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IMPACT FACTOR 4.047

Peer Review File

Article information: https://dx.doi.org/10.21037/tp-22-220

First round of peer review

This study reported a case of Shashi-Pena Syndrome caused by a pathogenic variant in ASXL2, and summarized the clinical characteristics of 11 probands, including 10 patients reported previously. Authors made a good job of diagnosing the SHAPNS in the neonatal period by trio-WES, which is the earliest age of diagnosis so far. This provides and retains more clinical details early around the neonatal period.

However, there are several major issues need to be addressed.

Comment 1: As mentioned in title, authors claimed that this report expanded the phenotype of SHAPNS. Please highlight the new findings and differences of this case, such as the first application of Octreotide in SHAPNS, in Abstract and Discussion with a separate paragraph. Doing so will reflect innovation and uniqueness of this paper.

Reply 1: Thank you for your helpful suggestion. We have modified our text as advised. We have highlighted the new findings and differences of this case in Abstract and Strengths Limitations (see page 3, lines 65-68; page 18, lines 372-380)

Comment 2: Considering the case of SHAPNS is extremely rare, detailed descriptions of key clinical findings is imperative. Although results of a series of investigations were given in Line 127-132, please interpret the meaningful results exhaustively, instead of only “showed evidence of …”. And we recommend to add more information about the size of “A small cerebellum” in Fig.2, and to specify in the legend.

Reply 2: Thank you for your helpful suggestion. We have interpreted the meaningful results exhaustively, added more information about the size of “A small cerebellum” in Fig.2, and to specify in the legend. (page 7, lines 135-139; page 8, lines 144-148; page 24, line 493)

Comment 3: Taking into account the relevant reports and information are very limited, please give the reason for the exclusive criterion in Line 211-212, “For patients from one family, only the proband was included in this review”.

Reply 3: Thank you for your suggestion. The medical records of the other patients from the proband’s family were incomplete, thus only the proband was included. We described this reason in the text. “For patients from one family, the medical records of the other patients from the proband’s family were incomplete, thus only the proband of this family was included in this review” (page 11, lines 217-219).

Comment 4: As Sanger sequencing is the gold standard for DNA sequencing, authors wrote "Variants were confirmed in the proband and the parents, if available, using Sanger sequencing" in Line 166-167. But the corresponding results, which need to be clarified, are not available in Results.

Reply 4: Thank you for your suggestion. We added Figure 4 of Sanger sequencing analysis and results in Results part. (page 9, line 174; page 24, line 497)

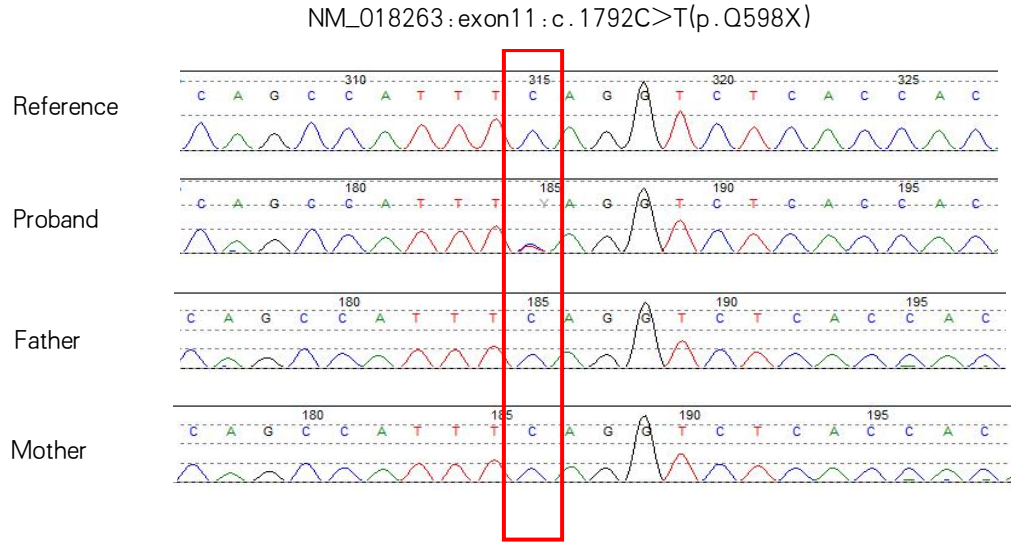

Figure 4. Analysis and results of sanger sequencing of the family.

Comment 5: As for the analysis of pathogenicity, please present the interpretation of the variant classified as PVS1_Strong level, rather than quote the "Criteria for LoF disease mechanism" directly in Line 198-201.

