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A novel homozygous synonymous variant further expands the phenotypic spectrum of *POLR3A*-related pathologies

Davor Lessel[1] | Katrin Rading[1] | Susan E. Campbell[2] | Holger Thiele[3] | Janine Altmüller[3,4,5] | Leslie B. Gordon[6,7,8] | Christian Kubisch[1]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[2]Center for Gerontology and Healthcare Research, Brown University, Providence, Rhode Island, USA

[3]Cologne Center for Genomics, University of Cologne, Cologne, Germany

[4]Berlin Institute of Health, Charité—Universitätsmedizin Berlin, Core Facility Genomics, Berlin, Germany

[5]The Genomics unit, Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

[6]Department of Pediatrics, Division of Genetics, Hasbro Children's Hospital, Providence, Rhode Island, USA

[7]Warren Alpert Medical School, Brown University, Providence, Rhode Island, USA

[8]Department of Anesthesiology, Perioperative and Pain Medicine, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence
Davor Lessel, Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany.
Email: d.lessel@uke.de

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Abstract

Pathogenic biallelic variants in *POL3RA* have been associated with different disorders characterized by progressive neurological deterioration. These include the 4H leukodystrophy syndrome (hypomyelination, hypogonadotropic hypogonadism, and hypodontia) and adolescent-onset progressive spastic ataxia, as well as Wiedemann–Rautenstrauch syndrome (WRS), a recognizable neonatal progeroid syndrome. The phenotypic differences between these disorders are thought to occur mainly due to different functional effects of underlying *POLR3A* variants. Here we present the detailed clinical course of a 37-year-old woman in whom we identified a homozygous synonymous *POLR3A* variant c.3336G>A resulting in leaky splicing r.[3336ins192, =, 3243_3336del94]. She presented at birth with intrauterine growth retardation, lipodystrophy, muscular hypotonia, and several WRS-like facial features, albeit without sparse hair and prominent scalp veins. She had no signs of developmental delay or intellectual disability. Over the years, above characteristic facial features, she showed severe postnatal growth retardation, global lipodystrophy, joint contractures, thoracic hypoplasia, scoliosis, anodontia, spastic quadriplegia, bilateral hearing loss, aphonia, hypogonadotropic hypogonadism, and cerebellar peduncles hyperintensities in brain imaging. These manifestations partially overlap the clinical features of the previously reported *POLR3A*-associated disorders, mostly mimicking the WRS. Thus, our study expands the *POLR3A*-mediated phenotypic spectrum and suggests existence of a phenotypic continuum underlying biallelic *POLR3A* variants.

KEYWORDS
leaky splicing, phenotypic continuum, POLR3A, synonymous variant, Wiedemann-Rautenstrauch syndrome

1 | INTRODUCTION

RNA polymerase (Pol) III is one of three eukaryotic nuclear RNA polymerases, responsible for transcription of small noncoding genes such as ribosomal 5S RNAs, transfer RNAs (tRNAs), signal recognition particle (SRP) RNAs, mitochondrial RNA-processing RNAs (RMRP), H1 RNAs (RPPH1), U6 and 7SK small nuclear RNA (snRNA) (Jarrous et al., 2021; Yeganeh & Hernandez, 2020). Pol III is composed of

17 protein subunits, and the largest subunit is encoded by *POLR3A* (#MIM 614258) (Sepehri & Hernandez, 1997; Yeganeh & Hernandez, 2020).

