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Revisiting Wiedemann-Steiner Syndrome: Novel *KMT2A* Variants and Broadened Clinical Spectrum

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Background: Wiedemann-Steiner syndrome (WDSTS) is a rare autosomal dominant neurodevelopmental disorder caused by heterozygous pathogenic variants in *KMT2A*. Although several large international cohorts have helped define its broad clinical spectrum, data from underrepresented populations remain limited.

Aims: To characterize the molecular and phenotypic spectrum of Turkish patients with WDSTS and compare these findings with previously published cohorts.

Study Design: Multicenter retrospective cohort study.

Methods: Sixteen individuals from 15 unrelated families were recruited across Türkiye. Clinical information was obtained through medical records and systematic phenotyping. Molecular analyses included next-generation sequencing or targeted variant testing, and the variants were classified according to the American College of Medical Genetics and Genomics guidelines.

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Results: Fifteen distinct *KMT2A* variants were identified, including nine novel alleles. Most variants were predicted to result in loss of function; only one was a missense substitution. Neurodevelopmental involvement was prominent, with developmental and speech delays, intellectual disability, and behavioral comorbidities such as autism spectrum disorder and attention-deficit/hyperactivity disorder. Endocrine evaluation revealed growth hormone deficiency in approximately half of the tested patients. Ophthalmologic, cardiac, and dental abnormalities, including delayed tooth eruption, further expanded the known phenotype. Additional systemic features included skeletal, genitourinary, and immunological findings. Comparison with previously reported cohorts displayed no statistically significant genotype-phenotype correlations, although truncating variants appeared to be associated with more pronounced neurodevelopmental and behavioral manifestations.

Conclusion: This report presents the largest Turkish WDSTS cohort to date, expands the known *KMT2A* variant spectrum with nine novel alleles, and highlights several underreported clinical features. Beyond its immediate clinical relevance, the study further supports *KMT2A* as a key chromatin regulator and an "umbrella gene" within the chromatinopathy spectrum. Growing recognition of these disorders underscores the need for systematic, multidisciplinary surveillance and contributes to the expanding global understanding of their shared pathogenic mechanisms.

INTRODUCTION

Wiedemann-Steiner syndrome (WDSTS; OMIM #605130) is a rare autosomal dominant neurodevelopmental disorder caused by heterozygous pathogenic variants in *KMT2A* (also known as *MLL).* *KMT2A* encodes a histone lysine methyltransferase that plays a central role in epigenetic regulation, early embryonic development, and transcriptional control of key developmental pathways.[1,2] Clinically, WDSTS is characterized by developmental delay or intellectual disability, distinctive facial features, hypertrichosis cubiti, short stature, and behavioral abnormalities. Its phenotypic spectrum is broad and may include additional manifestations such as seizures, growth hormone (GH) deficiency, congenital heart defects, and urogenital anomalies.[3,4]

With increasing accessibility of exome sequencing, the number of diagnosed WDSTS cases has risen, allowing clearer delineation of genotype-phenotype relationships and improved understanding of functional domains within *KMT2A*. Large international cohorts have contributed substantially to defining the clinical variability of the syndrome.[5,6] Recent studies have also highlighted the clustering of pathogenic missense variants within specific protein domains and proposed functional modeling strategies to assist variant interpretation.[7,8] Despite these advances, the literature remains heavily skewed toward European and East Asian populations, leaving significant gaps in data from underrepresented groups.

In this context, the present study aimed to characterize the molecular and phenotypic features of individuals diagnosed with WDSTS across multiple centers in Türkiye. By reporting 16 previously unreported patients and identifying nine novel *KMT2A* variants, this study contributes to expanding the global variant spectrum. Detailed clinical phenotyping further enables comparison with existing cohorts, exploration of genotype-phenotype correlations, and identification of underreported clinical features within this population.

MATERIALS AND METHODS

Ethical considerations

This study protocol followed the principles of the Declaration of Helsinki. Written consent was obtained from the patients' parents for genetic testing and the disclosure of genetic and clinical data for scientific purposes. Ethical approval was obtained from the Non-Interventional Clinical Research Evaluation Committee of the Faculty of Medicine at Aydın Adnan Menderes University (protocol number: 19, date: 11/07/2025).

