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A Novel de novo Frameshift Mutation in the *BCL11A* Gene in a Patient with Intellectual Disability Syndrome and Epilepsy

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BCL11A · Dias-Logan syndrome · Epilepsy · Novel mutation · Trio whole-exome sequencing

Abstract
Intellectual disability syndrome (IDS) associated with a hereditary persistence of fetal haemoglobin (HbF), also known as Dias-Logan syndrome, is commonly characterised by psychomotor developmental delay, intelectual disability, language delay, strabismus, thin upper lip, abnormalities of external ears, microcephaly, downslanting palpebral fissures. Sporadically, autism spectrum disorders and blue sclerae in infancy have been reported in IDS. Rarely, IDS-affected patients present with epilepsy and/or epileptic syndromes. It has been shown that a haploinsufficiency of the B cell leukaemia/lymphoma 11A gene (*BCL11A*) is responsible for IDS. Herein, we identified a novel de novo frameshift deletion (c.271delG; p.E91Afs*2) in the *BCL11A* gene in a boy affected with IDS. Interestingly, this heterozygous loss-of-function *BCL11A* mutation was also associated with a generalised idiopathic epilepsy and severe language delay observed in the patient. Moreover, our study showed that the combination of molecular genetic analyses with the monitoring of HbF was essential to make the final diagnosis of Dias-Logan syndrome. Because our patient suffered from well-controlled epilepsy, we propose to include the *BCL11A* gene in routinely used molecular genetic epilepsy-related gene panels. Additionally, many of the clinical features of IDS overlap with symptoms observed in patients with suspected alcohol spectrum disorders. Therefore, we also suggest monitoring HbF levels in patients with these syndromes to further facilitate clinical diagnosis.

Intellectual disability (ID) is characterised by below-average intelligence and deficits in adaptive behaviour [Marrus and Hall, 2017]. It is a highly complex neurodevelopmental disorder showing different degrees of intellectual delay. The worldwide ID prevalence was estimated to about 1% with the highest frequency reported in children and adolescents [Maulik et al., 2011].

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More than 820 genes are related to different childhood neurodevelopmental disorders with ID [Kochinke et al., 2016].

Furthermore, the ID syndrome (IDS) associated with a hereditary persistence of fetal haemoglobin (HbF) has been described as the Dias-Logan syndrome (OMIM 617101) [Dias et al., 2016]. It is characterised by a wide spectrum of clinical symptoms, including psychomotor developmental delay, intellectual disability, speech motor delay, strabismus, and thin upper lip, abnormalities of external ears, microcephaly, downslanting palpebral fissures, autism spectrum disorder, and blue sclerae in infancy [Dias et al., 2016]. Many clinical features of IDS overlap with a chromosome 2p16.1p15 microdeletion syndrome (OMIM 612513) [Rajcan-Separovic et al., 2007]. The smallest microdeletion encompassing the B cell leukaemia/lymphoma 11A gene (*BCL11A*; OMIM 606557) has been described in a paediatric patient with hypotonia, mild intellectual delay, speech disorder, and gross motor impairments [Peter et al., 2014]. It has also been shown that de novo heterozygous mutations in the single *BCL11A* gene cause IDS [Dias et al., 2016]. Genetic studies performed on large cohorts of patients with infantile epileptic encephalopathies recently described 2 children with de novo mutations in the *BLC11A* gene [Yoshida et al., 2018]. These findings emphasise that epileptic phenotypes are sporadically related to this gene [Peron et al., 2019].

The *BCL11A* gene is highly expressed in the fetal brain, especially in cortex, caudate, hippocampus, and putamen, but also in B lymphocytes, and in the adult erythroid lineage [Funnell et al., 2015]. It acts as a transcriptional repressor in B lymphocytes, and its downregulation in primary adult erythroid cells results in a high expression of HbF [Sankaran et al., 2008]. The *BCL11A* gene plays a role in silencing of the human gamma-globulin and has been proposed as a therapeutic target for reactivation of HbF in inherited blood disorders [Sankaran et al., 2008]. The persistence of HbF in blood has been observed in rarely reported *BCL11A*-affected patients [Dias et al., 2016].