Pathogenic biallelic variants in *POL3RA* were first identified in individuals affected by a hypomyelinating leukodystrophy and variable degree of tremor-ataxia, oligo- and hypodontia, and hypogonadotropic hypogonadism (Bernard et al., 2011). This neurodegenerative condition, which is also known as the 4H leukodystrophy syndrome (hypomyelination, hypogonadotropic hypogonadism, and hypodontia) (Bernard et al., 2011), is characterized by high inter- and intra-familial variability regarding the age of onset and progression of neurologic symptoms (Wolf et al., 2014). Noteworthy, hypomorphic biallelic *POLR3A* variants were also identified in individuals affected by adolescent-onset progressive spastic ataxia with no overt hypomyelinating leukodystrophy (Minnerop et al., 2017). Moreover, Jay et al. identified compound heterozygosity for two null variants in *POLR3A* in an individual that resembled Wiedemann–Rautenstrauch syndrome (WRS) (Jay et al., 2016), a neonatal/congenital segmental progeroid syndrome (Lessel & Kubisch, 2019; Paolacci et al., 2017). WRS, first described in 1977 by Thomas Rautenstrauch and Friedemann Snigula (Rautenstrauch & Snigula, 1977) followed by Hans Rudolph Wiedemann in 1979 (Wiedemann, 1979), is characterized by recognizable facial features, intrauterine growth retardation with subsequent severe failure to thrive, natal teeth followed by hypodontia or anodontia of permanent teeth, thin and atrophic skin, global developmental delay, progressive neurological deterioration, generalized lipodystrophy with local fat pads, and associated cachectic appearance (Lessel & Kubisch, 2019; Paolacci et al., 2017). We and others independently confirmed that biallelic loss-of-function variants in *POLR3A* cause WRS (Lessel et al., 2018; Paolacci et al., 2018; Wambach et al., 2018). In addition, heterozygous missense variants in *POLR3A* and *POLR3C*, as well as combination of variants in both genes, have been associated with increased susceptibility to severe varicella zoster virus (VZV) infection in otherwise unremarkable individuals (Ogunjimi et al., 2017).

Here, we report a detailed clinical course in a 37-year-old woman harboring a *POLR3A* homozygous synonymous variant, which results in aberrant RNA splicing and leads to a combination of clinical signs and symptoms previously associated with *POLR3A*-pathologies although mostly resembling WRS.

2 | MATERIALS AND METHODS

2.1 | Human subjects

All biological samples and images were obtained following written informed consent from the affected individual and her family members. Initial patient contact was made with The Progeria Research Foundation (PRF) International Patient Registry (www. Progeriaresearch.org), this study has ongoing institutional review board approval from the Hasbro Children's Hospital. In addition, the study was performed in accordance with protocols approved by the Ethics Committee of the Hamburg Chamber of Physicians (PV 3802). The study was performed in accordance with the Declaration of Helsinki protocols.

2.2 | Genetic analyses

DNA samples from whole blood were isolated by standard procedures. Trio-whole exome sequencing with DNA samples of both healthy parents and the index patient was performed at the Cologne Center for Genomics on two lanes of an Illumina GAIIx Sequencer using a single read 150 bp protocol after enrichment of exonic and splice-site sequences with the Agilent SureSelect Human All Exon 50 Mb kit as previously described (Lessel et al., 2018). Variant annotation and bioinformatic filtering of identified variants was performed as described before (Lessel et al., 2018). Sanger sequencing was performed to validate candidate variants and perform segregation analyses within the family. All primer pairs are available upon request.

2.3 | *POLR3A* transcript analysis

Total RNA was extracted using the PAXgene Blood RNA Kit IVD (Qiagen, Hilden, Germany) and RT-PCR was performed as previously described (Girisha et al., 2016). Briefly, RT-PCR was performed using the OneStep RT-PCR Kit (Qiagen). Primer sequences were as follows: Forward primer 5′-GACCCTGAAGACTTTCCACTTTGC-3′ and reverse primer 5′-TCTCAGCGTTCACTTCCAGTCTCA-3′. Resulting products were electrophoresed, gel-extracted using the QIAquick Gel Extraction Kit (Qiagen), and subjected to Sanger sequencing. Sequence traces were assembled, aligned and analyzed with the Seqman software (DNASTAR Lasergene, Madison, WI, USA). Image Lab™ Software was used to calculate the relative abundance of *POLR3A* transcripts.

