DOI: XXX

CASE REPORT

A novel *de novo* splice-site variant in *RPS26* identified by exome sequencing in a patient with suspected Diamond-Blackfan Anemia

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ABSTRACT

Background: Diamond-Blackfan anemia (DBA) is a rare inherited bone marrow failure syndrome characterized by pure red cell aplasia and is commonly caused by variants in ribosomal protein genes. Variants in *RPS26* are relatively uncommon, and splice-site changes in this gene are still underreported.

Case Presentation: We describe a 17-month-old female child who presented with severe anemia with clinical features suggestive of Diamond-Blackfan anemia. Whole-exome sequencing (WES) identified a novel heterozygous *de novo* canonical splice-site variant, NM_001029.5:c.182-1G>A, in the *RPS26* gene. The variant was absent from population databases, including gnomAD, and was also confirmed by Sanger sequencing. *In silico* analysis using SpliceAI predicted disruption of the canonical splice acceptor site along with activation of a cryptic splice site, suggesting a deleterious effect on normal splicing. However, RNA studies are needed to confirm the exact splicing outcome and to better understand how this variant affects *RPS26* function. Based on ACMG/AMP guidelines, the variant was classified as likely pathogenic.

Conclusion: This case contributes to the known mutational spectrum of *RPS26* and highlights the value of exome sequencing in the molecular evaluation of patients with suspected DBA.

Keywords: Diamond-Blackfan anemia, *RPS26*, splice-site variant, Whole Exome sequencing.

Introduction

Diamond-Blackfan anemia (DBA; OMIM: 613309) is a congenital bone marrow failure disorder characterized by pure red cell aplasia (PRCA), macrocytic anemia, and reticulocytopenia, typically presenting within the first year of life [1]. Approximately 30%-50% of affected individuals exhibit congenital anomalies, including craniofacial, upper limb, cardiac, and urogenital malformations. The clinical presentation is variable, even among individuals carrying the same genetic variant [2,3].

DBA is primarily caused by heterozygous variants in genes encoding ribosomal proteins (RPs), which play essential roles in ribosome biogenesis and protein synthesis. To date, pathogenic variants have been identified in multiple RP genes, including *RPS19*, *RPL5*, *RPS10*, *RPL11*, *RPL35A*, *RPL26*, *RPS24*, *RPS17*, *RPS7*, *GATA1*, and *RPS26* [4]. Despite advances in sequencing technologies, 40%-45% of patients remain without a molecular diagnosis [5].

The *RPS26* gene, located on chromosome 12, encodes a component of the 40S ribosomal subunit and is involved in ribosome biogenesis and regulation of its own pre-mRNA splicing [6,7]. Pathogenic variants in *RPS26* have been identified in a subset of patients with DBA [5]. Most reported variants include start-loss, nonsense, and frameshift mutations, while splice-site variants are less frequently described. However, the functional consequences of splice-site variants in *RPS26* remain incompletely understood.

Given the established role of *RPS26* in ribosome biogenesis, disruption of normal splicing is expected to result in haploinsufficiency, which is consistent with the pathogenic mechanism underlying DBA. Here, we report a 17-month-old female child with clinical features suggestive of DBA, in whom whole-exome sequencing identified a novel de novo canonical splice-site variant in *RPS26*. This finding expands the known mutational

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Received: 09 May 2026 |**Accepted:** 26 July 2026

spectrum of *RPS26* and supports the utility of molecular evaluation of patients with suspected DBA.

Methods

Ethics and consent

The study was approved by the Ethical Review Committee (ERC) of Dr. Lal Pathlabs. Written informed consent was obtained from the patient's parents for participation and publication of clinical and genetic data.