To the best of our knowledge, less than 20 IDS-related *BCL11A* mutations have been reported worldwide [Dias et al., 2016; Cai et al., 2017; Soblet et al., 2018; Yoshida et al., 2018]. Most of them are represented by loss-of-function (LoF) mutations, leading to haploinsufficiency of the *BCL11A* gene. Very few missense variants have also been identified in the *BCL11A* gene [Dias et al., 2016; Cai et al., 2017; Soblet et al., 2018; Yoshida et al., 2018].

Herein, we present a young male patient with a novel frameshift deletion in the *BCL11A* gene. In addition to the commonly observed IDS symptoms, the patient also manifested a generalised idiopathic epilepsy and severe language delay.

Case Presentation

The patient was a 13 year-old-boy, the only affected child of non-consanguineous parents from the northwest of Germany (online suppl. Fig.1; see www.karger.com/doi/10.1159/000508566 for all suppl. material). There was no history of intellectual disability, epilepsy, or alcohol consumption in this family. The patient was born spontaneously at term, showing normal birth weight (3.780 g), length (52 cm), head circumference (35 cm), and Apgar values (10/10).

He was admitted to a local children's hospital at the age of 9 months due to muscle hypotonia and motor development delay. Examination of cerebral MRI, electroencephalography, echo- and electrocardiography, and cerebrospinal fluid screening for inborn metabolic disorders were normal. A persistent elevation of serum creatine kinase (300–400 U/L, reference <180) initially suggested a neuromuscular disorder in this patient. At the age of 12 months, the patient showed a decreased head circumference to the 3rd percentile (45 cm). The patient was able to roll without crawling; sitting independently was not possible. He showed axial and facial muscle hypotonia with hypersalivation and connective tissue weakness with pronounced joint hyperlaxity. However, tendon reflexes were normal. He presented with delayed speech and language development, e.g., very weak syllabification and speech understanding. He manifested discrete dysmorphic signs (Fig. 1A, top). At the age of 3 years, the patient began to walk independently. He was able to pronounce 4 words, without speech understanding. At the age of 40 months, myoclonic astatic epilepsy with febrile motor tonic-clonic seizures was diagnosed (Fig. 1A, middle). Applying a combined therapy with valproic acid and ethosuximide effectively discontinued the seizures. After tapering and stopping of the antiepileptic therapy at the age of 9 years, the patient did not show any recurrence of epilepsy. A cerebral MRI at the age of 46 months showed slightly enlarged ventricles and external cerebrospinal fluid spaces frontal and enlarged fossa posterior (Fig. 1A, bottom). At the age of 8 years, the patient spoke 25–30 words in a slurred, weak manner. He manifested a cognitive developmental delay with temporary autistic behaviour. Over years, the patient showed a sleep disorder with night terrors.

Material and Methods

Cytogenetic analyses, including karyotyping, subtelomere chromosome testing, and oligo-array-comparative-genomic-hybridization (array-CGH) did not reveal any genetic abnormalities in the patient. A panel diagnostic of genes associated with intellectual disability (CEGAT, Tübingen) performed in the patient revealed heterozygous variants in 2 candidate genes, the glutamate receptor ionotropic N-methyl-D-aspartate subunit 2B (*GRIN2B*; OMIM 613970; c.3993G>A, p.M1331I) and the lysine-specific de-

