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ORIGINAL ARTICLE

Expanding the phenotype of Wiedemann-Steiner syndrome: Craniovertebral junction anomalies

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Abstract

Wiedemann–Steiner syndrome (WDSTS) is a rare autosomal dominant condition caused by heterozygous loss of function variants in the *KMT2A* (*MLL*) gene, encoding a lysine N-methyltransferase that mediates a histone methylation pattern specific for epigenetic transcriptional activation. WDSTS is characterized by a distinctive facial phenotype, hypertrichosis, short stature, developmental delay, intellectual disability, congenital malformations, and skeletal anomalies. Recently, a few patients have been reported having abnormal skeletal development of the cervical spine. Here we describe 11 such individuals, all with *KMT2A* de novo loss-of-function variants: 10 showed craniovertebral junction anomalies, while an 11th patient had a cervical abnormality in C7. By evaluating clinical and diagnostic imaging data we characterized these anomalies, which consist primarily of fused cervical vertebrae, C1 and C2 abnormalities, small foramen magnum and Chiari malformation type I. Craniovertebral anomalies in WDSTS patients have been largely disregarded so far, but the increasing number of reports suggests that they may be an intrinsic feature of this syndrome. Specific investigation strategies should be considered for early identification and prevention of craniovertebral junction complications in WDSTS patients.

KEYWORDS
cervical C2/C3 vertebral fusion, Chiari malformation, craniovertebral junction, *KMT2A*, small foramen magnum, Wiedemann–Steiner syndrome

1 | INTRODUCTION

Wiedemann-Steiner syndrome (WDSTS, OMIM 605130) is a rare autosomal dominant disorder, characterized by hypertrichosis (cubiti—hairy elbow—or generalized), short stature, intellectual disability (ID) and distinctive facial features, including round and flat face, thick eyebrows, long eyelashes, downslanted and vertically narrow palpebral fissures, hypertelorism, wide nasal bridge, broad nasal tip, low-set ears, long philtrum, high narrow palate, abnormalities of the dentition. Since it was first described by Wiedemann, Kunze, Grosse, and Dibbern (1989), several authors—most notably Steiner and Marques (2000)—have reported individuals with a similar phenotype over the years, but it was only in 2012 (Jones et al., 2012) that the genetic defect responsible for the disease was identified by whole exome sequencing (WES).

WDSTS is caused by heterozygous variants in the *KMT2A* gene (Lysine-Specific Methyltransferase 2A, OMIM 159555), previously known as *MLL* (Mixed Lineage Leukemia) because of its recognition as a frequent target of somatic rearrangements in acute leukemia. *KMT2A* encodes a histone methyltransferase involved in the regulation of chromatin-mediated transcription and is widely expressed in most human tissues. The majority of WDSTS-associated variants reported so far are de novo loss-of-function (LoF) mutations, predicted to result in premature termination of translation and nonsense-mediated decay of the corresponding transcripts. However, rare missense variants have also been detected and associated with the impairment of essential protein domains that would also lead to a LoF (Lebrun et al., 2018).

With the application of WES and WGS (whole genome sequencing), more WDSTS patients have been identified and described, expanding the phenotypic spectrum of the syndrome with clinical features such as strabismus and other ocular abnormalities, recurrent infections of the genitourinary or respiratory tract, cardiac and urogenital malformations, skeletal abnormalities (delayed skeletal maturation, hip dysplasia, short palm, brachydactyly, clinodactyly of the fifth finger, short foot) (Aggarwal, Rodriguez-Buritica, & Northrup, 2017; Stellacci et al., 2016; Strom et al., 2014; Sun et al., 2017).

We have recently resolved the diagnosis of a patient who, in addition to typical WDSTS features, presented with cervical C2/C3 vertebral fusion and small foramen magnum. At the same time, anomalies of the craniovertebral junction (CVJ) have been reported in a few other WDSTS patients (Baer et al., 2018; Feldman, Dlouhy, Lah, Payne, & Weaver, 2019; Lebrun et al., 2018), suggesting that they may warrant inclusion among the WDSTS-associated features.

The CVJ is a definite anatomical entity marking the transition between the skull and the spine. It is composed of the occiput, the foramen magnum and the first two cervical vertebrae, and encloses the brainstem and upper cervical spinal cord. The foramen magnum and C1 vertebral arches form two rings moving around a central pivot comprising the vertebral body and odontoid process of C2, allowing the elaborate motions of the head; ligaments bind these elements to

provide stability. During embryogenesis, starting at the fourth gestational week, the CVJ originates from a process of separation and resegmentation of the somites that is far more complex than in the subaxial spine (as detailed in Pang & Thompson, 2011). Ossification begins from multiple centers, proceeds through cartilage intermediates and is complete at the age of 10–13 years. This complicated origin makes the CVJ a common seat of congenital anomalies in genetic syndromes. Abnormal segmentation can lead to hemi-vertebrae, fusion of the cervical C1 vertebra to the occiput, or Klippel-Feil anomaly, that is, fusion of the bodies and/or spinous processes of at least two cervical vertebrae.[1] Ossification defects can lead to hypoplasia of the odontoid process or ring structures, a frequent cause of CVJ instability. Hypoplasia of the occiput may result in basilar invagination, the prolapse of the vertebral column into the skull base, marked by the projection of the odontoid process above the foramen magnum. Both CVJ instability and bone abnormalities themselves may limit neck movement and can cause compression of the neural structures. A further risk listed among CVJ anomalies is Chiari malformation type I, defined by the herniation of the cerebellar tonsils through the foramen magnum; its development is considered dependent on predisposing bone anomalies such as small posterior fossa and is unrelated to Chiari types II and III, which result from neural tube defects. Neural involvement may range from asymptomatic to a variety of clinical manifestations, including neck pain, recurrent headaches, bulbar or motor neuron palsy. When required, surgical treatments usually consist of bone fusion to provide stability and/or spinal decompression through bone reduction or removal (Machnowska & Raybaud, 2014; Morota, 2017).