Case presentation

The proband is a 17-month-old female child who presented with severe anemia and clinical features reported as consistent with pure red cell aplasia. There was no significant family history, and no congenital anomalies were observed. Detailed hematological parameters, including hemoglobin level, MCV, reticulocyte count, white blood cell and platelet counts, fetal hemoglobin, erythrocyte adenosine deaminase activity, and bone marrow findings, were not available for retrospective review. Transfusion history, steroid treatment and response, and the exact age at onset of anemia were not documented in the available records. The clinical diagnosis of DBA was based on the referring clinician's assessment of severe anemia with features suggestive of pure red cell aplasia, rather than on comprehensive diagnostic evaluation. These limitations preclude a definitive diagnosis of DBA on clinical grounds alone. Consequently, the diagnosis of DBA remains suggestive in this case.

DNA extraction and quality assessment

Genomic DNA was extracted from peripheral blood using the Qiagen DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA quality and quantity were assessed using standard laboratory methods, a NanoDrop 2000 spectrophotometer followed by more accurate quantification using a Qubit 3.0 fluorometer.

Library preparation and WES

Exome library preparation was performed using the Ion AmpliSeq™ Exome RDY Panel (Thermo Fisher Scientific) with approximately 100 ng of input DNA. Sequencing was performed on the Ion Proton platform (Thermo Fisher Scientific).

Data processing and variant calling

Reads were aligned to the human reference genome GRCh37/hg19. Variant calling and annotation were performed using Torrent Variant Caller and Ion Reporter software (Thermo Fisher Scientific), respectively. Variant filtering and prioritization were performed using a phenotype-driven approach, as previously described, with minor modifications [8,9]. Visualization of sequencing reads was performed using the Integrative Genomics Viewer (IGV). Variant classification was carried out according to the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines [10].

Sanger sequencing validation

The candidate variant identified by WES was validated by Sanger sequencing. PCR amplification was performed using the following primers: Forward: 5′-TGTGCTCAGGTATTGGGCTG-3′ and Reverse: 5′-TCACCGCAGGTCTAAATCGG-3′. PCR products were analyzed on a 1% agarose gel, purified, and sequenced using an ABI 3500Dx Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific). Segregation analysis was performed in both parents.

Results

Genetic findings

WES identified a heterozygous canonical splice-site variant, NM_001029.5:c.182-1G>A, in the *RPS26* gene. The *RPS26* gene contains four exons, and the c.182-1G>A variant is located at the canonical splice acceptor site of intron 2, immediately upstream of exon 3. This variant is absent from the population database, gnomAD (v4.1.1), and has not been previously reported in the literature or other databases (HGMD, ClinVar, and LOVD). *In silico* splicing analysis using SpliceAI [11] predicted complete loss of the canonical splice acceptor site at the intron 2/exon 3 boundary (Δ score = 1.0), predicted to cause skipping of exon 3. Additionally, activation of two cryptic splice acceptor sites within intron 2, located 18 bp upstream (Δ score = 0.96) and 62 bp upstream (Δ score = 0.28) from the canonical splice acceptor site, was also predicted. These predictions were visualized using MobiDetails (Figure 1). Although the exact splicing outcome cannot be determined without RNA validation, all predicted outcomes are expected to disrupt normal *RPS26* function. Exon 3 skipping or utilization of either cryptic splice acceptor site is predicted to alter the reading frame and may introduce a premature termination codon, suggesting a deleterious effect on normal splicing.

Sanger sequencing confirmed the presence of the c.182-1G>A variant in the proband. Segregation analysis revealed that the variant was absent in both parents, consistent with a *de novo* occurrence (Figure 2). These findings support the disease-causing role of the variant in the patient's phenotype. Based on ACMG/AMP guidelines, the variant was classified as likely pathogenic using the criteria summarized in Table 1.