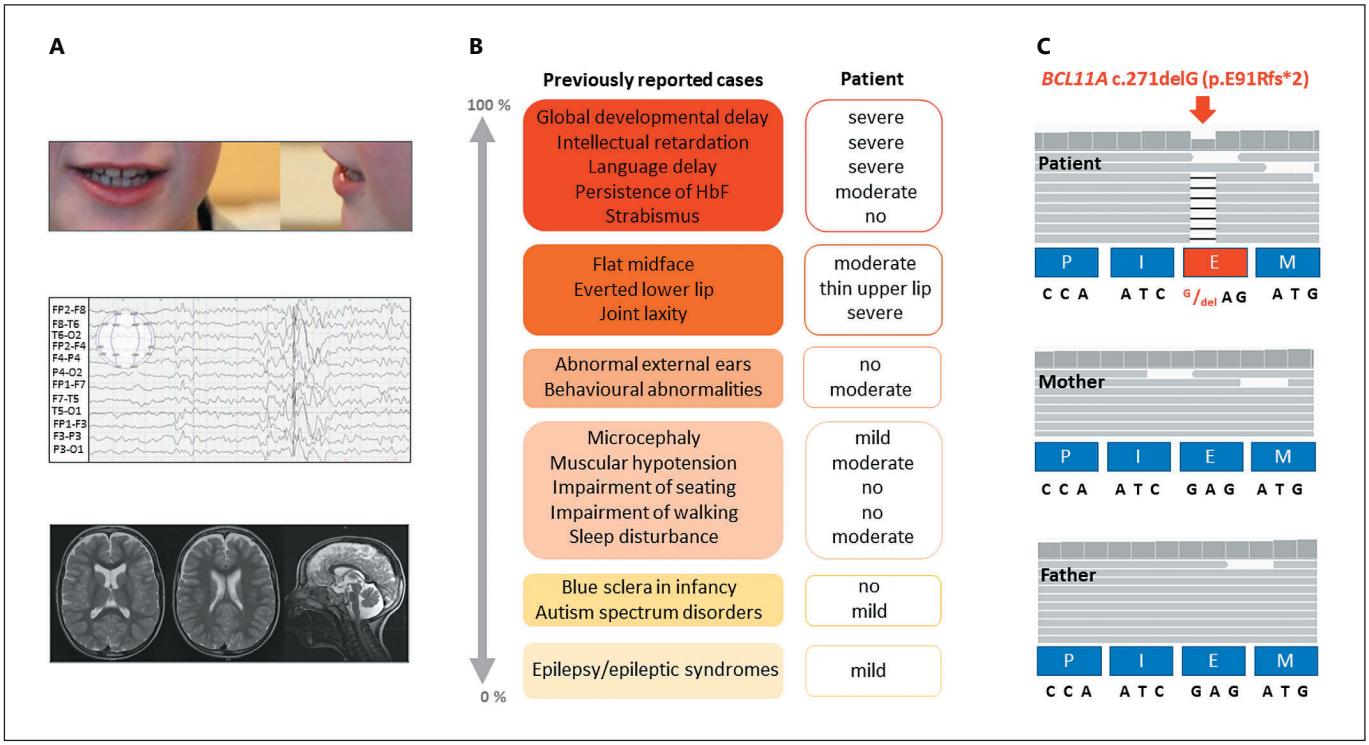

Fig. 1. Clinical manifestations of the patient with the newly identified *BCL11A* mutation. **A** Top: The patient at the age of 11 years and 4 months with a small upper lip and a head circumference of 52.3 cm (10–25th percentile). Middle: EEG of the patient performed at the age of 40 months showing generalised paroxysms of irregular spike waves. Bottom: Brain MRI of our patient achieved at the age of 46 months with slightly enlarged ventricles and external cerebrospinal fluid spaces frontal enlarged fossa posterior. **B** A comparison of the previously described IDS-symptoms (left) with the clinical features observed in our patient (right). An incidence of the previously described *BCL11A*-related clinical symptoms ranges from the most commonly (red) to sporadically reported (light yellow). **C** Trio-WES reads alignments. The de novo frameshift mutation (c.271delG; p.E91Rfs*2) was identified in the patient affected with IDS, whereas both heathy parents did not show any sequence abnormalities. Amino acids are displayed under each panel of sequencing reads.

methylase 5C (*KDM5C*; OMIM 300534; c.2517–9_2517–7dupACT, p.?). However, further familiar co-segregation analyses excluded these sequence variants.

Long-lasting clinical investigations and routine molecular diagnostics did not reveal a cause of the retardation epilepsy syndrome. Therefore, the family was directed to the Human Genetics Department at the University of Oldenburg (Germany) to find the cause of the disease.

Trio-Based Whole-Exome Sequencing

Genomic DNA was isolated from peripheral blood samples from the patient, the mother, the father, and the sister using QIAamp DNA Blood Maxi Kit (QIAGEN, Hilden, Germany).

DNA libraries from the patient and his parents were generated with TruSeq® Rapid Exome Kit (Illumina) and used for trio-based whole-exome sequencing (trio-WES). Paired-end sequencing (2× 75 bp) was performed at NextSeq500 platform (Illumina). The raw WES data were demultiplexed and mapped to the human genome reference hg19. Variant calling and annotations were made with the Verfeed™/Varvis™ bioinformatics (VARVIS Version 1.12, Limbus Medical Technologies GmbH, Rostock) (online suppl. Table 1). Sequence variants with high/moderate impacts were filtered with allele frequency ≤1.5 and sequencing reads quality was verified with the Integrated Genomics Viewer [Robinson et al., 2011]. Based on the familiar anamnesis, we considered autosomal recessive and X-linked modes of inheritance and de novo mutations.

