Peer Review File

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**Reviewer A**

Comment 1: *The manuscript by Yongxue Lyu et al. reports an INAD case in a 16-month-old male and associates the genetic disorder to the homozygous mutation NM_003560.2: c.1778C>T in PLA2G6 gene. The association is based on the possibility to investigate the proband's healthy parents and brother, which all carried the mutation at the heterozygous state. This is quite convincing, even though the genetic workup identified many other heterozygous mutations that could play a role in determining the clinical phenotype. I suggest the authors analyse the status of the parents and brother for the mutations that are potential cause of AD disorders with a phenotype similar to INAD and discuss them in more details.*

**Reply 1: We thank the reviewer for this good suggestion. We've discussed the clinical symptoms of those AD diseases and the differential diagnoses in detail. In fact, no one in the proband's family showed the clinical phenotypes of those AD diseases. Nonetheless, since we've not thoroughly investigated those AD-related variants in the proband's parents and brother, we can't exclude the possibly that those variants may be de novo in the proband and results in AD diseases in the future. We've also discussed this in the revised article. In addition, we've added more information about the AD/AR variants in Table 1. We hope that the revised version will satisfy the reviewer.**

**Changes in the text: Please see Page 12 & 13, line 202 to 220. Changes in Table 1, Please see Page 18, line 332.**

Comment 2: Minor errors (typos or sentences to be corrected)

*In figure 1, the dead brother is indicated as a black square. This means that he was homozygous for the mutation and affected by INAD. The manuscript lets infer that he was not investigated for INAD nor for the presence of the mutation. The figure should be corrected.*

**Reply 2: We apologize for the mistake. Yes, we correct the Figure and Figure Legend.**

**Changes in the text: Please see Page 17, line 319 and 320. In Figure 1A, Ⅱ-1 is now marked with a question mark. Its meaning is also discussed in the legend.**

Comment 3: 22 genotype–phenotype association is still lacking;72...However, clinical evidence is still lacking for the; 73 INAD phenotype relationships with a majority of PLA2G6 variants. Please explain this concept more extensively.

**Reply 3: We have modified our text as advised and explained the concept "genotype–phenotype association". We thank the reviewer for this suggestion.**

**Changes in the text: Please see Page 2, line 19 to 21. 'genotype–phenotype association is still lacking' has been changed to 'The clinical symptoms of INAD patients display considerable diversity, and many *PLA2G6* variants are still not thoroughly investigated in relation to their associated clinical presentations'. Please see Page 4, line 64 to 66. 'However, clinical evidence is still lacking for the INAD phenotype relationships with a**

**majority of PLA2G6 variants' has been changed to 'However, the association between an individual's genetic makeup (genotype) and the clinical characteristics (phenotype), i.e., genotype-phenotype association, is still lacking for many INAD patients attributed to *PLA2G6* variants.'.**

Comment 4: 42 INAD is an ultra-rare early-onset neurological disorder that caused by PLA2G6 variants.
**Reply 4: Yes, we have modified our text as advised. Thanks for pointing out the error.**
**Changes in the text: Please see Page 3, Line 39. 'that' is deleted.**

Comment 5: 65 ….of life [2, 3]. A Growing body of evidence.
**Reply 5: Yes, we have modified our text as advised. Thanks for pointing out the error.**
**Changes in the text: Please see Page 4, Line 58. 'A Growing body of evidence' has been changed to 'There is growing evidence that'.**

Comment 6: 67 (PLA2G6) gene on chromosome 22q are the predominant causative of INAD.
**Reply 6: Yes, we have modified our text as advised. Thanks for pointing out the error.**
**Changes in the text: Please see Page 4, Line 58 to 59. '(PLA2G6) gene on chromosome 22q are the predominant causative of INAD' has been changed to 'INAD is primarily caused by various loss-of-function mutations in the phospholipase A2 group VI (PLA2G6) gene on chromosome 22q'.**

Comment 7: 91 acquired motor and speech abilities that had been persisted for 4 months.
**Reply 7: Yes, we have modified our text as advised. Thanks for pointing out the error.**
**Changes in the text: Please see Page 5, Line 79. 'been' is deleted.**