Discussion

DBA is a ribosomopathy characterized by defective ribosome biogenesis and impaired erythropoiesis. Pathogenic variants in ribosomal protein genes disrupt ribosome assembly, resulting in nucleolar stress, activation of p53-dependent pathways, and impaired survival of erythroid progenitor cells. Variants in *RPS26* have been established as a cause of DBA, although splice-site variants remain relatively uncommon compared to start-loss, nonsense, and frameshift variants [5]. In the present study, we identified a novel de novo canonical

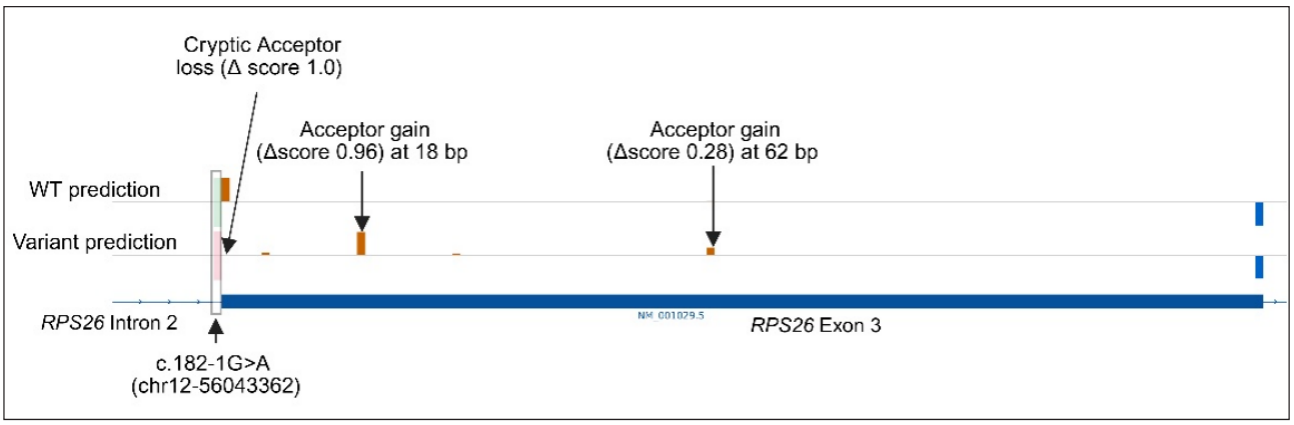

Figure 1. *SpliceAI prediction of the RPS26:c.182-1G>A variant. SpliceAI analysis predicts complete loss of the canonical splice acceptor site (Δ score = 1.0) and activation of nearby cryptic splice acceptor sites located 18 bp upstream (Δ score = 0.96) and 62 bp upstream (Δ score = 0.28), indicating a likely deleterious effect on normal splicing. Visualization was performed using MobiDetails.*