Reply 5: Thank you for your suggestion. We added a flow chart to illustrate the filtering strategy of variants is given (Figure 5 Filtering strategy of variant). (page 10, lines 197-198; page 24, line 498)

We added the description of PVS1[Clinical validity classification of gene is definitive and 13 pathogenic null variants were reported in ClinVar for this gene, across 2 different exons, of which 5 variants in this exon; And this nonsense variant predicted to undergo nonsense-mediated decay and exon is present in biologically-relevant transcript] (page 10-11, lines 205-208).

Comment 6: Considering this is a case report, we have some suggestions according to CARE checklist below.

(1) Title: The title should include the words "case report". And the repetition of gene name "ASXL2" is unnecessary. So we suggest change the title to "A newborn with a pathogenic variant in ASXL2 expanding the phenotype of SHAPNS: a case report and literature review".

Reply: Thank you for your helpful suggestion. We have modified our title to "A newborn

with a pathogenic variant in ASXL2 expanding the phenotype of SHAPNS: a case report and literature review" as advised. (page 1, lines 3-4)

(2) Key words: Likewise, key words should include the words "case report", and the number is limited within 3-5.
Reply: Thank you for your suggestion. We have added the words "case report" and removed "genetics" as advised.(page 4, lines 73-74)

(3) Patient Information: Relevant family history, such as the growth and development of family members, should be specified in Case Presentation.
Reply: Thank you for your suggestion. We have added relevant family history as advised. We added "Both mother and father were in good medical conditions. Antenatal care was unremarkable. There was no family history of inheritable disorders" (page 7 , lines 124-125)

(4) Timeline: We suggest a timeline to present relevant events, symptoms and treatment of the patient from birth to four-month-old. Readers will be able to acquire key points of the patient's medical history from this timeline. Meanwhile, authors need to present the case chronologically, better with specific date and time (like Sep. 14th, 2021).
Reply: Thank you for your suggestion. We added a timeline to present relevant events (figure 3) symptoms and treatment. Meanwhile, we modified the description to present the case chronologically with specific date and time. (page 5, line 116; page 6, line 120; page 8, lines150-160)

- **Sep.9th, 2021** Born with feeding difficulty, recurrent hypoglycemia and cyanosis
- **Sep.10th,2021** Admission and started on intravenous dextrose
- **Sep.11th,2021** Partial enteral feeding together with intravenous dextrose
- **Sep.18th,2021** Full enteral feeding without cyanosis but with recurrent hypoglycemia
- **Sep.19th,2021** Partial enteral feeding together with intravenous dextrose
- **Sep.27th,2021** Continuous glucagon infusion (12ug/kg/hour)
- **Oct.3rd,2021** Continuous glucagon infusion (10ug/kg/hour)
- **Oct.4th,2021** Continuous glucagon infusion (9.6ug/kg/hour)
- **Oct.5th,2021** Continuous glucagon infusion (8.4ug/kg/hour)
- **Oct.6th-12th,2021** Continuous glucagon infusion (7.2ug/kg/hour)
- **Oct.13th,2021** Subcutaneous octreotide injection (0.002mg/kg every 8 hours)
- **Oct.15th,2021** Subcutaneous octreotide injection (0.004mg/kg every 8 hours)
- **Oct.19th,2021** Full enteral feeding without intravenous dextrose
- **Oct.23rd,2021** Discharge home on subcutaneous octreotide injection

Figure 3. Timeline of the relevant events and specific treatment.

(5) Assessment&Intervention: Clinical indices and therapeutic intervention should be showed specifically, such as dosage, strength, duration, etc. Several places need to be refined below.
Line 127: “continuous intravenous fluid infusion”;
Line 143: “intravenous dextrose and glucagon”;
Line 146-147: “inappropriately high levels of insulin”;
Line 147&150-151: “octreotide injection”.
Reply: Thank you for your suggestion. Clinical indices and therapeutic intervention had been showed specifically, such as dosage, strength, duration, etc. and we had added some specific information as advised. (page 7, lines132-133; page 8, lines144-148, lines 150-155, lines 157-160)

(6) Strengths&Limitations: Given that SHAPNS is an extremely rare hereditary disease, the limit number of patients does not work as a major limitation. Please provide deeper insights and substantial viewpoints about this study, such as the study design, future directions.
Reply: Thank you for your suggestion. We have modified our text as advised. (page 18, lines 372-380; page 19, lines383-385)

(7) A statement should be included at the end of the Introduction&Footnote. Specific details would be found in Guidelines for Authors of Translational Pediatrics (https://tp.amegroups.com/pages/view/guidelines-for-authors#content-2-3-1).
Reply: Thank you for your suggestion. We added “We present the following case in accordance with the CARE reporting checklist.” at the end of the Introduction (page 6, lines 106-107)

Other concerns:
Comment 1: We suggest removing “OMIM #617190” from Abstract. Just keeping it in Introduction is fine.
Reply: Thank you for your suggestion. We have deleted “OMIM #617190” from the Abstract as suggested.