Comment 8 & 9: 98 of age; started rolling over around 4 months. 100 "mama" started around 12 months.
**Reply 8 & 9: Yes, we have modified our text as advised. Thanks for pointing out those errors.**
**Changes in the text: Please see Page 5, Line 85 to 87. 'around 4 months' has been changed to 'at four months old'; 'around 12 months' has been changed to 'at twelve months old'.**

Comment 10: 104 ... The motion.
**Reply 10: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 6, Line 91. 'motion' has been changed to 'motor'.**

Comment 11: 110 response (ABR) indicated moderately severe hearing loss (HL) with the left ear 60 dB 111 HL and the right ear 70 dB HL.
**Reply 11: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 6, Line 96 to 97. 'response (ABR) indicated…' has been changed to 'moderately severe hearing loss (HL). His left ear had 60 decibels (dB) HL and the right ear had 70 dB HL'.**

Comment 12: 126... Therefore, we next performed.
**Reply 12: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 7, Line 110. 'next' is deleted.**

Comment 13: 132 within the later ROH region (Supplementary Table 2).
**Reply 13: Yes, we have modified our text as advised. Thanks for pointing out the error.**
**Changes in the text: Please see Page 7, Line 115. 'the later ROH region' has been changed to 'the ROH region of 22q12.2-q13.31'.**

Comment 14: 145 ACMG (spell out) guideline.
**Reply 14: Yes, we have modified our text as advised.**
**Changes in the text: Please see Page 8, Line 125. 'ACMG' has been changed to 'the American College of Medical Genetics and Genomics (ACMG) guideline'.**

Comment 15: 158 with INAD, mainly contributed by a rare inherited
**Reply 15: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 8, Line 136 to 137. 'mainly contributed by a rare inherited' has been changed to 'which was potentially caused by a rare inherited'.**

Comment 16 & 17: 171 life span. Studies speculated that iPLA2-β enzymatic activity alteration caused by the; 172 same PLA2G6 mutation is distinctive from individuals, which…
**Reply 16 & 17: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 9, Line 145 to 149. This sentence has been changed to 'This phenomenon is likely attributed to the fact that INAD-related PLA2G6 variants may result in more severe impairment of iPLA2-β enzymatic activity compared to PLA2G6 variants linked to other PLAN disorders. It is also noteworthy that the same PLA2G6 variant can lead to distinct clinical phenotypes among individuals.'.**

Comment 18: 177 PLA2G6 c.991G>T homozygous variant reportedly was insusceptible to PLAN
**Reply 18: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 9, Line 152 to 153. This sentence has been changed to 'his younger sister (34 years old) with the same homozygous variant displayed no symptoms of PLAN'.**

Comment 19 & 20: 183 reported the PLA2G6 c.1778C>T variant-associated INAD child, who also harbored; 184 PLA2G6 c.1974C>A variant, while clinical information was limited [9].
**Reply** 19 & 20**: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 10, Line 166 to 167. This sentence has been changed to '… reported the co-occurrence of PLA2G6 c.1778C>T variant and PLA2G6 c.1974C>A variant in an INAD patient. Nevertheless, clinical information of this patient was limited.'**

Comment 21: 186 ….. In this study, we detect the PLA2G6
**Reply 21: Yes, we have modified our text as advised. Thanks.**

**Changes in the text: Please see Page 10, Line 170 to 171. 'detect' has been changed to 'detected'; 'describe' has been changed to 'described'.**

Comment 22 & 23: 194 … the early and accurate diagnosis of ultra-rare INAD was challenging, which; 195 poorly relies on a spectrum of unspecific clinical phenotypes.
**Reply 22 & 23: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 10, Line 176. This sentence has been changed to 'the diagnosis of INAD mainly relied on a spectrum of unspecific clinical phenotypes.'**

Comment 24: 203 stage of INAD [2, 17]. In particular, for previous patient with PLA2G6.
**Reply 24: Please see Page 11, Line 183. Yes, we have modified our text as advised. Thanks.**
**Changes in the text: 'In particular, for previous' has been changed to 'In the'.**