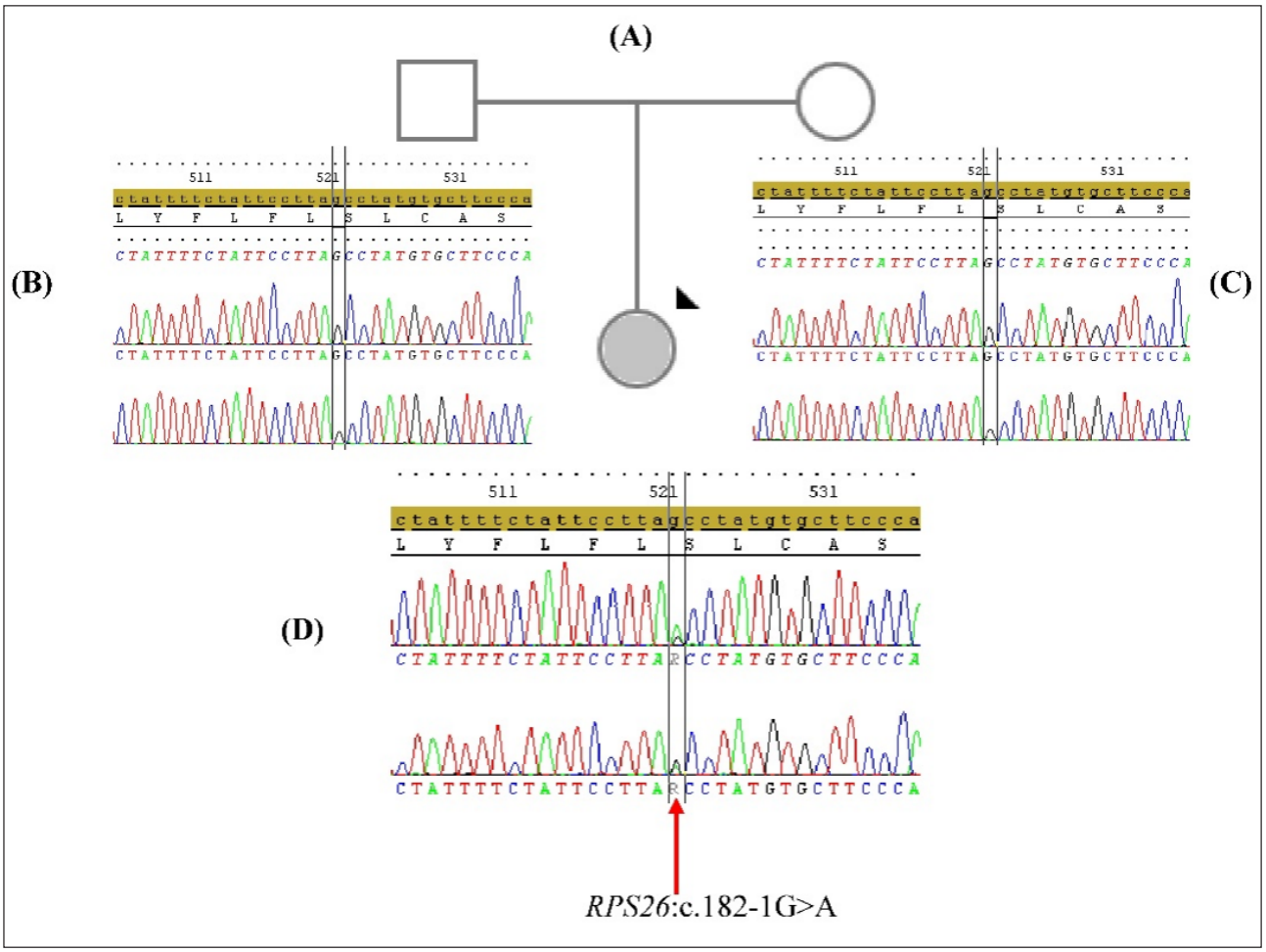

Figure 2. *Family pedigree and Sanger sequencing chromatograms for the RPS26:c.182-1G>A variant. (A) Family pedigree showing the proband (arrow) as the only affected individual. (B-C) Sanger sequencing chromatograms from the unaffected father (B) and mother (C) showing absence of the variant. (D) Sanger sequencing chromatogram from the proband showing the heterozygous c.182-1G>A variant.*

splice-site variant, NM_001029.5:c.182-1G>A, in a patient with clinical features suggestive of DBA.

In silico analyses strongly support disruption of normal splicing by this variant. SpliceAI predicted complete loss of the canonical splice acceptor site together with activation of nearby cryptic splice acceptor sites, suggesting abnormal transcript processing. These predicted outcomes are expected to alter the reading frame and may introduce a premature termination codon, consistent with a loss-of-function mechanism. However,

Table 1. *ACMG criteria applied to RPS26:c.182-1G>A according to ACMG guidelines***.**

Evidence code	Supporting evidence	Points
PVS1_Moderate	Canonical splice acceptor variant, NM_001029.5:c.182-1G>A, located at the intron 2/exon 3 boundary of *RPS26*. SpliceAI predicts complete loss of the canonical acceptor site (Δ = 1.0) with activation of cryptic acceptor sites at 18 bp (Δ = 0.96) and 62 bp (Δ = 0.28) upstream. Loss of function is an established disease mechanism for *RPS26*-associated DBA. However, RNA analysis was not performed, and the exact transcript consequence remains uncertain; therefore, PVS1 was conservatively applied at Moderate strength according to ClinGen SVI recommendations [12].	2 points
PS2_Strong	*De novo* occurrence in the proband, with absence of the variant in both parents.	4 points
PM2_Supporting	Variant absent from population database, gnomAD v4.1.1.	1 point
Final Classification	Based on the combined ACMG/AMP evidence criteria (PVS1_Moderate + PS2_Strong + PM2_Supporting), the variant was classified as Likely Pathogenic according to ACMG/AMP guidelines.	7 points

RNA studies were not performed, and the precise transcript consequence remains uncertain. According to ClinGen SVI recommendations [12], PVS1 was therefore conservatively applied at Moderate strength because experimental RNA evidence was unavailable.