3 | RESULTS

3.1 | Clinical report

The proband (Figure 1), a female born at 40 weeks of gestation, was the firstborn of healthy parents who are likely distantly related. She has a younger unaffected sister. Her birthweight was 2600 g (−2.07 SD), length 53 cm (+0.59 SD), and occipitofrontal head circumference (OFC) 31 cm (−3.00 SD). At birth, she had a triangular face, sparse eyebrows, deep set and closely spaced eyes with low positioning of the eyeballs (lower eyelid covering part of the cornea), bilateral microphthalmia, coloboma on left eye, small mouth, two natal teeth in the upper jaw, high-arched palate, malformed and low-set ears, small mouth, pointed chin, and mandibular underdevelopment. She also had global lipodystrophy, hip dysplasia, muscular hypotonia, and adducted thumbs. Karyotyping gave unremarkable result. Milestones of motor development and language development were unremarkable. Head and neck control was achieved at 3 months, she sat without support at 8 months and made first steps at 11 months of age. She spoke

FIGURE 1 Clinical characteristics of the proband. Proband at the age of 12 months (a), 3 years (b), 8 years (c), 12 years (d), and 34 years (e and f). Please note the triangular face, sparse eyebrows, deep set and closely spaced eyes with low positioning of the eyeballs, bilateral microphthalmia, small mouth, malformed and low-set ears, small and pointed chin. Progressive global lipodystrophy, thin arms and legs, and severely cachexic appearance at the age of 34 years (f)

short sentences at 2 years of age. The proband reported that her whole early postnatal development was characterized by poor weight gain and severe growth retardation. At the age of 6 and 8 years, she underwent surgical procedures to remove large subdural hematomas that both occurred after a fall and head injury. The proband reports on deterioration of motor abilities and general muscular weakness following subdural hematomas, she could not run as fast as previously but could still walk independently for around 5 km. Routine laboratory analyses performed after the second surgery, at the age of 8 years, revealed a mild aminoaciduria, and elevated cholesterol and triglyceride values. X-rays of the spine and pelvis revealed ankylosing spondylitis and thoracic hypoplasia. By the age of 8 years, she had complete anodontia in the lower jaw. Intelligence quotient (IQ) was measured 116. Endocrinology workup at the age of 15 years showed elevated levels of growth hormone and IGF-1, as well as low levels of progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Analysis of testosterone, prolactin, and estradiol as well as thyroid function test gave normal results. X-ray of the wrist suggested a bone age of 12 years. In addition, audiometry confirmed a progressive conductive hearing loss. Moreover, she developed a seborrhoeic dermatitis on the scalp. At the age of 20 years, she had an almost complete absence of secondary sexual characteristics including the absence of menarche. At the age of 26 years, she had a long lasting febrile infection with recurrent diarrhea of unknown origin. The proband reports further major deterioration of motor capacities after this infection of unknown origin. She was not able to climb stairs or walk larger distances anymore. Her weight had dropped from 31 to 22 kg, and she experienced gradual voice changes and could barely speak. Afterwards she took special measures, primarily avoiding unnecessary personal contacts, during the flu season and especially since onset of the SARS-Cov-2 pandemics. She reports no severe infections after her 26th birthday. At the age of 27 years, she was diagnosed with

Type 2 hyperlipoproteinemia. Her motor problems progressed to a spastic quadriplegia and she was unable to walk unaided. Brain MRI at the age of 33 years revealed cortical lesions of the angular and fusiform gyri, likely consequences of traumatic brain injuries, increased T2 signal intensity in the dorsal spinal cord, as well as corticospinal tract (CST) and cerebellar peduncles hyperintensities on MRI-fluid attenuated inversion recovery (FLAIR). Currently at the age of 37 years, her weight is 24 kg and height 148 cm. She exhibits a prematurely aged facial appearance, with a triangular face, small and deep set eyes, a beaked nose, complete anodontia, malformed and low-set ears, and mandibular underdevelopment. She has thin and sparse hair on the head, global lipodystrophy, thin arms and legs, spastic quadriplegia, scoliosis, a bilateral conductive hearing loss and is aphonic (Figure 1). Noteworthy, despite the abovementioned severe health issues, she gained university education.

