravis because the family member was never clinically diagnosed with the disease or did not live long enough for the disease to manifest. Similarly, other studies have found an overestimation of some autoimmune diseases, especially thyroid disease and rheumatoid arthritis, related to self-reporting.[23]"

In the paragraph on patient ascertainment it is mentioned that first degree relatives have 50% of DNA in common, which is more or less correct. However, the values given for second- and third-degree relatives are average values. For single individuals this may have a very large range because segregation of maternal and paternal chromosomes is random.

We have added approximation signs to indicate that the percent DNA in common for second- and third-degree relatives are average values. Specifically, in Paragraph 1 of the Methods section, we have added the following highlighted text:

> "A positive family history was defined as having a first-degree (~50% of DNA in common), second-degree (~25% of DNA in common), or third-degree (~12.5% of DNA in common) relative with the disease."

3. In the methods section it is mentioned that DNA samples were collected, but this study does not include any genetic data obtained from these samples.

We aim to be transparent to the reader as to the context in which this cohort was collected.
For that reason, it is appropriate to include that statement.

4. It is recognized that self-reporting of autoimmune diseases in family members is a serious limitation, but this is in particular true for thyroid and rheumatic diseases. This should be discussed in relation to existing literature on the overestimation of these diseases due to self-reporting.

We have added the following paragraph near the end of the Discussion:

> "While our study has some notable strengths, it also has an inherent limitation related to its reliance on self-reported family histories, which could have over- or underestimated the prevalence of the diseases studied. For example, it is plausible that a patient could self-report as not having a family history of myasthenia gravis because the family member was never clinically diagnosed with the disease or did not live long enough for the disease to manifest. Similarly, other studies have found an overestimation of some autoimmune diseases, especially thyroid disease and rheumatoid arthritis, related to self-reporting.[23]"

RESPONSE TO REVIEWER TWO

Major comment

1. The major strength of this article is inclusion of a large cohort of myasthenia gravis patients who were confirmed with anti-AchR antibodies.

We thank the reviewer for their comment.

2. The major weakness of this article is the lack of ascertainment of MG and other autoimmune diseases of the familial members of the patients. Considering the limitation to obtain the confirmed diagnosis of MG of familial members of the patients, I recommend the author to more specifically describe how to obtain the information of familial history of MG and other autoimmune disease from the patients and it would be better to provide the composition of a simple structured questionnaire the author mentioned.

The questionnaire used to collect the demographic and clinical information has been added to the supplementary material. Paragraph 1 of the Methods section has been modified as follows:

> "Family histories of myasthenia gravis and other autoimmune diseases were systematically obtained for each subject using a simple structured questionnaire (Table S1)."

Of fifty-eight patients who had family history of MG, the author reported parent-child (32.8%) type was the most common. The author needs to mention the presence of neonatal MG cases in the parent-child type, because neonatal MG is not the evidence of genetic contributive factor for MG.

Response: Per the Reviewer's suggestion, we have added the following to Paragraph 2 of the Results section:

> "Sibling-sibling (31.0%) and parent-child (32.8%) were the most common types of familial relationships, followed by uncle/aunt-nephew/niece (13.8%), cousin-cousin (12.1%) and grandparent-grandchild (5.2%) relationships. Of note, the indicated percentage of parent-child cases might be inflated because neonatal myasthenia gravis cases (which are not believed to be genetic) could not be discerned from non-neonatal cases."

4. The author compared the rate of other autoimmune disease in this cohort patients (26.6%) with the prevalence of autoimmunity in the general population (~3~9%). Because

this result is meaningful and significant in this article, it's better to show it with p-value like other authoritative journals.

While we appreciate what the Reviewer is saying, it would not be appropriate for us to apply a statistical test between our cohort and the general population. We mentioned this comparison in the manuscript to make the point that people with myasthenia gravis are more likely to have other autoimmune diseases. This difference is of sufficient magnitude to be self-evident to the readership of the journal.

5. The cohort used in this study was the same as GWAS study of MG published on JAMA neurology in 2015. So, the genotype and imputed variant data might be already established. I recommend analyzing whether the patients with familial history of MG have a tendency to show polymorphism in CTLA4 or other variants.