Inclusion criteria

Patients were deemed eligible for inclusion if they met all of the following criteria: (i) had pathogenic or likely pathogenic variant in *KMT2A*, classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG);[9] (ii) their clinical features were consistent with WDSTS, as determined through a comprehensive clinical evaluation; (iii) did not have other variants in multigene panel analyses that could account for their phenotypic findings.

Clinical information

This retrospective study utilized clinical data extracted from patients' existing medical records. Collected information included demographic characteristics (age and sex), family history, particularly the presence of consanguinity and similar conditions among relatives, and perinatal data, such as pregnancy and delivery details, birth weight and length, head circumference, and perinatal complications. Comprehensive physical examination findings were reviewed with emphasis on growth parameters, craniofacial dysmorphism, hair and skin anomalies, and additional system-specific abnormalities. Relevant laboratory investigations, including hematological, biochemical, and hormonal assessments (when available), were examined. Imaging studies, such as brain magnetic resonance imaging, echocardiography, and skeletal radiographs, were evaluated when performed. Follow-up records were analyzed to document longitudinal changes and the evolution of clinical features over time.

Genetic analysis and variant annotation

Genetic analyses were conducted in 15 unrelated families. Genomic DNA was extracted from peripheral venous blood samples using standard protocols. Fifteen individuals underwent next-generation sequencing (NGS), including clinical exome or whole-exome sequencing, while one patient underwent targeted testing following identification of a familial pathogenic *KMT2A* variant in an affected offspring (Table 1).

Sequence reads were aligned to the GRCh37/hg19 human reference genome and processed using validated bioinformatic pipelines. Variant annotation and filtering incorporated allele frequency thresholds (≤ 0.01% for final prioritization) with reference to population databases, including gnomAD, the 1,000 Genomes Project, Turkish Variome, and ExAC. Additional quality control criteria included a minimum read depth of 30× and an alternate allele fraction of at least 20%. Variants present in more than 10 unrelated individuals in the in-house database were excluded to minimize the inclusion of common polymorphisms and platform-specific artifacts. Candidate pathogenic and likely pathogenic variants were interpreted and classified according to the ACMG guidelines,[9] incorporating evidence from ClinVar, HGMD, and relevant published literature. All pathogenic or likely pathogenic variants were independently confirmed, and parental segregation analyses were performed in all cases.

RESULTS

Patient demographics

The cohort included 16 individuals from 15 unrelated families (11 males and 5 females) diagnosed with WDSTS across multiple centers in Türkiye (Figure 1). One father-son pair was included; the father received his diagnosis after the pathogenic *KMT2A* variant was identified in his child. The median age at last clinical evaluation was 10.87 years (range: 13 months-34 years). Consanguinity was documented in 3 of 16 (19%) individuals, including two families with third-degree parental relationships and one with a fifth-degree relationship (Supplementary 1).

Molecular findings

All individuals carried heterozygous pathogenic or likely pathogenic variants in *KMT2A*. A total of 15 distinct variants were identified, of which 14 (93%) were unique to a single individual. The only recurrent variant, *c.478C > T*, occurred in two related individuals (patients #1 and #2). Fourteen (93%) of these variants were predicted to result in loss-of-function (LoF), and one was a missense substitution located in exon 5 within the Cys-X-X-Cys (CXXC) domain (Figure 2). All truncating variants were predicted to undergo nonsense-mediated decay (NMD), except for the frameshift variant c.11832_11835del identified in P10. Fourteen individuals had *de novo* variants; paternal inheritance was confirmed in patients #1 and #9. Nine (60%) variants were novel (Supplementary 2), absent from population databases such as gnomAD. Eight had not been previously cataloged in ClinVar. One variant, *KMT2A* c.2569G > T, appears in ClinVar (Variation ID: 1335981) but has not yet been described in the literature. Six variants had been previously reported in published WDSTS cohorts (Table 1).[4-6,10-15]

Segregation analysis revealed that the splice-acceptor variant c.10901-1G > *T* in P9 was inherited from his father. The variant was not detected in paternal NGS data, likely due to low read depth or allelic dropout, and was subsequently confirmed via Sanger sequencing. The father, who carried the same heterozygous variant, had a history of mild motor developmental delay but lacked dysmorphic or syndromic features. According to the ACMG criteria, the variant was classified as a variant of uncertain significance (PVS1 + PM2_Supporting).