By querying other clinicians, family associations and a rare diseases registry, we were notified that a large number of WDSTS patients who had undergone radiological examinations of the cervical area presented with CVJ anomalies, for the most part fused cervical vertebrae. We were able to collect detailed information about 11 of these patients, including our original proband. Here we present a summary of their clinical and radiological features, with the aim to characterize some of the CVJ anomalies that seem to be recurring in patients with WDSTS and that may be part of the phenotypic spectrum of the syndrome.

2 | MATERIALS AND METHODS

2.1 | Recruitment and clinical overview of patients with WDSTS

Patient 1 (Pt.1) was in follow-up at our Medical Genetics unit, where a full clinical and genetic evaluation was performed in collaboration with other units of our institution and with Centro Nacional de Análisis Genómico of Barcelona, Spain (see Appendix S1).

In order to identify further WDSTS patients with CVJ anomalies, we adopted a threefold approach:

1. We consulted the international CoRDS registry (Coordination of Rare Diseases at Sanford, Sanford Research, Sioux Falls, SD, USA), which includes the largest data collection for individuals diagnosed with WDSTS. Specifically, we were granted access to its 2018 update, hosting self-reported clinical data of 61 WDSTS patients from different countries (mostly from the USA, but also 11 from Europe).
2. We made inquiries through associations of patients' families that included our proband's parents, most prominently the "WSS Foundation" (Sacramento, CA, USA).
3. We contacted collaborating pediatricians and geneticists directly.

Eleven patients gave consent to be enrolled in the study: Pt.1 was in follow-up at our Medical Genetics Unit and was also on the CoRDS 2018 update; Pt.2, Pt.5, and Pt.6 were recruited via CoRDS, through invitations sent out by the registry curators; Pt.7 and Pt.10 were recruited through the WSS Foundation; all others were referred to us directly by collaborating clinicians. Inclusion criteria were: diagnosis of WDSTS with *KMT2A* variation confirmed by gene testing; concurrent diagnosis of Klippel-Feil syndrome, or other well-documented indication of CVJ anomalies; receipt of informed consent from the participants' families. Clinical, molecular, and radiological data were entered by the participants' caregivers, with assistance from the families, in a Case Report Form specific for this study and recapitulating the main features of WDSTS. Particular emphasis was placed on information about CVJ anomalies and on diagnostic imaging investigations or clinical interventions concerning these features. Copies of cervical X-rays, CT-scans and/or brain MRIs were provided, if available. Collected clinical data and radiological findings were then compared in order to extensively describe the observed WDSTS-associated CVJ anomalies. Anonymized information about the *KMT2A* variants was entered in the Leiden Open Variation Database v.3.0 (https://www.lovd.nl/), except for Pt.10 whose data had already been entered in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

2.2 | Editorial policies and ethical considerations

This study was approved by the AVEN Ethics Committee (AUSLRE protocol 2018/0053224) and conducted in accordance with medical professional codes (Helsinki Declaration of 1996), Good Clinical Practice criteria (DL 06/11/2007), Italian Privacy Law (DL 196/2003) and European Regulations on personal data protection (EU 2016/679). All participants—or their parents/legal caretaker—provided written informed consent for study participation and publication of photographs.

3 | RESULTS

3.1 | Clinical highlights and molecular data of the 11 participants

Table S1 reports the clinical and molecular data collected from the 11 participants with CVJ anomalies we recruited, including our original proband (who is also detailed in Appendix S1). There is a good correspondence with the features described in larger, random cohorts

(cf. Table S2; Baer et al., 2018). All participants showed developmental delay and nine were diagnosed with ID, mostly mild to moderate. Craniofacial features were quite distinctive (Figure 1a–n), the most notable and recurrent concerning the orbital region with wide nasal bridge, hypertelorism, downslanted and vertically narrow palpebral fissures. Interestingly, hypertrichosis (Figure 1r–t), as already noted in recent works (Baer et al., 2018), was not limited to the elbows (6/11 participants), but was often generalized or noted on the back and legs (9/11).

All participants had de novo LoF defects in the *KMT2A* gene, either a nonsense/frameshift variant or a missense variant disrupting a known functional domain (Figure 2). Most individuals (9 of 11) had been investigated through WES/WGS, including our original proband (Pt.1) who had a novel, heterozygous nonsense variant: (GRCh 37) NM_001197104.2:c.[2513G > A];[=], NP_001184033.1:p.[(W838*)];[=] (Appendix S1). No other relevant variants were reported, except for Pt.6, who had a variant in the *KRIT1* gene (OMIM 604214), of maternal origin and considered responsible for the multiple cavernous hemangiomas detected by MRI, and a 450 Kb duplication in the 8q13.2 region, also of maternal origin and—given the lack of possible association with specific phenotypes of Pt.6—unlikely to be clinically significant.

3.2 | CVJ anomalies of the 11 participants

CVJ findings in our cohort are summarized in Table 1. Figure 3 shows representative examples of CVJ anomalies from the available X-rays, CT scans and MRIs of the recruited individuals.