3.2 | Genetic analyses

In order to identify the putative genetic cause, we performed trio whole-exome sequencing (trio-WES) with a DNA sample of the proband and her both parents. Approximately 96% of target sequences were covered at least 10-fold with a mean coverage of 88x. Bioinformatic filtering identified no de novo variants and no putatively compound heterozygous variants with a minor allele frequency (MAF) < 0.001 according to gnomAD. We next searched for rare, homozygous variants. This analyses revealed seven homozygous variants in six genes, all of which were within regions of homozygosity in line with distant consanguinity of the parents. (Tables S1 and S2). Notably, we identified a synonymous variant c.3336G>A, p.(Glu1112Glu), affecting the last nucleotide in Exon 25 of *POLR3A* (NM_007055.4). Sanger sequencing-based, subsequent segregation analyses with DNA samples of the proband, her both parents and unaffected sister, excluded four of these variants (Table S2). Due to similarities to previously reported *POLR3A*-cases (Jay et al., 2016; Lessel et al., 2018; Paolacci et al., 2018; Wambach et al., 2018) and as the *Splice Site Prediction by Neural Network* (Berkeley Drosophila Genome Project; http://www.fruitfly.org/seq_tools/splice.html) predicted a weaker splice donor site with a score of 0.83 compared to a score of 1.00 that is assigned to the wild-type sequence, we further analyzed the impact of this synonymous *POLR3A* variant.

To test for the predicted splicing defect of the variant c.3336G>A, we performed RT-PCR analysis with RNA isolated from lymphocytes of the proband and one control individual. This analysis revealed in the control individual only one amplicon of the expected wild-type size (~300 bp). Analysis of the proband RNA yielded three amplicons, one amplicon of the expected wild-type size (~300 bp) with a relative abundance of ~30%, one stronger and larger amplicon of ~500 bp with a relative abundance of ~65%, and one very weak amplicon of ~200 bp with a relative abundance of ~5%. Sanger sequencing revealed that the larger amplicon (~500 bp) resulted from insertion of intron 25 whereas the lower amplicon (~200 bp) resulted from skipping of exon 25 (Figure 2). Thus we conclude that the identified synonymous variant results in leaky splicing at least in blood. It preferentially results in intron 25 insertion, r.[3336ins192] on the RNA level, which is predicted to lead to a premature termination codon p.(Glu1112Glu*fs**7) on the protein level. The less abundant alternatively spliced transcript resulting from skipping of Exon 25 leads to r.[3243_3336del94] on the RNA level, and also likely leads to a premature termination codon p.(Ser1081Arg*fs**28). Noteworthy, in addition to these alternatively spliced transcripts we also identified the wild-type transcript of intermediate abundancy.