If there is relevance between familial history of MG and the presence of genetic variants, it would be strong evidence for genetic contribution to MG.

While we appreciate the possible value of such analyses, we aimed to focus on epidemiological analyses for the current study and not to extend into genetic investigations. To address this comment, we have added the following highlighted text to Paragraph 4 of the Discussion:

> "The increased prevalence of familial myasthenia gravis and increased prevalence of other autoimmune disorders both suggest that these autoimmune diseases may share a common predisposition that may be genetic in origin. Future studies focusing on specific genes and genomic variants encountered in patients with familial myasthenia gravis offer the promise to identify more precisely any such genetic contributions to the disease."

6. The author suggested the fact that males had a slightly higher prevalence of familial MG compared to the females (1:1.32) reflected the different age distribution of MG cases between two genders (mean onset age for females =45.6; mean onset age for males=59.8). I need a logical explanation about the relationship between higher prevalence of familial history of MG in male patients and the older age of symptom onset in male patients, because

some reports including this article showed that age at symptom onset was younger among patients with familial history of MG than patients without familial history of MG.

There is no empirical evidence that explains why females tend to have an earlier age of onset of MG. To clarify this point, we have added the following highlighted text to Paragraph 3 of the Discussion:

> "This observation may reflect the different age distribution of myasthenia gravis cases in males versus females observed across all myasthenia gravis cases. The reason for this is unclear, though we note that our previous genome-wide association study of myasthenia gravis indicated that the genetic architecture was different among younger and older age groups.[10]"

7. According to this article, thyroid disease is the most common autoimmune disease concomitant in MG patients and in the families of MG patients. There is no in-depth discussion about this result. I recommend to describe further discussion about the pathologic relationship between thyroid disease and MG. We have commented on this issue in the 4th paragraph of the Discussion, which now reads as follows:

> "Myasthenia gravis is an immunological disorder and, generally speaking,
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> autoimmune diseases are known to have heritable components.[21] Likewise, approximately one-third of our cohort had a personal history and/or a family history of another autoimmune disease, which is much higher than the prevalence of 3-9%, which has been historically reported in the general
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> population.[17] We found that 19% (11/58) of the familial myasthenia gravis cases also had a personal history of autoimmune diseases; this was more than the sporadic myasthenia gravis cases in which only 10% (98/974) had a personal history of autoimmune diseases. Similar to previous reports, we found thyroid disease, rheumatoid arthritis, systemic lupus erythematous, and type 1 diabetes
>
> to be the most commonly identified comorbidities (figure 2C).[18, 21] Interestingly, the frequency of thyroid disease in this cohort (11.4%) is similar to the frequency
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> range of thyroid disease reported in a study by Kiessling et al.[22] The increased prevalence of familial myasthenia gravis and increased prevalence of other autoimmune disorders both suggest that these autoimmune diseases may share a common predisposition that may be genetic in origin. Future studies focusing on specific genes and genomic variants encountered in patients with familial myasthenia gravis offer the promise to more precisely identify any genetic contributions to the disease."

8. The author mentioned the familial MG patients were more likely to have a personal history of autoimmune disease than the sporadic cohort, but there is no numerical data about this. Considering the importance of this result, I recommend the author to show the number of patients with concomitant autoimmune disease in patients with familial history of MG and without familial history of MG, respectively.

We have added the following highlighted sentence to Paragraph 3 of the Discussion to address this issue:

> "We found that 19% (11/58) of the familial myasthenia gravis cases also had a personal history of autoimmune diseases; this was more than the sporadic myasthenia gravis cases in which only 10% (98/974) had a personal history of autoimmune diseases."

Minor comment

1. In table 1, the term of familial or sporadic MG seems inappropriate. The term of 'patients with or without familial history of MG' is more appropriate, because the presence of self-reported familial history of MG is not equivalent to 'familial MG'. To the best of our knowledge, there is no semantic difference between "familial MG" and "patients with family history of myasthenia gravis". Furthermore, we have defined what we mean by familial and sporadic disease in the manuscript.