In Pt.1, a 24-years-old female, brain MRI at the age of 6 years 5 months to investigate the cause of her epilepsy confirmed developmental brain anomalies (cf. Appendix S1 and Table S1) and also revealed the presence of CVJ anomalies. Subsequent spine X-ray and CT-scan clearly displayed cervical C2/C3 vertebral fusion, small foramen magnum, midline cleft of the posterior arch of C1 with inturned hemiarches, slightly retroflexed odontoid process with mild basilar invagination (Figure 3a–g). Follow-up suggestions included periodic imaging to monitor the risk of spinal compression and occasionally wearing a cervical collar to avoid possible shocks during travel or stressful situations. At the age of 13 years, despite the absence of symptoms, CT-scan showed a worsening of the vertebral impression on the spinal canal and the dysmorphic posterior arch of C1 was surgically removed (Figure 3h). Over the following months, some of Pt.1's clinical symptoms improved: her walking became less clumsy and in a couple of years she acquired control of the sphincters.

In Pt.2, a 4-years-old female, brain and spine MRI at 20 months revealed a segmentation anomaly in the upper cervical region involving the C2 and C3 vertebrae, which was subsequently confirmed by cervical spine X-rays (Figure 3k). The occipital condyles appeared dysmorphic and hypoplastic; spinal canal stenosis was considered nonsignificant. She was also missing two sets of ribs. The CVJ anomalies were regarded to be responsible for the obstructive sleep apnea observed in the participant, for which she is being treated with nocturnal oxygen (0.25 L/min).

Pt.3, a 6-years-old male, performed a brain and brainstem MRI at 2 years 3 months for pituitary gland evaluation, because of a

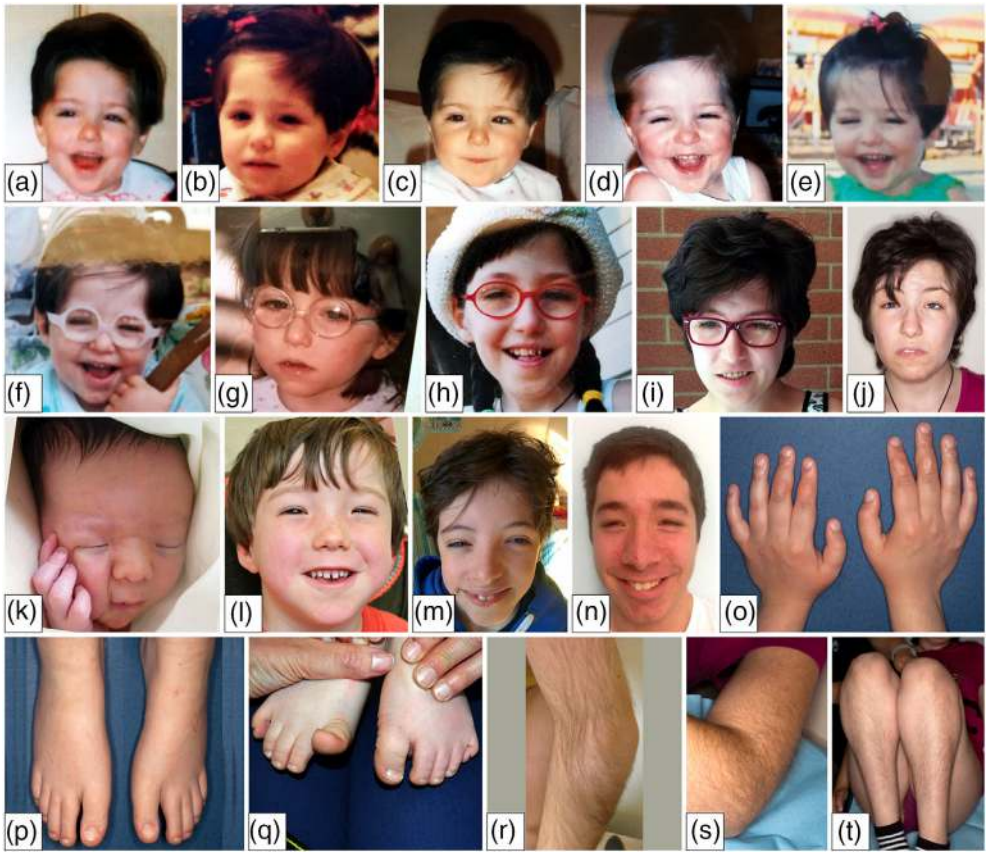

FIGURE 1 Craniofacial appearance and general characteristics of the described WDSTS patients. (a–n) facial appearance: (a–j) Pt.1 at age 1 through 16 years, (k–l) Pt.6 at age 1 week and age 3 years, (M) Pt.4, (n) Pt.10 at age 16 years. (o, p) Pt.1 hands (with tapered fingers) and feet, age 16 years. (q) Pt.6 ft, age 3 years. (r) Pt.4 hypertrichosis cubiti. (s,t) Pt.1 hypertrichosis cubiti and of the lower limbs [Color figure can be viewed at wileyonlinelibrary.com]