Comment 25: 329 carriers have a semisolid mark. (B) The timeline of the proband's main motion
**Reply 25: Yes, we have modified our text as advised. Thanks.**
**Changes in the text: Please see Page 17, Line 316 to 318. 'a semisolid mark' has been changed to 'marked with semisolid color'; 'motion' has been changed to 'motor'.**

**Reviewer B**

The authors report the case of a proband with infantile onset of neurodegeneration and identification of a likely pathogenic homozygous variant of the PLA2G6 gene. The results are convincing, and the methods are sound. I have some remarks for improving the manuscript:

Comment 1: The figure of the pedigree must be revised. Carriers must be marked with a dot, like healthy carriers. The older brother is marked as affected, but it is not certain that he had the same disease and the genetic analysis was not done, so he should be marked with a question mark or a lighter black and discussed in the legend.
**Reply 1: We apologize for the mistake. Yes, we correct the Figure and Figure Legend.**
**Changes in the text: Please see Page 17, Line 319. In Figure 1A, Ⅱ-1 is now marked with a question mark. Its meaning is also discussed in the legend.**

Comment 2: I think there is not much doubt about the causality of the PLA2G6 variant and the proband's phenotype. However, for completeness, I would mention the possibility that some of the identified heterozygous variants could play a role if they occurred de novo in this patient. Clearly, it would be incompatible with the hypothesis that his brother had the same disease, but this is not known.
**Reply 2: Yes, we thank the reviewer for this good suggestion. Please also see the Reply 1 to Reviewer A. In the revised article, we've discussed variants related to AD diseases.**
**Changes in the text: Please see Page 12 & 13, line 202 to 220.**

Comment 3: It would be useful to show at least one MRI T2 ore GRE sequence showing the

absence of iron accumulation in the SN and GP

**Reply 3: Yes, we have provided the brain MRI SWI images in Figure 1C (iii, iv), which show the absence of iron accumulation more clearly.**

**Changes in the text: Please see Page 17, Line 324.**

Comment 4: Line 140: I would replace with INAD unrelated genes

**Reply 3: Yes, we have modified our text as advised. Thanks.**

**Changes in the text: Please see Page 7, Line 121. 'such as PDE10A, ATP13A2, and VPS53,' is deleted.**

**Reviewer C**

I reviewed the manuscript entitled "A Rare Inherited Homozygous Missense Variant in PLA2G6 Influences Susceptibility to Infantile Neuroaxonal Dystrophy: A Case Report." The authors analyzed the gene of a patient with infantile neuroaxonal dystrophy (INAD) and found the pathogenic homozygous missense variant of PLA2G6 (1778C>T), which is the gene responsible for INAD. Although the 1778C>T variant has been suggested as a suspected pathogenic variant in 2016, this study confirmed the variant causes INAD. The relationship between PLA2G6 and neuronal disease has been collected attention but has not been well understood yet. Therefore, this report can be helpful for the research field. I found several points that should be fixed and added. My specific comments are as follows.

Comment 1: The authors argued that the 1778C>T variant might severely impact the enzymatic activity of iPLA2 in lines 179 to 181. However, in this study, this seemed to be an overstatement without any molecular biological experiments. As the authors mentioned, some PLA2G6 variants that maintain catalytic activity cause PLA2G6-related neurodegeneration. Therefore, it would be better to discuss more about the structural position/feature of Pro593Ile. Also, comparing the 1778C>T variant and other variants would be helpful since several variants have been tested their activity and characteristics.