Familiar Co-Segregation Analyses and in silico Predictions of BCL11A Protein Domains

The de novo variant (c.271delG; p.E91Rfs*2) in *BLC11A* was verified by Sanger sequencing. Primers located in intronic regions encompassing exon 2 of the *BCL11A* gene (fwd: 5′-atgatgtggtgggatggcat-3′, rev: 5′-acaactccttactgcttggc-3′) were designed. The PCR conditions, enzymatic purifications of amplicons, and bilateral sequencing were performed as previously described [Reiff et al., 2016].

Human BCL11A protein domains were in silico predicted with the SMART database (http://smart.embl-heidelberg.de).

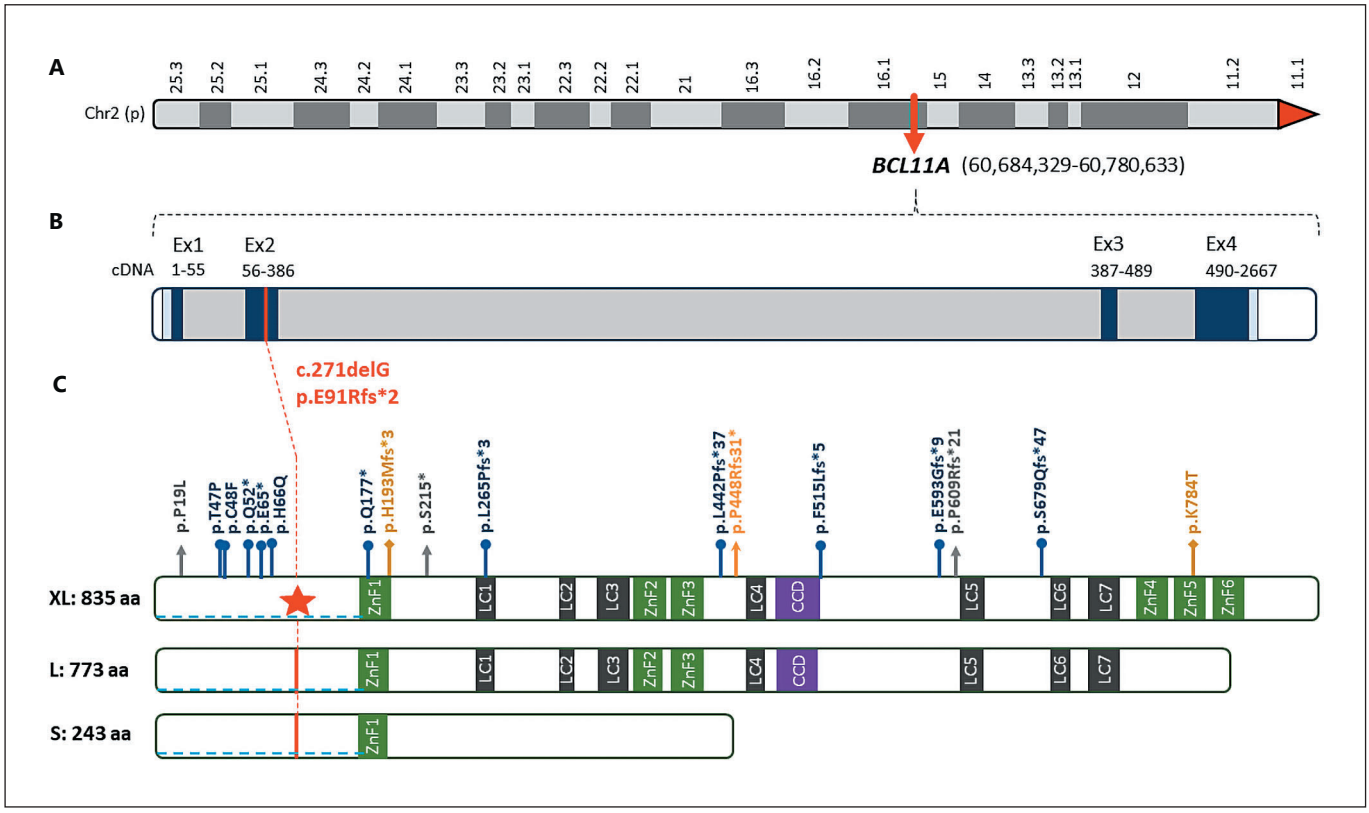

Fig. 2. Graphic presentation of the novel *BCL11A* mutation. **A** Chromosomal position of the *BCL11A* gene. **B** Structure of the *BCL11A* gene. The largest transcript isoform (BCL11A-XL, NM_022893.3) consisting of 4 exons. Colour code: dark blue (coding), light blue (non-coding), introns (grey), white (untranslated regions), and red bar (exonic position of the mutation). **C** The 3 major isoforms of BLC11A protein (XL: NP_075044.2; L: NP_060484.2; S: NP_612569). The figure was modified from Dias et al., [2016] and the cyan dashed lines indicate the putative dimerization region. The *BCL11A* mutations reported by Dias et al., [2016] (blue circles), Cai et al., [2017] (grey triangles), Yoshida et al., [2018] (yellow rhomb), Soblet et al., [2018] (orange arrow), and the newly identified frameshift mutation (red star/bars). Chr, chromosome; Ex, exons; cDNA, complementary DNA; ZnF, zinc finger motifs; CCD, coiled coil domain, and LC, low complexity regions.

Results

Trio-WES and Sanger Sequencing

In total, 4 genes (*BCL11A*: c.271delG; p.E91Rfs*2, *EGR4*: c.74T>C; p.L25R, *ST6GALNAC4*: c.799G>A; p.E267K, and *KIAA1468*: c.2002A>G; p.M668V) carried heterozygous sequence variants in the patient and passed the initial filtering criteria. The 3 missense variants were excluded for further analyses due to the coverage, sequencing reads quality, and an inappropriate disease association and/or inheritance. All coding exons of the *BCL11A* gene were sequenced in the probands. Additionally, 2 sequence variants, including synonymous and intronic variants were found in the *BCL11A* gene in the patient (online suppl. Table 2). The frameshift sequence variant (p.271delG; p.E91Rfs*2) located in the second