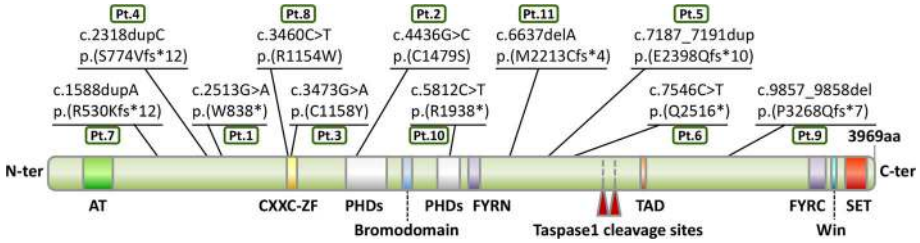

FIGURE 2 Graphic representation of KMT2A protein structure highlighting the germline variants in our cohort. AT, DNA binding AT hooks; CXXC-ZF, CXXC-motif zinc fingers; PHD, plant homeodomain zinc fingers; FYRN/FYRC, PheTyr-rich N/C-terminal domain; TAD, transactivation domain; Win, WDR5 protein interaction motif; SET, Su(var)3–9/Enhancer-of-zeste/Trithorax domain (catalytic methylation domain) [Color figure can be viewed at wileyonlinelibrary.com]

diagnostic suspect of Coffin-Siris syndrome (rejected the next year when WDSTS was ascertained). Besides mild developmental anomalies of the brain (cf. Table S1), imaging revealed cervical C2/C3 vertebral fusion and small foramen magnum, later confirmed by X-rays (Figure 3i,j). The posterior arch of C2 appeared dysmorphic and hypertrophic, causing mild spinal canal stenosis. Up to the last examination at 4 years of age, however, Pt.3 did not manifest any neck pain or other symptoms traceable to these CVJ anomalies; specific follow-up or interventions were not considered necessary.

In Pt.4, a 14-years-old male, spine MRI at both 4 and 12 years of age revealed a dysmorphic CVJ: reduced posterior occipitocervical angle, tendency of the Wackenheim (basilar) line to assume an horizontal orientation, retroflexed odontoid process with mild basilar invagination. A slight thickening of the posterior ligaments was also noted. These features, confirmed by X-ray, caused a slight bending of the brainstem, but apparently did not lead to any overt symptoms in the participant. However, follow-up MRI or CT scan was recommended.

In Pt.5, a 6-years-old male, spine MRI at 3 months of age revealed several anomalies: a short coccyx, curved caudally rather than anteriorly; tethering of the spinal cord, which was surgically corrected; the C2/C3 vertebral bodies and posterior elements were at least partially fused, leaving a hypoplastic disc space. Subsequent spinal X-rays at 2 years 3 months and at 5 years 1 month confirmed a stable cervical C2/C3 vertebral fusion, hinting at an evolution from a previous cartilaginous fusion in the first months of life. X-rays also revealed 11 thoracic vertebrae instead of 12 and fusion of C1 with the occiput. No abnormal motion or instability of the CVJ was noted on flexion and extension X-rays; the participant did not complain of any neck pain and showed no difficulty with range of motion. Follow-up diagnostic imaging was suggested every 3–5 years.

Pt.6, an 8-years-old male, recurrently reported strong, brief headaches (sharp pain at the back of head/neck) after increased pressure, for example, excessive running or laughing, for which he underwent cervical MRI at 4 years of age. This revealed cervical C1/C2 vertebral fusion, retroflexed odontoid process with basilar invagination, small foramen magnum and Chiari malformation type I. He is currently being monitored with MRI every 2 years, also due to the presence of multiple cavernous hemangiomas in his brain unrelated with WDSTS.

In Pt.7, a 5-years-old female, cervical CT scan and MRI at 3 years 11 months showed cervical C2/C3 vertebral fusion, a sligthly dysmorphic C3 vertebral body and the cleft of the posterior arch of C1 (Figure 3m,n). The ends of the posterior arch of C1 were inturned, causing a mild impression on the bulbospinal junction that reduced spinal canal width (Figure 3o,p). We were unable to obtain further information concerning planned treatments or follow-up.

In Pt.8, a 10-years-old female, cervical MRI was performed due to neck pain at 6 years 5 months of age, revealing a slight cleft of the posterior arch of C1 (Figure 3q). Her sensibility and strength were reported as normal and no specific follow-up plan was considered necessary.

In Pt.9, a 19-years-old male, spine X-rays performed at 18 years revealed fusion of the bodies and posterior elements of the cervical C2/C3 vertebrae (Figure 3l), in addition to mild scoliosis. The participant showed no telltale signs of this CVJ anomaly and no specific follow-up was required.

Pt.10 is a 20-years-old female with mild WDSTS-associated features (mild developmental delay, good expressive language at present) and a concurrent diagnosis of Hypermobile Ehlers-Danlos Syndrome (hEDS). X-rays at 8 years of age first identified cervical C2/C3 vertebral fusion and 11 pairs of ribs. Pt.10 later manifested episodic numbness of the lower legs; spine MRI at 15 years 10 months reported a mildly small posterior fossa and a borderline Chiari malformation type I, with the right cerebellar tonsil protruding ~3 mm below the level of the foramen magnum and mildly pointed, but there was capacious cerebrospinal fluid, with no evidence of neural crowding. On the other hand, degenerative disc disease (Schmorl's nodes) at T11-T12, T12-L1 and L1-L2 was observed, as well as mild kyphosis at L1-L2. After further episodes of leg numbness, occasional headaches in the suboccipital region (radiating anteriorly to the middle of the brain) and a sudden onset of back pain, at the age of 18 years she underwent cervical CT scan and flexion-extension MRI, which confirmed the borderline Chiari type I and also revealed a duplicated odointoid process causing borderline basilar invagination, although well below the pathological threshold (Figure 3r). There was no obvious brainstem or spinal compression, syringomyelia, or craniocervical instability, but pathological subluxation at C5–C6 and some laxity of the ligaments at C3–C4 and C4–C5 were noted. Overall, the referring clinicians did