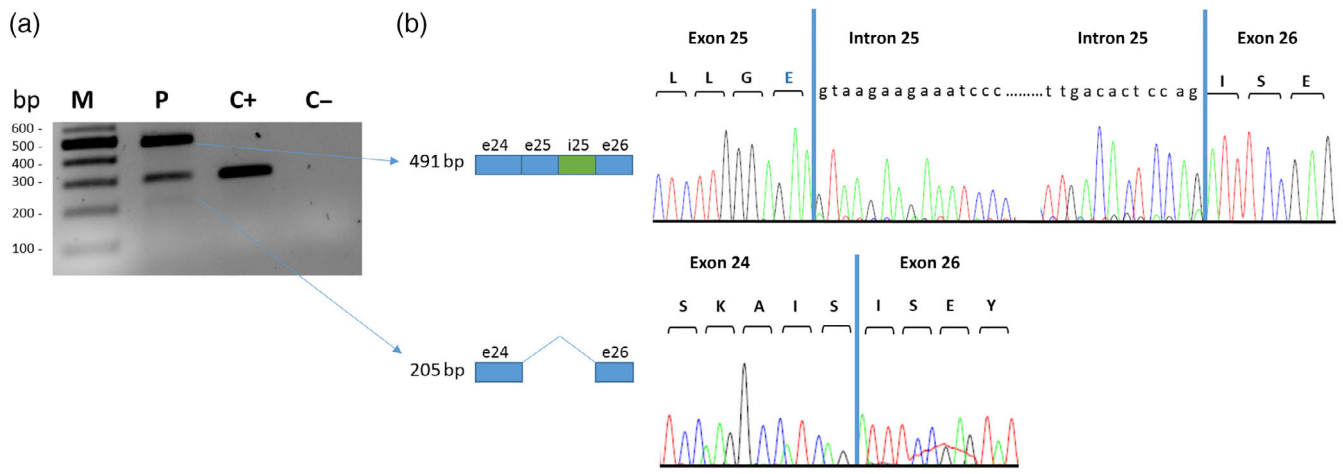

FIGURE 2 *POLR3A* transcript analysis. (a) *POLR3A* transcript analysis was performed using lymphocyte-derived RNA of the proband (P) and an unaffected individual (C+). The 299-bp wild-type RT-PCR amplicon was amplified in both the proband and the control (C+), while no product was yielded in the negative control (C−, no template). Two further PCR products were identified. One stronger and larger amplicon of ~500 bp, suggestive for Intron 25 inclusion (299 + 192 = 491), and one very weak amplicon of ~200 bp, suggestive for exon 25 skipping (299–94 = 205). Molecular marker (M) indicates the reference bands. (b) Sanger sequencing of the two alternative amplicons confirmed the intron 25 inclusion in the larger amplicon, and the skipping of exon 25 in the lower amplicon

TABLE 1 Comparison of clinical signs and symptoms of the here described individual and individuals affected by *POLR3A*-related disorders

	This study	WRS biallelic *POLR3A* variants[a,b,c,d]	WRS monoallelic *POLR3A* variants[b,c]	4H leukodystrophy *POLR3A* and *POLR3B*[e]	Late onset spastic ataxia *POLR3A*[f]
Sex	Female	10 females 9 males	5 females 1 males	53 females 52 males	14 females 15 males
Birth parameters					
Weight at birth <P3	+	17/19	4/5	?	?
Length at birth <P3	−	5/13	4/5	?	?
OFC at birth <P3	+	4/14	2/4	?	?
Craniofacial features					
Triangular face	+	16/19	5/5	?	?
Sparse scalp hair	−	16/19	5/5	?	?
Thin/translucent skin	−	18/18	5/5	?	?
Prominent scalp veins	−	17/17	5/5	?	?
Closely spaced eyes	+	3/9	5/5	?	?
Deeply set eyes	+	12/12	3/4	?	?
Microphthalmia	+	−	−	?	?
Coloboma	+	−	−	?	?
Lower eyelid covering part of the cornea	+	19/19	5/5	?	?
Ear abnormalities	+	17/18	4/5	?	?
Small mouth	+	15/18	4/5	?	?
Natal teeth	+	17/19	3/5	18/94	?
Pointed chin	+	13/18	5/5	?	?
Mandibular underdevelopment	+	6/9	1/1	?	?
Neurological and neurodevelopmental abnormalities					
Motor development delay	−	4/8	?	54/103	1/29
Intellectual disability	−	8/13	?	54/103	5/29
Ataxia	+	2/11	1/3	+*	29/29
Tremor	−	3/11	?	+*	15/29
Loss of independent walking	+	?	?	28/54	20/20
Hearing loss	+	2/13	?	−	0/29
Dysarthria	+	?	?	+*	16/29
Neurologic deterioration after infection	+	?	?	42/97	?
Brain anomalies US/MRI					
Structural brain anomalies	−	2/7	1/1	+**	1/21
Hypomyelination	−	0/4	?	93/97	0/20
SCP hyperintensity (FLAIR)	+	?	?	−	11/14
Metabolic anomalies					
Lipodystrophy	+	19/19	6/6	−	?
Hyperlipoproteinemia	+	?	?	?	?
Hypertriglyceridaemia	+	2/4	?	?	?
Hypogonadism	+	?	?	47/62	?
Amenorrhea	+	?	?	+	?
Growth hormone	Elevated	?	?	Deficiency 5/10	?