Clinical findings

Prenatal and perinatal findings

Prenatal assessments revealed heterogeneous findings, including oligohydramnios, intrauterine growth restriction (IUGR), and the risk of preterm delivery. Half of the individuals were born preterm (median gestational age: 36.5 weeks).

Growth parameters

Short stature was noted in 8 of 15 (53%) individuals, while microcephaly was documented in 6 of 12 (50%). Endocrine abnormalities were observed, with GH deficiency identified in approximately half of those evaluated.

Craniofacial and dental features

Typical WDSTS craniofacial features, including thick eyebrows, long eyelashes, synophrys, a broad nasal bridge, and a thin upper lip, were observed across the cohort. Dental anomalies were present in 8 of 16 (50%) individuals, with variable patterns of tooth eruption.

Dermatologic findings

Hypertrichosis cubiti was present in 7 of 16 (44%) individuals, and several patients exhibited generalized hypertrichosis.

Neurodevelopmental and neurological findings

All patients showed neurodevelopmental impairment of varying severity. Developmental delay (11/16; 69%) and speech delay (9/16; 56%) were frequent. Cognitive assessments (available for 12 individuals) revealed mild-to-moderate intellectual disability in all evaluated cases. Behavioral comorbidities were common, including autism spectrum disorder (ASD) (10/15; 67%), attention-deficit/hyperactivity disorder (ADHD) (8/15; 53%), and other behavioral disturbances (9/15; 60%). Seizures or seizure-like events occurred in 5 of the 16 (31%) patients. Neuroimaging abnormalities, including hypoplastic corpus callosum and white matter changes, were identified in 5 of 13 (38%) individuals (Figure 3).

Systemic findings

Ophthalmological abnormalities were documented in nearly all individuals. Skeletal findings, including scoliosis and pectus excavatum, were frequent. Cardiac, genitourinary, and gastrointestinal findings were variably present across the cohort. One individual demonstrated recurrent infections and elevated immunoglobulin E (IgE) levels, raising the possibility of immune dysregulation. A summary of key clinical features is provided in Supplementary 3.

To assess potential genotype-phenotype associations, data from four previous large WDSTS cohorts[4,6,14,16] were combined with the present study. In total, 73 individuals with truncating variants and 18 with