TABLE 1 Craniovertebral junction anomalies in the eleven individuals described in this study and in WDSTS-affected individuals from other sources

	Individuals recruited in our cohort												WDSTS parent support group (n=81)‡	CoRDS (WDSTS 2018 update) (n=61)‡	Cases in literature†		
	Pt.1	Pt.2	Pt.3	Pt.4	Pt.5	Pt.6	Pt.7	Pt.8	Pt.9	Pt.10	Pt.11	Total†			Baer et al., 2018	Lebrun et al., 2018	Feldman et al., 2019
Sex	F	F	M	M	M	M	F	F	M	F	M	5F 6M	NA	25F 36M	11F 22M	1F 3M	1F 1M
Age (Age at last examination)	24y	4y (20m)	6y (4y)	14y (12y)	6y (5y)	8y (4y)	5y (3y 11m)	10y (6y 5m)	19y (18y)	20y (18y)	17y						
***KMT2A* variant**																	
[NM_001197104.2]	c.2513G>A	c.4436G>C	c.3473G>A	c.2318dup	c.7187_7191dup	c.7546C>T	c.1588dup	c.3460C>T	c.9857_9858del	c.5812C>T	c.6637del						
[NP_001184033.1]	p.(W838*)	p.(C1479S)	p.(C1158Y)	p.(S774Vfs*12)	p.(E2398Qfs*10)	p.(Q2516*)	p.(R530Kfs*12)	p.(R1154W)	p.(P3286Qfs*7)	p.(R1938*)	p.(M2213Cfs*4)						
Craniovertebral junction anomalies																	
C1/occiput fusion	-	-	-	-	+	-	-	-	-	-	-	1/11	3			1/4	
Fused cervical vertebrae	+	+	+	-	+	+	+	-	+	+	-	8/11	30	(6)§	5/11		
C2/C3 fusion	+	+	+	-	+	-	+	-	+	+	-	*7/11*	*19*		*4/11*	*1/4*	
C1/C2 fusion	-	-	-	-	-	+	-	-	-	-	-	*1/11*	*3*				
Other / non specified	-	-	-	-	-	-	-	-	-	-	-	*0/11*	*8*		*1/11*		
Vertebral dysmorphisms	+	-	+	+	-	+	+	+	-	+	-	7/11	8		1/11		1/2
Cleft C1 (posterior arch)	+	-	-	-	-	-	+	+	-	-	-	*3/11*					
Inturned C1 posterior arch	+	-	-	-	-	-	+	-	-	-	-	*2/11*					
Abnormality of odontoid process	retroflexion	-	-	retroflexion	-	retroflexion	-	-	-	duplication	-	*4/11*					
Other	-	-	posterior arch of C2	-	-	-	C3 body	-	-	-	-	*2/11*					
Occipital bone hypoplasia	-	dysmorphic condyles	-	-	-	-	-	-	-	-	-	1/11	2				
Basilar invagination	mild	-	-	mild	-	+	-	-	-	mild	-	4/11	2			1/4	
Small foramen magnum	+	-	+	-	-	+	-	-	NA	-	-	3/10	9				
Chiari malformation	-	-	-	-	-	type I	-	-	NA	type I (borderline)	-	2/10	11	4	1/29	2/4	
Symptoms possibly related to CVJ anomalies	-	sleep apnea?	-	-	-	head and neck pain	-	neck pain	-	headaches, episodic leg numbness	-	4/11					
Follow-up (incl. treatments, surgery)	periodic MRI/CT, cervical collar; removal of posterior arch of C1	-	-	repeat MRI/CT scan	imaging every 3-5y	MRI every 2y	NA	-	-	periodic imaging; posture exercises, cervical collar	-						
Other anomalies of the axial skeleton																	
Abnormality of the ribs	-	10 pairs of ribs	-	-	-	-	-	-	-	11 pairs of ribs (T1 ribs hypoplasia)	cervical ribs in C7	3/11		10	4/17		
Abnormality of the vertebral column (excluding the CVJ)	-	-	-	scoliosis	11 thoracic vertebrae, short coccyx	-	-	-	scoliosis, mild	kyphosis, mild; Schmorl's nodes	sacro-coccygeal cyst	5/11		(11)§			
Sacral dimple	-	+	+	+	+	+	+	-	+	-	+	8/11		30	8/25		

Note: The shades of grey and Italics helps to distinguish the two subsections: one for "Fused cervical vertebrae", one for "Vertebral dysmorphisms".

Abbreviations: CoRDS, Coordination of Rare Diseases at Sanford; CVJ, craniovertebral junction; F, female; M, male; m, months; NA, data not available; WDSTS, Wiedemann-Steiner Syndrome; y, years.