TABLE 1 (Continued)

	This study	WRS biallelic *POLR3A* variants[a,b,c,d]	WRS monoallelic *POLR3A* variants[b,c]	4H leukodystrophy *POLR3A* and *POLR3B*[e]	Late onset spastic ataxia *POLR3A*[f]
Skeletal anomalies					
Abnormal dentition	+	10/10	?	71/98	11/20
Delayed bone age	+	?	?	?	?
Short stature	+	12/14	?	47/91	?
Kyphosis/scoliosis	+	1/2	?	?	?
Other anomalies					
Cardiac anomalies	−	1/3	1/1	?	?
Joint contractures	+	3/3	?	?	?

Abbreviations: +, present; −, absent; ?, unknown; +*, reported as common without exact numbers; +**, thin corpus callosum is present in all cases after the age of 17 years; WRS, Wiedemann–Rautenstrauch syndrome.
[a]Jay et al. (2016).
[b]Lessel et al. (2018).
[c]Paolacci et al. (2018).
[d]Wambach et al. (2018).
[e]Wolf et al. (2014).
[f]Minnerop et al. (2017).

4 | DISCUSSION

Wiedemann–Rautenstrauch syndrome (WRS), caused by biallelic loss-of-function variants in *POLR3A* (Jay et al., 2016; Lessel et al., 2018; Paolacci et al., 2018; Wambach et al., 2018), is a neonatal/congenital segmental progeroid syndrome accompanied by a decreased life expectancy. Literature makes note of only a few individuals who were still alive at the age of 20 years (Paolacci et al., 2017; Wambach et al., 2018). We present here a detailed clinical course of a 37-year-old woman whose clinical characteristics and facial features mostly resembled those of individuals affected by the *POLR3A*-associated WRS (Table 1). In line with the clinical assumption trio-WES analysis identified a homozygous synonymous variant in *POLR3A* that affects the last nucleotide in Exon 25 and leads to leaky splicing defects. In more detail, her clinical presentation during the perinatal and early childhood period are highly similar to what is documented in WRS (Lessel et al., 2018; Paolacci et al., 2017; Wambach et al., 2018). Albeit, she did not have sparse scalp hair, nor thin and translucent skin with prominent scalp veins, findings that were observed in almost all previously described WRS-cases bearing *POLR3A* biallelic pathogenic variants. Moreover, as compared to other WRS cases she had no global developmental delay, nor intellectual disability. Actually, despite severe health issues she gained a university degree. Postnatal growth retardation with poor weight gain, global lipodystrophy, joint contractures, anodontia and progressive deterioration of neurological functions, including muscle weakness that progressed to spastic quadriplegia, progressive bilateral hearing impairment, and aphonia, are mostly in line with previously documented WRS cases that survived beyond the early childhood (Lessel et al., 2018; Paolacci et al., 2017; Paolacci et al., 2018; Wambach et al., 2018).

Of note the presence of natal teeth and subsequent dental anomalies, progressive motor regression and spasticity, dysarthria, short stature, and hypogonadotropic hypogonadism with primary amenorrhea observed in the here presented individual are also common clinical findings in 4H leukodystrophy syndrome, another *POLR3A*-associated disorder (Paolacci et al., 2017; Wolf et al., 2014). 4H leukodystrophy syndrome is further characterized by hypomyelinating leukodystrophy, initial tremor and developmental delay, in ~50% of affected individuals, none of which have been observed in the here presented individual (Wolf et al., 2014). Furthermore, whereas individuals with 4H leukodystrophy syndrome commonly have a growth hormone deficiency (Wolf et al., 2014), the here presented individual actually had elevated levels of both growth hormone and IGF-1. Interestingly, the here observed hyperintensities along the cerebellar peduncles on MRI-fluid attenuated inversion recovery (FLAIR) without hypomyelination have been observed in individuals affected by adolescent-onset progressive spastic ataxia harboring hypomorphic biallelic *POLR3A* variants (Minnerop et al., 2017).