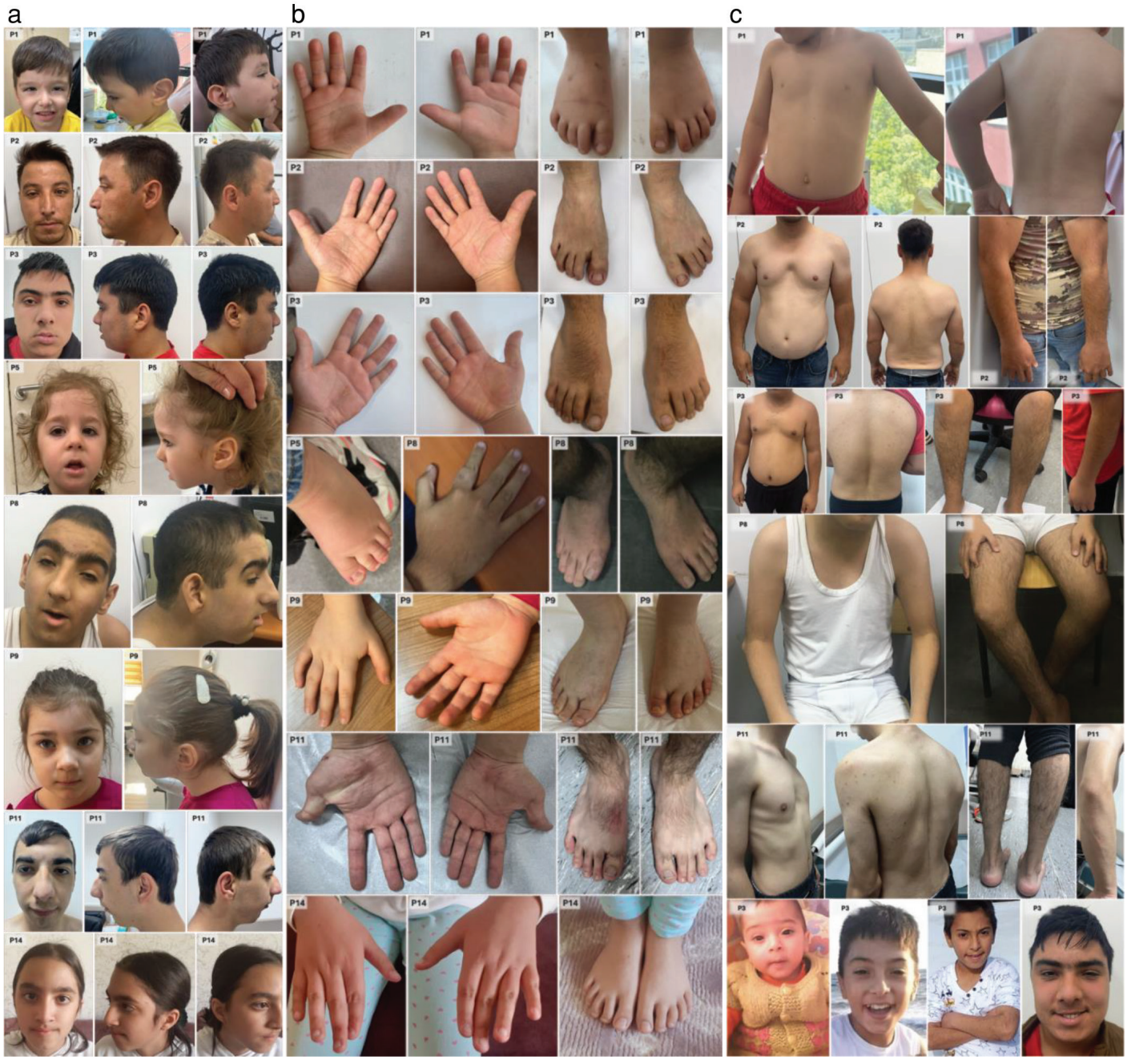

FIG. 1. Dysmorphic features in individuals with Wiedemann-Steiner syndrome from our cohort who provided consent for photographic documentation. (a) Facial features of the patients. (b) Hand and foot photographs of the patients. (c) Photographs of trunks and extremities of the patients, and serial photographs of patient 3 (P3) at different ages, demonstrate the phenotypic evolution over time.

non-truncating variants were analyzed (Table 2). The prevalence of major clinical features, including neurodevelopmental delays, growth abnormalities, and systemic findings, was broadly similar between the two groups. Fisher's exact test revealed no statistically significant genotype-phenotype correlations for any evaluated feature (all $p > 0.05$).

DISCUSSION

This multicenter cohort of 16 Turkish individuals with WDSTS represents the largest series reported from Türkiye and adds to the growing body of international data on the disorder. Baer et al.[6] described 33 French patients and identified 29 novel *KMT2A* variants, highlighting the wide clinical variability. Li et al.[14]