†Totals refers to the number of individuals presenting with the listed features out of the number of participants that were formally evaluated for the respective characteristics

‡'n' refers to the population size, but the number of cases effectively evaluated for each feature (e.g. via appropriate imaging) is unknown; the columns below indicate the absolute number of reports recorded in the CoRDS registry or received through the WDSTS parents support group

§Out of a total of 11 reports of vertebral fusion (location not specified), at least 6 concerned the CVJ (they were either annotated as Klippel-Feil in the registry or ascertained through enrollment in our study); see Supporting Information File 4 for further details

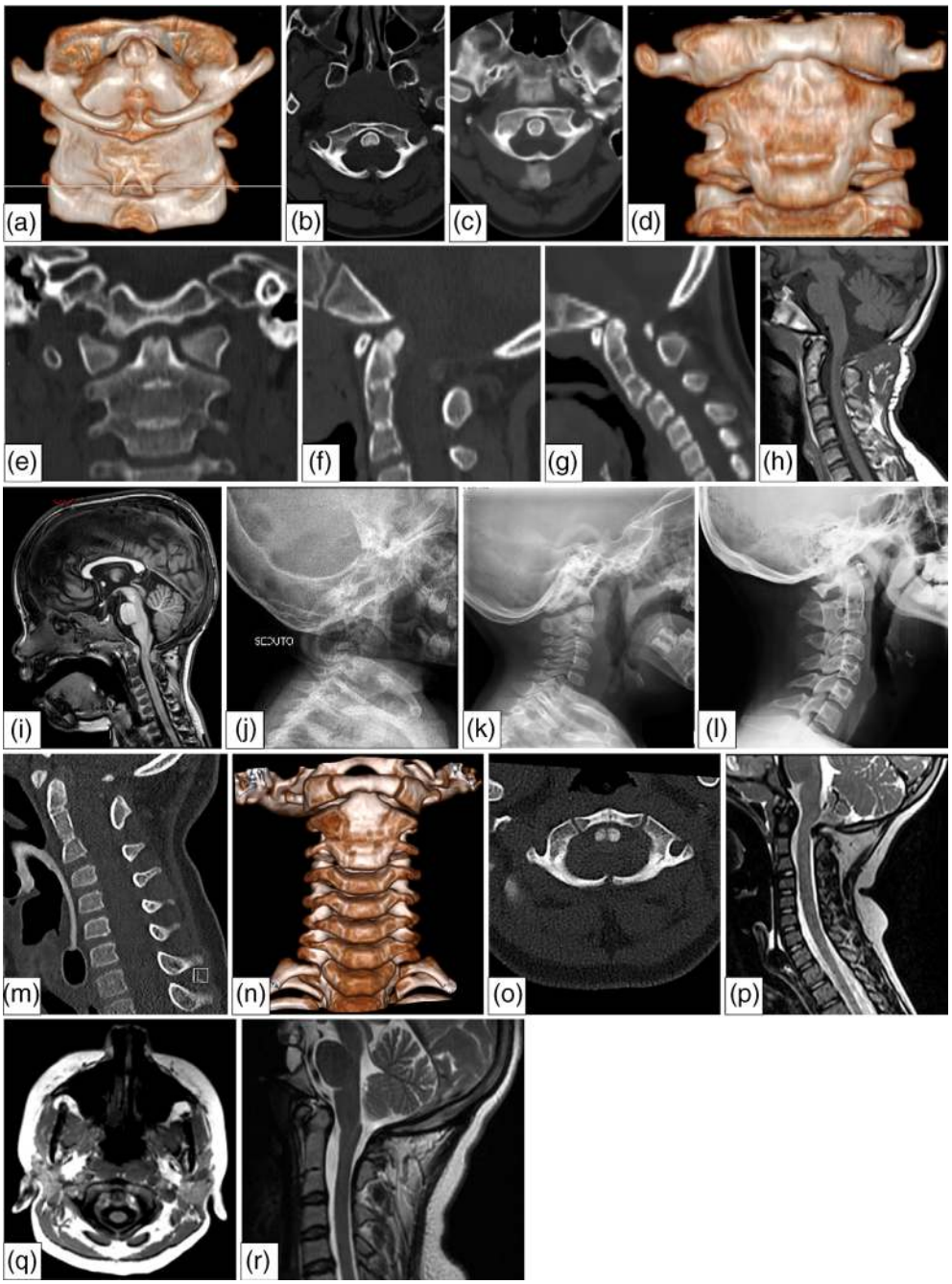

FIGURE 3 Radiological and neuroradiological imaging in the described WDSTS patients. (a–g) Pt.1, Cervical CT scans: (a) cleft and inturning of the posterior arch of C1, at age 7 years (oblique view, 3D reconstruction); (b,c) cleft and inturning of the posterior arch of C1, at 7 and 13 years of age (axial view); (d,e) cervical C2/C3 vertebral fusion at age 7 years (coronal view and 3D reconstruction); (f,g) cervical C2/C3 vertebral fusion, mild retroflexion and basilar invagination of the odontoid process at 7 and 13 years of age (sagittal view). (h) Pt.1, age 15 years, Cervical MRI (sagittal view) after surgical excision of the posterior arch of C1. (i) Pt.3, Cervical MRI (sagittal T1 view): small foramen magnum and spinal canal stenosis. (j–l) Pt.3, Pt.2, and Pt.9, Cervical X-rays: cervical C2/C3 vertebral fusion. (m–p) Pt.7, age 3 years: (m,n) Cervical CT scan (multiplanar reconstruction, sagittal view, and 3D reconstruction): cervical C2/C3 vertebral fusion; (o) Cervical MRI (axial T1 view): cleft of the posterior arch of C1; (p) Cervical MRI (sagittal T2 view): mild impression of the posterior arch of C1 on the bulbospinal junction. (q) Pt.8, Cervical MRI (axial T1 view): cleft of the posterior arch of C1. (r) Pt.10, Cervical MRI (sagittal T2 view): cervical C2/C3 vertebral fusion, mild impression of the odontoid process [Color figure can be viewed at wileyonlinelibrary.com]

not consider the neurological and radiological findings suggestive of a significant pathology of the CVJ; they viewed weakness and pain of neck and limbs as due to the premature disc degeneration caused by hEDS and in part as due to a iatrogenic origin. Follow-up recommendations included isometric exercises for neck strengthening, correct posture and sagittal balance; wearing a cervical collar while traveling or during periods of neck pain; monitoring the cervical region, particularly the C5–C6 instability, for the appearance of any sign that may require surgery.