The NM_001029.5 transcript is the principal protein-coding transcript of *RPS26* and encodes a component of the 40S ribosomal subunit. *RPS26* is ubiquitously expressed and has an established role in erythropoiesis. Functional studies have demonstrated that *RPS26* deficiency impairs erythroid differentiation, globin synthesis, and hemoglobin production in HUDEP-1 cells [13]. In addition, lymphoblastoid cells derived from patients with *RPS26* variants show defective pre-rRNA processing and impaired 40S ribosomal subunit biogenesis [5]. The pathogenic mechanism of *RPS26*-associated DBA is consistent with haploinsufficiency, and the reported mutation spectrum predominantly includes loss-of-function variants such as start-loss, nonsense, frameshift, and splice-site changes [5]. The high intolerance of *RPS26* to heterozygous loss-of-function variation in population databases further supports this mechanism.

The *de novo* occurrence of the variant and its absence from population and disease databases, including gnomAD, ClinVar, HGMD, and LOVD, further support its clinical significance. Based on ACMG/AMP criteria, the variant was classified as likely pathogenic.

The clinical presentation of our patient is compatible with previously reported *RPS26*-related DBA cases. Although congenital anomalies are common in DBA overall, they are not universally present in *RPS26*-associated disease, and isolated anemia without physical malformations has been described previously [14,15]. The patient's presentation at 17 months of age is also within the recognized clinical spectrum of DBA [1]. Nevertheless, we acknowledge that the available phenotype data are limited. Detailed hematological parameters, bone marrow findings, treatment history, and follow-up information were unavailable for retrospective review, which limits definitive clinical confirmation of DBA in this case.

Several alternative diagnoses should also be considered in a child presenting with severe anemia and suspected pure red cell aplasia. These include transient erythroblastopenia of childhood, nutritional anemia, hemolytic anemia, congenital infections such as parvovirus B19, acquired red cell aplasia, mitochondrial disorders including Pearson syndrome, and other inherited bone marrow failure syndromes. Because complete clinical records were unavailable, these conditions could not be formally excluded. However, the identification of a likely pathogenic de novo variant in *RPS26* provides strong molecular evidence supporting DBA as the underlying diagnosis.

Overall, this report expands the mutational spectrum of *RPS26* and highlights the value of exome sequencing in the evaluation of genetically heterogeneous disorders such as DBA. At the same time, the study emphasizes the importance of integrating genomic findings with detailed clinical and functional validation. Further RNA-based and functional studies will be necessary to confirm the precise impact of the c.182-1G>A variant on *RPS26* splicing and function.

Conclusion

We report a novel *de novo* splice-site variant in *RPS26* in a patient with suspected DBA. This finding broadens the spectrum of pathogenic *RPS26* variants and underscores the utility of exome sequencing in the molecular evaluation of DBA.

List of Abbreviations

ACMG	American College of Medical Genetics and Genomics
AMP	Association for Molecular Pathology
ClinGen	Clinical Genome Resource
DBA	Diamond-Blackfan anemia
DNA	Deoxyribonucleic acid
ERC	Ethical Review Committee
gnomAD	Genome Aggregation Database
HGMD	Human Gene Mutation Database
IGV	Integrative genomics viewer
LOVD	Leiden open variation database
OMIM	Online Mendelian Inheritance in Man
PRCA	Pure red cell aplasia
SVI	Sequence variant interpretation
WES	Whole-exome sequencing

Acknowledgments

The authors are grateful to the patient and her family for their participation. We thank the management of Dr. Lal Pathlabs for providing the necessary facilities.

Authors' contribution

DP and AT conceived and designed the study. DP performed data analysis. DP drafted the manuscript. AT supervised the study. All authors reviewed and approved the final manuscript.