**Reply 1: Yes, we agree that the statement '*PLA2G6* c.1778C>T homozygous variant may severely impact iPLA2-β enzymatic activity' was exaggerated. In fact, this was only a speculation based on: 1. the severity of the proband's clinical presentations; 2. the association between iPLA2-β enzymatic activity and clinical presentations; 3. evolutionary conservation of the mutation site and the features of Pro593Leu. Nevertheless, without directly measuring the iPLA2-β enzymatic activity, no one knows for sure about the effect of *PLA2G6* variants. We thank reviewer for this advice. In addition, we thank the suggestion about discussing more about the structural position/feature of Pro593Leu. In the revised article, we have discussed more about the iPLA2-β catalytic domain and the potential impact of Pro593Leu, instead of the specific position, since study of 593 amino acid in iPLA2-β is limited. Furthermore, in previous version, we have compared the *PLA2G6* c.1778C>T and PLA2G6 c.991G>T homozygous variant in the first paragraph of Discussion. *PLA2G6* c.991G>T homozygous variant results in 70% reduction in iPLA2-β enzymatic activity and patients with this variant**

**developed AREP phenotypes with relatively later onset ages and slower disease progression compared to our patient with *PLA2G6* c.1778C>T. These findings made us speculate that PLA2G6 c.1778C>T homozygous variant may cause more severe impact on iPLA2-β enzymatic activity. We hope those changes/discussion meet your requirements.**
**Changes in the text: Please see Page 9, Line 145 to 153; Page 9&10, Line 154 to 164.**

Comment 2: The authors utilized the software and online tools (e.g. SIFT, PolyPhen2, and ClinPred) to analyze the results, but the references for them are lacking. Please insert adequate references for these software and tools.
**Reply 2: Yes, we now provide the Research Resource Identifiers (RRIDs)/references for those tools according to your kind suggestion and the policy of TP journal. Thank you.**
**Changes in the text: Please see Page 8, Line 135 to 136.**

Comment 3: Please revise all Pro583 to Pro593 in the Molecular Analysis paragraph.
**Reply 3: We apologize for the mistake. Yes, we correct them. Thank you.**
**Changes in the text: Please see Page 8, Line 130 to 134.**

**Reviewer D**

This is a case report of a 16-month-old male with Infantile neuroaxonal dystrophy (INAD) carrying a rare homozygous missense variant, Pro593Leu in PLA2G6. Homozygosity mapping, whole exome sequencing, segregation analysis, predicted pathogenicity of the variant by different pathogenicity prediction tools, the occurrence at amino acid positions that are conserved across species, and the predicted altered 3D structure, all these arguments are in favor of the contribution of this pathogenic PLA2G6 variant to INAD, at least for this patient. The manuscript is well documented, and well written. However, I have a few minor comments:
Comment 1: Since more than 100 mutations in PLA2G6 were found to be associated with either INAD/atypical neuroaxonal dystrophies (ANAD) or atypical parkinsonism (PARK14), the authors should moderate/re-edit their statement in the Abstract and Discussion "We report the FIRST INAD child with a rare PLA2G6 C.1778c>t homozygous missense variant with detailed clinical characteristics".
**Reply 1: We apologize for the misunderstanding. Yes, we correct the statement as advised. Thank you.**
**Changes in the text: Please see Page 2, Line 29 and 35; Page 13, Line 230.**

Comment 2: Some sentences deserve to be re-edited: 1) page 6, "However, an individual with the PLA2G6 c.991G>T homozygous variant reportedly was insusceptible to PLAN." Moreover, the reference 15 seems incorrect and can't be found in PubMed; 2) page 7, "Innovative therapies to correct iPLA2-b enzyme activity, such as enzyme replacement therapy and gene therapy, are in their infancy."
**Reply 2: We apologize for those mistakes. Yes, we have modified our text as advised. Thanks.**
**Changes in the text: 1) Please see Page 9, Line 152 and 153. This sentence has been**

**changed to ‘i his younger sister (34 years old) with the same homozygous variant displayed no symptoms of PLAN’. 2) R**eference 15 is deleted. **3) Please see Page 12, Line 196 and 197. This sentence has been changed to ‘Enzyme replacement therapy and gene therapy, as innovative approaches to rectify iPLA2-b enzyme activity, are still in the early stages of development.’.**

Comment 3: Page 4, Fig. 1C was not cited in the main text or there might be a typo error since Fig. 1B was cited twice.

**Reply 3: We apologize for the mistake. Yes, we have modified our text as advised. Thanks for pointing out the error.**

**Changes in the text: Please see Page 6, Line 95.**