Comment 2: A flow chart to illustrate the filtering strategy of variants described in “Molecular studies” is suggested. And based on this, authors also need to modify this section to present more succinctly.
Reply: Thank you for your suggestion. We have added a flow chart to illustrate the filtering strategy of variants (Figure 5) and modify this section as suggested. (page 10, line 199; page 24, line 506)

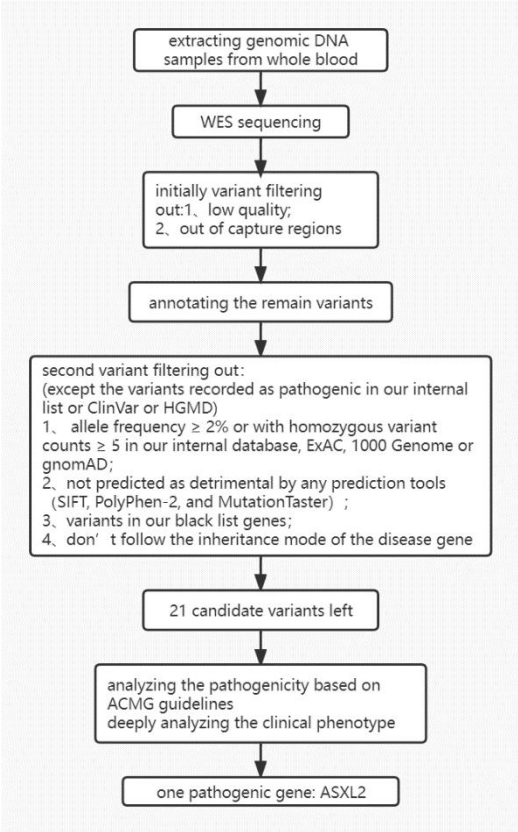

Figure 5. Filtering strategy of variant.

Comment 3: Please modify Tables for a clearer appearance, especially Table 1, such as presenting in the normative three-part table form, using "N/A" instead of "/" or ".", and labelling the unit only in the first column, etc. There is a example for reference (Table 1 in https://tp.amegroups.com/article/view/64402/html).

Reply: Thank you for your suggestion. We have modified table 1 as suggested. We spliced table 1 into 2 tables (table 1 and table 2), and used "N/A" instead of "/" or "." (page 12, line 241; page 13, line 264; Supplemental Table S2)

Table 1 Clinical synopsis of 11 patients with *ASXL2*-related SHAPNS

Classification	Number (Percentage)
Facial and skin features	
Hypertelorism	11 (100)
Broad nasal tip	10 (90.9)
Arched eyebrows	9 (81.8)
V-shaped glabellar nevus flammeus on the forehead	9 (81.8)
Low-set ears	8 (72.7)
Posteriorly rotated ears	7 (63.6)

Proptosis	6 (54.5)
Broad forehead	5 (45.5)
Hirsutism	5 (45.5)
Retrognathia	4 (36.4)
Ptosis	4 (36.4)
Capillary malformations	3 (27.3)
Long face	3 (27.3)
Flat face	1 (9.1)
Other abnormal eye findings	4 (36.3)
Skeletal and/or extremity manifestations	
Deep palmar creases	6 (54.5)
Overlapping toes	3 (27.3)
Brain MRI findings	
Normal	2 (18.2)
Enlarged extra-axial spaces	2 (18.2)
White matter volume loss	2 (18.2)
Ventriculomegaly	2 (18.2)
Small cerebellum	1 (9.1)
Choroid plexus papilloma	1 (9.1)
Cardiovascular findings	
Normal	1 (9.1)
Atrial septal defect	4 (36.4)
Patent foramen ovale	3 (27.3)
Patent ductus arteriosus *	1 (9.1)
Mitral and tricuspid regurgitation	1 (9.1)
Tricuspid insufficiency *	1 (9.1)
Pericardial effusion*	1 (9.1)
Ventricular ectopy bradycardia	1 (9.1)

*One patient had evidence of patent ductus arteriosus, patent foramen ovale, mild tricuspid insufficiency, mild pericardial effusion and pulmonary hypertension at 2 months

of age and died of heart disease at 16 months of age.