exon of the *BCL11A* gene was exclusively present in the patient (Fig. 1C). This de novo heterozygous frameshift deletion was likely to explain the IDS symptoms observed in the patient. Extended familiar co-segregation analyses confirmed the de novo occurrence of the frameshift deletion (c.271delG; p.E91Rfs*2) in the *BCL11A* gene in the patient (online suppl. Fig.1). According to the classification of sequence variants recommended by the American Collage of Medical Genetics and Genomics (ACMG), the novel de novo frameshift deletion in the *BCL11A* gene fulfilled criteria for a very strong and pathogenic sequence variant [Richards et al., 2015]. Among others, this sequence variant (i) is a frameshift variant in a gene of known LoF pathomechanism, (ii) its de novo appearance was confirmed only in the patient, and (iii) a damaging effect of the *BCL11A* gene was con-

firmed in well-established functional studies [Dias et al., 2016].

In silico Predicted BCL11A Protein Domains

The de novo frameshift deletion (c.271delG; p.E91rfs*2) was located in the N-terminal region of the BCL11A protein. This protein region is responsible for a recruitment of small ubiquitin-related modifier 1 (SUMO1) and involved in nuclear transport, transcriptional regulation, protein stability, and protein modification [Kuwata and Nakamura, 2008]. The single guanine deletion was also located in a putative dimerization region [Dias et al., 2016] and predicted to cause a premature termination codon (Fig. 2A–C).

Discussion and Conclusion

The *BCL11A* gene encodes a Krüppel zinc finger protein that regulates transcription by interacting with COUP-TF proteins and/or sequence-depending DNA binding [Satterwhite et al., 2001]. Three major human transcriptional isoforms of the *BCL11A* gene, including XL (GenBank: NM_022893.3), L (GenBank: NM_018014.3), and S (GenBank: NM_138559) have been described [Satterwhite et al., 2001]. The BCL11A protein shows a high evolutionary conservation across different species [Satterwhite et al., 2001]. It has been described that the murine *Evi9* gene (also known as *Bcl11a* and *Ctip1*) also encodes 3 tissue-specific protein isoforms, including Evi9a (773 aa), Evi9b (486 aa), and Evi9c (239 aa). The murine Evi9a and the Evi9c correspond to the human BCL11A-L and BCL11A-S isoforms, respectively [Satterwhite et al., 2001].