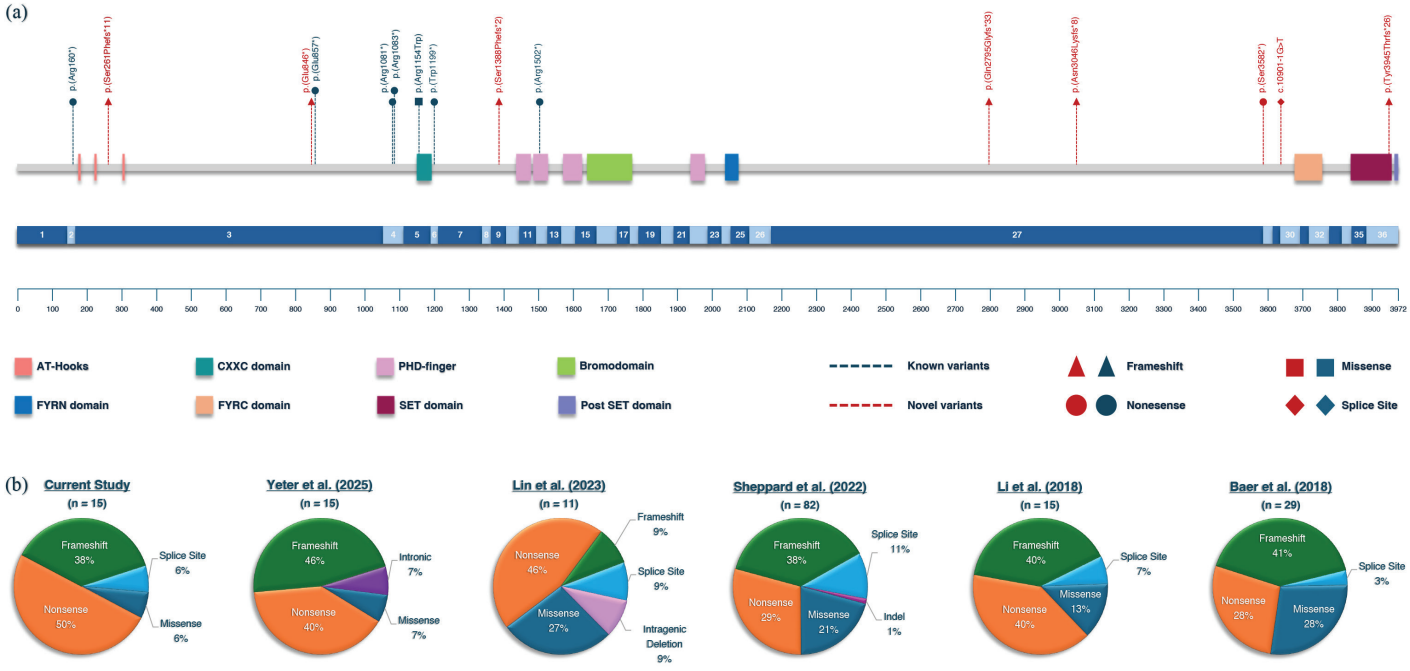

FIG. 2. (a) Schematic representation of the 15 *KMT2A* variants identified in our cohort, mapped to the protein structure and the corresponding exons. Novel variants detected in this study are highlighted in red (dashed lines), whereas previously reported variants are shown in blue (dashed lines). Different shapes indicate various types: triangles for frameshift, circles for nonsense, squares for missense, and diamonds for splice site mutations. The schematic also illustrates the known functional domains of the KMT2A protein, including the AT-Hooks (Adenine-Thymine hook), CXXC domain (Cysteine-X-X-Cysteine domain), PHD-finger (Plant Homology Domain finger), Bromodomain, FYRN (Phenylalanine-Tyrosine Rich N-terminal domain), FYRC (Phenylalanine-Tyrosine Rich C-terminal domain), SET [Su(var)3-9 Enhancer-of-zeste Trithorax domain], and Post-SET domains. Protein domain boundaries were defined based on the UniProt database (UniProtKB-Q03164). (b) The distribution of variant types in our cohort is presented as pie charts and compared with previously published WDSTS cohorts (Yeter et al.,[16]; Lin et al.,[4]; Sheppard et al.,[5]; Li et al.,[14]; Baer et al.,[6]).

WDSTS, Wiedemann-Steiner syndrome

reported 14 Chinese patients who demonstrated that missense variants within the CXXC domain may be associated with more severe neurodevelopmental phenotypes. Sheppard et al.[5] expanded the spectrum in a diverse cohort of 104 individuals, delineating long-term outcomes and genotype-phenotype correlations, including an association between non-LoF variants and seizures. More recently, Lin et al.[4] and Yeter et al.[16] described 11 Chinese and 15 Turkish patients, respectively, extending the molecular spectrum and adding novel phenotypic observations.

The demographic composition of our cohort (11 males, 5 females, and one father-son pair) and the predominance of *de novo* variants align with an autosomal-dominant, primarily sporadic condition. The paternally inherited variant in our study underscores the relevance of cascade testing after identification of an index case and illustrates intra-familial variability. In our series, the variant spectrum was dominated by predicted LoF alleles (93%), with only one missense variant. Consequently, our dataset was underpowered to test the effects of domain-specific missense variants proposed by the literature. Nevertheless, the predominance of LoF variants was found to be consistent with previous cohorts and supports haploinsufficiency as the primary disease mechanism in WDSTS.[5,6,16] The observation of seizures in a patient without a missense variant further supports that epileptology in WDSTS is not restricted to specific variant classes. The high rate of novel alleles (60%) underscores substantial allelic heterogeneity and extends the known variant spectrum in *KMT2A*; the observation that c.2569G > T is cataloged in the ClinVar database but remains unpublished in the primary literature further illustrates gaps between variant databases and peer-reviewed reports. When comparatively evaluated with previously reported WDSTS cases from the Turkish population, no mutational hotspot was detected; however, the variants appeared to cluster predominantly within exons 3–10.[16-18] The identification of nine novel *KMT2A* variants in this cohort provides new insights into the mutational landscape and pathogenic mechanisms underlying WDSTS. *In silico* analyses using Combined Annotation Dependent Depletion (CADD; v1.7) revealed that all variants possess high pathogenicity potential and are consistent with the LoF mechanism. Specifically, six frameshift, two nonsense, and one canonical splice-acceptor variant were identified, all predicted to induce premature termination or splice-disruption leading to NMD. Their CADD-PHRED scores (> 30 in seven variants) place them within the most deleterious 0.5% of the human genome, whereas strong evolutionary conservation (PhastCons ≈ 1.0, PhyloP ≈ 5.7) supports their functional importance. These findings collectively fulfill the ACMG PVS1 criterion, further reinforcing the likelihood of their pathogenic nature (Supplementary 4).