Consent for publication

Due consent was obtained from the guardians of the patient to publish this case report.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this case report.

Funding

The authors declare that this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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References

1. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Diagnosing and treating Diamond-Blackfan anemia: results of an international clinical consensus conference. Br J Haematol. 2008;142(6):859–76. https://doi.org/10.1111/j.1365-2141.2008.07269.x
2. Ball SE, Mcguckin CP, Jenkins G, Gordon-Smith EC. Diamond-Blackfan anaemia in the U.K.: analysis of 80 cases from a 20-year birth cohort. Br J Haematol. 1996;94(4):645–53. https://doi.org/10.1046/j.1365-2141.1996.d01-1839.x
3. Lipton JM, Atsidaftos E, Zyskind I, Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond-Blackfan anemia: an update from the Diamond-Blackfan Anemia Registry. Pediatr Blood Cancer. 2006;46(5):558–64. https://doi.org/10.1002/pbc.20642
4. Boria I, Garelli E, Gazda HT, Aspesi A, Quarello P, Pavesi E, et al. The ribosomal basis of Diamond-Blackfan anemia: mutation and database update. Hum Mutat. 2010;31(12):1269–79. https://doi.org/10.1002/humu.21383
5. Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Clinton C, et al. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2010;86(2):222–8. https://doi.org/10.1016/j.ajhg.2009.12.015
6. Ivanov AV, Malygin AA, Karpova GG. Human ribosomal protein S26 suppresses the splicing of its pre-mRNA. Biochim Biophys Acta. 2005;1727(2):134–40. https://doi.org/10.1016/j.bbaexp.2004.12.011
7. Min EE, Roy B, Amrani N, He F, Jacobson A. Yeast Upf1 CH domain interacts with RPS26 of the 40S ribosomal subunit. RNA. 2013;19(8):1105–15. https://doi.org/10.1261/rna.039396.113
8. Panwar D, Lal V, Thatai A. Identification of a novel c.3080delC JAG1 gene mutation associated with Alagille syndrome: whole exome sequencing. JMIR Bioinf Biotechnol. 2022;3(1):e33946. https://doi.org/10.2196/33946
9. Panwar D, Singh KG, Mathur S, Prasad B, Joshi A, Lal V, et al. Heterozygous missense variant in the TTN gene causing tibial muscular dystrophy. Egypt J Med Hum Genet. 2022;23:65. https://doi.org/10.1186/s43042-022-00284-y
10. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24. https://doi.org/10.1038/gim.2015.30
11. Jaganathan K, Kyriazopoulou Panagiotopoulou S, Mcrae JF, Darbandi SF, Knowles D, Li YI, et al. Predicting splicing from primary sequence with deep learning. Cell. 2019;176(3):535–48. https://doi.org/10.1016/j.cell.2018.12.015
12. Walker LC, Hoya M, Wiggins GAR, Lindy A, Vincent LM, Parsons MT, et al. Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing: recommendations from the ClinGen SVI Splicing Subgroup. Am J Hum Genet. 2023;110(7):1046–67. https://doi.org/10.1016/j.ajhg.2023.06.002
13. Piantanida N, La Vecchia M, Sculco M, Talmon M, Palattella G, Kurita R, et al. Deficiency of ribosomal protein S26, which is mutated in a subset of patients with Diamond Blackfan anemia, impairs erythroid differentiation. Front Genet. 2022;13:1045236. https://doi.org/10.3389/fgene.2022.1045236
14. Li J, Su Y, Chen L, Lin Y, Ru K. Identification of novel mutations in patients with Diamond-Blackfan anemia and literature review of RPS10 and RPS26 mutations. Int J Lab Hematol. 2023;45(5):766–73. https://doi.org/10.1111/ijlh.14126
15. Shi X, Huang X, Zhang Y, Cui X. Identification of a novel RPS26 nonsense mutation in a Chinese Diamond-Blackfan Anemia patient. BMC Med Genet. 2019;20(1):120. https://doi.org/10.1186/s12881-019-0848-1