Pt.11, a 17-years-old male, did not present with true CVJ anomalies, but X-rays showed congenital bilateral cervical ribs in C7, as well as a sacrococcygeal cyst. No related symptoms were noted and no specific follow-up was planned.

4 | DISCUSSION

To date, there are only three reports of CVJ anomalies in WDSTS, and although two provide a detailed description, none includes any associated imaging. In a cohort of 33 French patients, Baer et al. (2018) mentioned five cases of fused cervical vertebrae and one case of hypoplasia of the posterior arch of C1. Lebrun et al. (2018) described

a 10-year-old female with radiological evidence of Chiari malformation type I, basilar impression, cervical C2/C3 vertebral fusion and C1/occiput fusion. In a further 2-years-old male patient, head and cervical CT scans and MRI revealed narrowing at the CVJ with mild posterior indentation at the upper cervical cord and possible C1 compression (Feldman et al., 2019).

Consistent with these reports, the detailed radiological data of the 11 patients we enrolled showed CVJ anomalies that typically involve the cervical C1–C3 vertebrae. The predominant findings were vertebral segmentation defects (9/11), involving different cervical vertebrae and various degrees of fusion of the vertebral bodies and/or posterior elements. The most common was cervical C2/C3 vertebral fusion (7/11), also reported in Baer et al., 2018 and Lebrun et al., 2018; one individual had a more severe C1/C2 vertebral fusion, while another had a combination of C2/C3 and C1/occiput fusion. Chiari malformation type I, already noted in a few patients in the literature (Lebrun et al., 2018), was observed in only two of our participants, one of them borderline. However, half of our cohort showed mild spinal cord compression or spinal canal stenosis, in one case even complete effacement of cerebrospinal fluid (Pt.6), as the result of a variety of cervical dysmorphisms: midline cleft of the posterior arch of C1 (3/11) also with inturning of the ends (2/11), retroflexed or duplicated odontoid process with basilar invagination (4/11), small foramen magnum (3/11), dysmorphic occipital condyles (1/11). These features clearly imply a developmental origin (Connor, Chandler, Robinson, & Jarosz, 2001; Machnowska & Raybaud, 2014), and the presence of multiple patients with similar findings (some quite uncommon, such as C1 posterior arch cleft) suggests an inherent aspect of WDSTS rather than an effect secondary to this condition. In further support, no other gene variants potentially causative for CVJ anomalies were reported in the participants tested through WES/WGS. In particular, we were able to directly review the WES data of Pt.1 and they did not reveal any clinically relevant variant in the genes specifically associated with isolated Klippel-Feil syndrome: *GDF6* (OMIM 601147), *GDF3* (OMIM 606522), *MEOX1* (OMIM 600147) and *MYO18B* (OMIM 607295).

The association between LoF variants of the *KMT2A* gene and CVJ anomalies such as fused cervical vertebrae and/or Chiari type I is not surprising. This gene encodes a histone-lysine N-methyltransferase that participates in specific complexes mediating the methylation of lysine 4 of histone H3 (H3K4me) and the acetylation of lysine 16 of histone H4 (H4K16ac), tags for epigenetic transcriptional activation (Dou et al., 2005; Milne et al., 2002). Known targets include the *HOX* genes, a family of transcription factors essential for normal embryonic development: they regulate segment specification along the body axis, thus also controlling the positional identity of prevertebral bodies at the CVJ (Pang & Thompson, 2011). *Kmt2a* heterozygous (+/−) knockout mice display defects in segment identity along the axial skeleton and abnormalities of the C1 and C2 vertebrae (Yu, Hess, Horning, Brown, & Korsmeyer, 1995), which resemble some of the anomalies observed in patients with WDSTS.

Furthermore, protein–protein interaction networks (https://string-db.org/) show known and predicted interactions with other proteins having a role in histone-mediated chromatin remodeling. CREBBP, an interactor of the KMT2A transactivator domain, supports the role of some of these proteins in CVJ anomalies: de novo LoF/deletion variants in the *CREBBP* gene are responsible for Rubinstein-Taybi syndrome 1 (RSTS1, OMIM # 180849), a severe multisystemic disorder in which Chiari malformation type I is common (Ajmone et al., 2018; Lee et al., 2015) and a high risk of cervical vertebral anomalies has been reported, including vertebral fusion and abnormalities of the odontoid process (Milani et al., 2015; Yamamoto et al., 2005). Similar findings in a few cases of Kabuki syndrome (OMIM # 147920 and # 300867; Ciprero et al., 2005)—and in particular in one case associated with a LoF variant of *KDM6A* (Cheon & Ko, 2015)—further point to alterations in histone acetylation and methylation as causative of Chiari type I.

It must be noted that the incidence of Chiari malformation type I in these severe syndromes may be higher than reported. Indeed, most publications are about little children who, because of the young age and the psychomotor delay, are likely unable to manifest persistent headache, neck pain, or other Chiari type I symptoms that would have prompted an MRI scan. For the same reasons, CVJ anomalies associated with *KMT2A* defects are probably under-recognized in the literature and in public databases. In the DECIPHER database, among the patients with interstitial or distal 11q deletions involving the *KMT2A* gene, no Chiari type I or other CVJ defects were reported (DECIPHER, GRCh37, Search results for "gene:KMT2A," copy-number variant losses). On the other hand, both CoRDS registry data and our informal inquiries into WDSTS family networks suggest that *KMT2A* defects are associated with CVJ anomalies more frequently than expected (see Appendix S2—Additional results, including Tables S3 and S4).