TABLE 1. Spectrum of *KMT2A* Variants İdentified in the Cohort.

Patient	Gene	RefSeq ID	Transcript change	Protein change	Exon number	Variant type	ACMG classification	Mutation taster	Gnomad	Turkish variome	Literature
P1-P2	*KMT2A*	NM_001197104.2	c.478C > T	p.(Arg160*)	2	Nonsense	Pathogenic PVS1, PS4_M, PM2_P, PM6	Deleterious	Not present	Not present	Known Baer et al.[6]
P3	*KMT2A*	NM_001197104.2	c.9138_9142del	p.(Asn3046Lysfs*8)	27	Frameshift	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P4	*KMT2A*	NM_001197104.2	c.8383_8384del	p.(Gln2795Glyfs*33)	27	Frameshift	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P5	*KMT2A*	NM_001197104.2	c.3596G > A	p.(Trp1199*)	6	Nonsense	Pathogenic PVS1, PS4_P, PM2_P, PM6	Deleterious	Not present	Not present	Known Di Fede et al.[10]
P6	*KMT2A*	NM_001197104.2	c.3247C > T	p.(Arg1083*)	4	Nonsense	Pathogenic PVS1, PS4_P, PM2_P, PM6	Deleterious	Not present	Not present	Known Dunkerton et al.[11]
P7	*KMT2A*	NM_001197104.2	c.4504C > T	p.(Arg1502*)	12	Nonsense	Pathogenic PVS1, PS2, PS4_M, PM2_P	Deleterious	Not present	Not present	Known Sheppard et al.[5] Lin et al.[4]
P8	*KMT2A*	NM_001197104.2	c.2535dup	p.(Glu846*)	3	Nonsense	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P9	*KMT2A*	NM_001197104.2	c.10901-1G > T	-	Intron 29	Splice acceptor	VUS PVS1, PM2_P	Deleterious	Not present	Not present	Novel
P10	*KMT2A*	NM_001197104.2	c.11832_11835del	p.(Tyr3945Thrfs*26)	36	Frameshift	Likely Pathogenic PVS1_M, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P11	*KMT2A*	NM_001197104.2	c.10745C > G	p.(Ser3582*)	27	Nonsense	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P12	*KMT2A*	NM_001197104.2	c.4162_4163del	p.(Ser1388Phefs*2)	9	Frameshift	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P13	*KMT2A*	NM_001197104.2	c.3460C > T	p.(Arg1154Trp)	5	Missense	Pathogenic PS4, PS2, PM2_P, PM5, PP2, PP3	Deleterious	Not present	Not present	Known Lebrun et al.[12] Baer et al.[6] Giangiobbe et al.[13]
P14	*KMT2A*	NM_001197104.2	c.779dup	p.(Ser261Phefs*11)	3	Frameshift	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel
P15	*KMT2A*	NM_001197104.2	c.3241C > T	p.(Arg1081*)	4	Nonsense	Pathogenic PVS1, PS2, PS4_M, PM2_P	Deleterious	Not present	Not present	Known Li et al.[14] Silveira et al.[15]
P16	*KMT2A*	NM_001197104.2	c.2569G > T	p.(Glu857*)	3	Nonsense	Pathogenic PVS1, PM2_P, PM6	Deleterious	Not present	Not present	Novel

FIG. 3. Distribution of major clinical features in Wiedemann-Steiner syndrome. The panels illustrate (a) neurological, (b) dysmorphic and skeletal, and (c) other clinical features, highlighting similarities and differences between the current cohort and previous reports.