Several studies have shown that frameshift and nonsense variants are equally spread across the entire *BCL11A* gene. These LoF mutations have been predicted to deleteriously influence the L and XL isoforms due to an early truncation and/or a nonsense-mediated degradation of the protein [Dias et al., 2016]. It has been postulated that missense mutations exclusively locate to a putative dimerization region in the N-terminal region of the BCL11A protein [Liu et al., 2006; Dias et al., 2016]. These mutations lead to mis-localization of BCL11A and affect its dimerization and transcriptional activity [Peron et al., 2019]. Recently, a missense substitution (p.K74T) was found in the C-terminal part of the protein in a young patient affected with West syndrome and severe developmental delay [Yoshida et al., 2018].

Our patient showed a de novo heterozygous deletion (c.271delG; p.E271Rfs*2) in the *BCL11A* gene. The newly identified frameshift mutation was located in the N-terminal region of the BCL11A protein. The patient showed clinical overlap with IDS-affected patients [Dias et al., 2016], but he also manifested the rarely *BCL11A*-associated epileptic phenotype (Fig. 1B). In contrast to the reported patients with severe and pharmaco-resistant epileptic encephalopathies, the myoclonic astatic epilepsy in our patient was well controlled with anticonvulsant therapy.

The murine-based studies have shown that the *Bcl11a* gene is a key regulator in a neurite arborization, and its proper expression is required for the development of neuronal networks [Kuo et al., 2009]. Therefore, the haploinsufficiency of the *BCL11A* gene caused by LoF mutations has been postulated to not only lead to IDS, but also to evoke epilepsies [Yoshida et al., 2018]. Nevertheless, modifier genes have also been suggested for epileptic components [Yoshida et al., 2018]. We also verified a panel of 387 epilepsy-related genes, but we did not find any plausible modifier variant (online suppl. Table 3). However, modifier variants located in uncovered coding and non-coding gene regions cannot be currently excluded.

BCL11A is responsible for transcriptional repression of HbF [Liu et al., 2018]. Many of IDS-affected patients showed hereditary persistence levels of HbF [Dias et al., 2016]. A complete blood count was retrospectively measured in the patient (HbF 7.5%, normal <2). Combining molecular analyses with HbF monitoring, we successfully confirmed the Dias-Logan syndrome in the patient.

Moreover, reported *BCL11A* patients showed dysmorphic signs with a thin upper lip and/or an everted lower lip, microcephaly, intellectual disability, speech impairment, and behaviour abnormalities. All these symptoms are often found in fetal alcohol spectrum disorder, suggesting that haemoglobin electrophoresis should also be performed in patients with suspected alcohol spectrum disorder. Considering the patient described herein manifested well-controlled seizures, we also postulate to include *BCL11A* in molecular diagnostic epilepsy-related gene panels to expand the spectrum of this rare retardation epilepsy syndrome.

In conclusion, we identified a novel de novo heterozygous LoF mutation (c.271delG; p.E91Afs*2) in the *BCL11A* gene in a boy affected with IDS, epilepsy, and severe language delay. Our clinical and molecular outcomes complemented sporadically observed phenotype-genotype correlations related to the *BCL11A* gene.

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Statement of Ethics

The study adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee (Hannover Medical School (MHH) Ethics Committee, OE9515; University of Oldenburg, Faculty VI – School of Medicine and Health Science, Medical Ethics Committee, 2018-097). All examined persons were informed in detail about the genetic testing and formally agreed to participate in the study by informed written consent. The parents signed the informed written consent for participation in the study for the underage patient.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contribution

All authors read and approved the manuscript. G.C. Korenke: performed clinical investigations, shared clinical data, and wrote clinical parts of the manuscript. B. Schulte: performed molecular diagnostics investigations and shared the data. S. Biskup: coordinated molecular diagnostics investigations and shared the data. J. Neidhardt: contributed to the manuscript and the coordination of the study. M. Owczarek-Lipska: performed molecular research investigations and analyses, major contribution to the coordination of the study, and wrote molecular parts of the manuscript.

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