Table 2 Prognosis of 11 patients with *ASXL2*-related SHAPNS at follow-up

Prognosis	Number (Percentage)
Feeding difficulties	10 (90.9)
Developmental delay	10 (90.9)
Macrocephaly at follow-ups	8 (72.7)
Skeletal and/or extremity manifestations	8 (72.7)
Hypotonia	8 (72.7)
Seizure activities	6 (54.5)
Hypoglycemia	6 (54.5)
Appendicular hypertonia	2 (18.2)
Growth retardation	2 (18.2)

Comment 4: Table 1 and the summary of previous reports in Results present the same information. We recommend to simplify the Table avoiding many explanatory words, and conclude the key topics in the text.

Reply: Thank you for your suggestion. We have modified our text and table 1 as suggested.

Comment 5: The No.4 reference only reported one male case, which is inconsistent with the information of Individual 7 in Table S1. Please explain it and its footnote "& Only the female proband of the family was included in the review".

Reply: Thank you for your comment. **In the supplemental table S2, the information of individual 4 [3] and individual 7 [4]&** came from reference [3](Alqaisi D, Hassona Y. Oral findings and healthcare management in Shashi-Pena syndrome. Spec Care Dentist 2021;1-5.) and reference [4] (Wang Y, Tan J, Wang Y, et al. Diagnosis of Shashi-Pena Syndrome Caused by Chromosomal Rearrangement Using Nanopore Sequencing. Neurol Genet 2021;7(6):e635.), respectively.

The information of gender was consistent of individual 7 (female) with reference 4 in Table S2.

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Supplemental Table S2

Characteristics	Summary	Individual 1 (this paper)	Individual 2 [1]#	Individual 3 [2]	Individual 4 [3]	Individual 5 [2]	Individual 6 [2]	Individual 7 [4]&
Demographic data								
Age at diagnosis	4.6 years (21day-31 years)*	21 days	6 months	10 months	3 years	4 years 1 month	4 years 7 months	6 years
ASXL2 variants	8 had de novo truncating mutations	c.1792C>T, p.Gln598*	c.2485C>T, p.Gln829*	c.2081dupG, p.Gly696Argfs*11	c.1217dup, p.Glu407*	c.2472delC, p.Ser825Valfs*16	c.1225_1228delCCAA, p.Pro409Asnfs*13	t(2;11)(p23;q23)
Sex	Male 72.7% (8/11)**	Male	Male	Male	Male	Male	Female	Female

This patient was reported to have minor ear abnormalities and congenital foot deformity but without details. He died of heart disease at 16 months of age.A65:M70

& The medical records of the other patients from the proband's family were incomplete, thus only the female proband of this family was included in this review.

* median (range)

** percentage (case/total number of patients)

References
[1] Jiao Z, Zhao X, Wang Y, et al. A de novo and novel nonsense variants in ASXL2 gene is associated with Shashi-Pena syndrome. Eur J Med Genet 2022;65(4):104454.
[2] Shashi V, Pena LDM, Kim K, et al. De Novo Truncating Variants in ASXL2 Are Associated with a Unique and Recognizable Clinical Phenotype. Am J Hum Genet 2017;100(1):179.
[3] Alqaisi D, Hassona Y. Oral findings and healthcare management in Shashi-Pena syndrome. Spec Care Dentist. 2021;1-5.
[4] Wang Y, Tan J, Wang Y,et al. Diagnosis of Shashi-Pena Syndrome Caused by Chromosomal Rearrangement Using Nanopore Sequencing. Neurol Genet 2021;7(6):e635.
[5] Cuddapah VA, Dubbs HA, Adang L, et al. Understanding the phenotypic spectrum of ASXL-related disease: Ten cases and a review of the literature. Am J Med Genet A 2021;185(6):1700-11.

Comment 6: The sentence "For variant calling, GATK best practice (V.3.2) was employed for single-nucleotide variants 165 (SNVs)/small indels" appeared twice in Line 165&171, respectively. Please recheck the full text to avoid this.

Reply: Thank you for your suggestion. We have modified our text as advised.(L198-199)

Second round of peer review

The authors have made great efforts in revising the manuscript. Only one minor issue left. In the "Strengths & Limitations" section, lines 373-381, there is no discussion of LIMITATIONS. All discussed are the strengths.

Response: Thank you for your suggestion. We have added limitation "So far, the long-term prognosis of this patient was lacked" under this part. (page 18, line 379)