Official data from the CoRDS registry 2018 update, combined with further details we received from the four CoRDS participants recruited in our cohort, revealed fused cervical vertebrae and/or Chiari malformation type I in eight out of 61 individuals with a confirmed *KMT2A* defect (13.1%) (Table S3). Informal inquiries into WDSTS family networks about the results of diagnostic imaging of the head and neck proved even more intriguing: out of 81 patients from a WDSTS parent support group, 15 stated that diagnostic imaging of the CVJ was normal and 38 reported CVJ anomalies, for the most part cervical C2/C3 vertebral fusion (Table S4). Since many individuals in this population did not reply (or provided non-relevant answers), a reporting bias favoring positive over negative answers had to be considered and the actual prevalence of CVJ anomalies could not be calculated. However, our estimates still indicated that CVJ anomalies concerned *at least* 38/81 patients (46.9%), suggesting that these features have been under-reported in the literature so far.

In fact, in our cohort, most CVJ anomalies were discovered as incidental findings during neuroradiological investigations performed in the context of developmental delay/ID. Only three participants complained about neck pain; none of the others reported clearly related symptoms, either because of their inability to express them, or because the manifestations were subclinical (e.g., C2/C3 vertebral fusion) and/or blended with the psychomotor delay inherent to WDSTS. Features such as cleft of the posterior arch of C1 are usually considered benign, but on occasion, especially in combination with

other cervical defects (e.g., vertebral fusion), they may lead to severe neurological deterioration (Connor et al., 2001). The evolution of the C1 posterior arch in Pt.1 at different ages, with the hemiarches progressively reducing the spinal canal, can be considered as evidence of these risks (Figure 3b,c). After surgical removal of the C1 hemiarches, Pt.1 acquired sphincter control and her motor coordination improved. For some participants, such as Pt.10, surgery was considered unnecessary, but other procedures were implemented to reduce the risk of spinal compression, such as postural exercises or wearing a cervical collar during stressful situations.

Acknowledgement of an increased incidence of CVJ anomalies in WDSTS patients is important to establish proper examination strategies. Clinical guidelines by Baer et al. (2018) suggested performing CT scan or MRI of the cervical spine upon diagnosis. Caregivers of WDSTS patients should be aware that clinical and neurological manifestations of CVJ anomalies—such as head or neck pain, reduced head mobility, torticollis, numbness of the extremities—may be very subtle. They should consider a multidisciplinary approach combining clinical and radiological information, for example, dynamic X-rays, in order to carefully look for these signs and plan appropriate neuroradiological investigations. An early diagnosis may allow caregivers to seek orthopedic assistance or neurosurgical correction before the appearance of overt symptoms, thus preventing possible complications ranging from motor impairment to life-threatening circumstances.

In conclusion, our data support the inclusion of CVJ anomalies in the phenotypic spectrum of WDSTS. Furthermore, there is some indication that developmental defects of the axial skeleton intrinsic to WDSTS may not be limited to the CVJ: two of our participants and an additional one in the CoRDS registry had scoliosis, one of our participants and a second one from CoRDS reported tethered cord, 8/11 individuals in our cohort as well as 30/61 in CoRDS and many more in the literature (e.g., 8/25 in Baer et al., 2018) had sacral dimple. Lastly—although this feature did not properly affect the CVJ—we included in our study an individual with a different cervical anomaly, supernumerary ribs in C7 (Pt.11), while two other participants had missing ribs, a finding also noted in four patients in Baer et al. (2018). Additional data will be needed to assess the exact incidence of these features in individuals affected with WDSTS, or with other syndromes associated with LoF/haploinsufficiency of genes involved in histone-mediated chromatin remodeling. However, these preliminary observations suggest the necessity of specific strategies for early identification and prevention of complications in affected individuals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

Livia Garavelli and Orsetta Zuffardi conceived the study and reviewed the data and the manuscript; Sara Giangiobbe and Stefano Giuseppe Caraffi carried out the investigations and drafted the manuscript; Ivan Ivanovski, Ilenia Maini, Marzia Pollazzon, Simonetta Rosato, Gabriele Trimarchi, Anna Lauriello, and Nancy J. Mendelsohn assisted with data collection and manuscript preparation; Maria Marinelli, Davide Nicoli, Chiara Baldo, Steven Laurie, and Aldesia Provenzano carried out molecular investigations; Josue Flores-Daboub, Elena Andreucci, Francesca Peluso, Renata Rizzo, Helen Stewart, Katherine Lachlan, Allan Bayat, Janice Baker, Alyssa Mendel, Gianluca Piatelli, Chiara Pantaleoni, Teresa Mattina, and Paolo Prontera provided and evaluated participants' data; Manuela Napoli, Giorgia Carboni, Gianluca Piatelli, and Sabrina Giglio assisted with data analysis, especially imaging data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request, except the data obtained from the CoRDS registry, which are subject to third-party restrictions and should be requested directly to CoRDS (https://research.sanfordhealth.org/rare-disease-registry).

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ENDNOTE

[1] Although Klippel-Feil syndrome originally indicated a distinct genetic condition (OMIM #118100, #214300, #613702, #616549), nowadays the term also refers to any congenital fusion of the cervical spine.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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