VERSION 2 – REVIEW

REVIEWER	Jan Damoiseaux Maastricht University Medical Center The Netherlands
REVIEW RETURNED	02-Jun-2020

GENERAL COMMENTS	Thank you for taking my comments in consideration and for the well-structured response to the reviewers; I do not have any further comments.

REVIEWER	Jung-Joon Sung Department of Neurology, Seoul National University Hospital, Seoul, Republic of Korea
REVIEW RETURNED	23-Jun-2020

GENERAL COMMENTS	Thank you for your responses to the comments. There are still a few things I recommend to supplement.

	4. The purpose of this journal is to provide the evidence for a hereditary contribution to myasthenia gravis with epidemiological data. The evidence is supported by objective data like statistical values, no matter how plausible it may seem. So, I suggested to add the p-value, because the prevalence of autoimmunity is one of the most important data in this article. (Refer to this journal; Namba, T. (1971). Familial Myasthenia Gravis. Archives of Neurology, 25(1), 49. doi:10.1001/archneur.1971.00490010059009) 5. I understand that you want to focus on epidemiological analyses for the current study. I didn't mean to newly analyze the genetic variants in your cohort, but if you already had genetic analysis data, I suggested that you could use it as supporting data for epidemiologic results. 7. Your response doesn't include what I commented. I requested to include the in-depth discussion why thyroid disease is the most common autoimmune disease concomitant in MG patients. You need to show your interpretation about the results what you've observed.

VERSION 2 – AUTHOR RESPONSE

RESPONSE TO REVIEWER TWO

4. The purpose of this journal is to provide the evidence for a hereditary contribution to myasthenia gravis with epidemiological data. The evidence is supported by objective data like statistical values, no matter how plausible it may seem. So, I suggested to add the p-value, because the prevalence of autoimmunity is one of the most important data in this article.
(Refer to this journal; Namba, T. (1971). Familial Myasthenia Gravis. Archives of Neurology, 25(1), 49. doi:10.1001/archneur.1971.00490010059009)

Response: While we understand the point that the reviewer is making, the co-authors of our paper have now discussed this issue and do not believe that including a p-value to show that 28.4% is statistically different from ~3-9% is needed in this case. Furthermore, we do not think that the Namba et al. paper actually uses a statistical test that would be appropriate in this context.

5. I understand that you want to focus on epidemiological analyses for the current study. I didn't mean to newly analyze the genetic variants in your cohort, but if you already had genetic analysis data, I suggested that you could use it as supporting data for epidemiologic results.

Response: To address the Reviewer's point, we have now added the following sentence to the Discussion section of the paper:

"Our previous genetic analysis of this cohort showed the heritability of myasthenia gravis to be 25.5%."

Note that this genetics study involving the same is already cited in our manuscript.

7. Your response doesn't include what I commented. I requested to include the in-depth discussion why thyroid disease is the most common autoimmune disease concomitant in MG patients. You need to show your interpretation about the results what you've observed.

Response: The Reviewer asks for an additional explanation about why thyroid disease is the most common autoimmune disease encountered in MG. We have now done so as follows: immediately after the relevant text in paragraph 3 of the Discussion (see yellow-highlighted sentence), we have now added the green-highlighted sentence.

"Similar to previous reports, we found thyroid disease, rheumatoid arthritis, systemic lupus erythematous, and type 1 diabetes to be the most commonly identified comorbidities (figure 2C).[18, 19] Interestingly, the frequency of thyroid disease in this cohort (11.4%) is similar to the frequency range of thyroid disease reported in a study by Kiessling et al.[20] The increased prevalence of familial myasthenia gravis and increased prevalence of other autoimmune disorders both suggest that these autoimmune diseases may share a common predisposition that may be genetic in origin. We speculate that the link between thyroid disease and myasthenia gravis may be due to a common genetic background or an immunological cross-reactivity against epitopes or auto-antigens shared by the thyroid and other tissues relevant to myasthenia gravis.[20,21]".

VERSION 3 – REVIEW

REVIEWER	Jung-Joon Sung Department of Neurology, Seoul National University Hospital, Seoul, Republic of Korea
REVIEW RETURNED	27-Jul-2020

GENERAL COMMENTS	The comments mentioned were well reflected.