Our RT-PCR analysis showed that c.3336G>A can lead to leaky splicing r.[3336ins192, =, 3243_3336del94]. Thus, above retaining some wild-type transcript two alternatively spliced variants are generated, resulting either in inclusion of Intron 25 or skipping of Exon 25 both of which are predicted to lead to a truncated protein. Previous studies identified several intronic variants, some of which are recurrent, that affect transcript processing in individuals affected by WRS (Jay et al., 2016; Lessel et al., 2018; Paolacci et al., 2018; Wambach et al., 2018). Moreover, c.1909+22G>A, shown to be a hypomorphic allele, was observed in >80% of individuals affected by the adolescent-onset progressive spastic ataxia (Minnerop et al., 2017). Similarly, intronic variants, mostly in combination with a missense variant on the other allele, were also identified in individuals affected by 4H leukodystrophy (Wolf et al., 2014). It was initially hypothesized that biallelic *POLR3A* null variants result in WRS, whereas biallelic hypomorphic variants cause a milder 4H leukodystrophy or late-onset progressive spastic ataxia (Jay et al., 2016). The

here presented individual harbors a variant, which results in leaky splicing, resembling a somewhat milder form of WRS, and displays some of the clinical features of both 4H leukodystrophy and late-onset progressive spastic ataxia providing further evidence for a phenotypic continuum between *POLR3A*-pathologies. Clearly, further functional studies aiming to decipher the impact of different *POLR3A* variants, especially those within introns, are needed to provide a molecular explanation for the observed differences in clinical presentation and outcomes.

Lastly, respiratory problems, mostly due to infections (pneumonia), are considered a major cause of death in WRS cases (Lessel & Kubisch, 2019; Paolacci et al., 2017). Although, the individual presented here had no history of severe respiratory infections, it is worth noting that her condition severely deteriorated, both in terms of motor abilities and weight loss, following an infection of unknown origin. Similarly almost half of the individuals affected by 4H leukodystrophy experienced neurologic deterioration following infections (Wolf et al., 2014). Interestingly, missense variants in *POLR3A* have been linked to enhanced susceptibility to severe outcome to VZV infection in otherwise unremarkable individuals, whereby leukocytes display poor IFN production and high replication of VZV upon infection, findings that can be restored upon expression of wild-type *POLR3A* (Ogunjimi et al., 2017). Taken together, these findings might warrant extra precautions for WRS cases in avoiding infections at least in the first years of life. Such preventive strategies could include strong hygiene measures and avoidance of unnecessary physical contacts.

In conclusion, we present here a detailed clinical course of a 37-year-old woman who harbors a homozygous synonymous *POLR3A* variant resulting in leaky splicing. She presented several facial features characteristic for WRS but later on developed a somewhat milder clinical outcome. In addition, she presented several phenotypic overlaps with other *POLR3A*-related disorders, that is, 4H leukodystrophy and adult-onset spastic ataxia. These findings provide further evidence for a phenotypic continuum between *POLR3A*-related disorders and may open the way to further studies aiming to provide a molecular understanding underlying pleiotropic effects of different *POLR3A* variants.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

Davor Lessel, Susan E. Campbell, Leslie B. Gordon, and Christian Kubisch performed clinical follow-up of the affected individual and revised the manuscript. Katrin Rading performed transcript analysis. Holger Thiele and Janine Altmüller performed whole-exome sequencing. Davor Lessel analyzed whole-exome sequencing data, performed Sanger sequencing and wrote the first draft of the manuscript.

DATA AVAILABILITY STATEMENT

All data are available by corresponding author upon reasonable request.

ORCID

Davor Lessel https://orcid.org/0000-0003-4496-244X

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SUPPORTING INFORMATION

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