N/A, not applicable.

TABLE 2. Comparison of Clinical Features Between Patients with Truncating and Non-Truncating Variants Across Five Cohorts.

	Current study		Yeter et al.[16]		Lin et al.[4]		Li et al.[14]		Baer et al.[6]		Total		
	Truncating (n = 15)	Non-truncating (n = 1)	Truncating (n = 13)	Non-truncating (n = 2)	Truncating (n = 8)	Non-truncating (n = 3)	Truncating (n = 14)	Non-truncating (n = 2)	Truncating (n = 23)	Non-truncating (n = 10)	Truncating (n = 73)	Non-truncating (n = 18)	*p* value
Microcephaly	5/11	1/1	5/13	2/2	0/8	1/3	8/14	0/2	7/20	3/10	25/66	7/18	1.0
Short stature	7/14	1/1	9/13	2/2	7/8	3/3	8/14	2/2	10/22	5/10	41/71	13/18	0.2944
Developmental delay	11/15	0/1	13/13	2/2	7/8	3/3	9/14	1/2	16/21	8/9	56/71	14/17	1.0
Intellectual disability	12/12	n.e.	12/13	1/2	5/8	3/3	13/14	1/1	23/23	10/10	65/70	15/16	1.0
Behavioural problems	12/15	n.e.	10/13	1/2	1/8	1/3	4/14	1/2	7/22	3/9	34/72	6/16	0.4266
Language delay	9/15	0/1	12/13	2/2	n.i.	n.i.	11/12	1/2	n.i.	n.i.	32/40	3/5	0.3057
Hypotonia	8/13	0/1	7/13	2/2	3/8	2/3	n.i.	n.i.	11/21	7/10	29/55	11/16	0.3909
Seizures	4/15	0/1	2/13	0/2	1/8	0/3	n.i.	n.i.	1/22	3/9	8/58	3/15	0.6857
Hypertrichosis cubiti	7/15	0/1	5/13	1/2	4/8	0/3	7/14	0/2	14/21	5/10	37/71	6/18	0.1917
Ocular abnormalities	14/15	1/1	6/13	2/2	1/8	1/3	5/14	0/2	14/22	5/10	40/72	9/18	0.7928
MRI findings	5/12	0/1	5/12	0/2	1/4	3/3	n.i.	n.i.	7/20	3/9	18/48	6/15	1.0
GH deficiency	5/10	n.e.	n.i.	n.i.	1/2	0/1	2/2	1/1	n.i.	n.i.	8/14	1/2	1.0
Cardiovascular defects	3/13	0/1	4/12	1/2	3/4	3/3	2/14	1/2	7/15	1/7	19/58	6/15	0.7610
Urogenital findings	5/15	0/1	8/13	1/2	3/8	2/3	n.i.	n.i.	5/13	2/10	21/49	5/16	0.5590
Hearing loss	1/14	0/1	n.i.	n.i.	1/8	1/3	n.i.	n.i.	n.i.	n.i.	2/22	1/4	0.4076

p values were calculated using Fisher's exact test, based on the number of individuals with available data for each clinical feature. None of the comparisons reached statistical significance (all $p > 0.05$), and results should be interpreted descriptively. "n.e." indicates that patients were not eligible for this assessment (e.g., due to age), while "n.i." denotes features not informative or not uniformly reported across cohorts.
GH, growth hormone; MRI, magnetic resonance imaging; n.e., not eligible; n.i., not informative.

From a broader perspective, these results strengthen the concept that haploinsufficiency of *KMT2A* is the principal pathogenic mechanism in WDSTS. Beyond their molecular novelty, the consistent clinical manifestations observed among patients carrying these LoF variants suggest that allelic context or modifier genes possibly contribute to the variability in systemic expressivity. Incorporating these variants into international reference databases is expected to refine diagnostic interpretation and support more accurate variant reclassification in the future. Ultimately, expanding the catalog of pathogenic *KMT2A* alterations can enhance the genotype-phenotype correlation frameworks, improving both clinical recognition and long-term management of individuals with WDSTS.

Identifying the canonical splice-acceptor variant c.10901-1G > T in both the patient and his mildly affected father in this case further supports variable expressivity and possible reduced penetrance in *KMT2A*-related disorders. This observation further illustrates that pathogenic *KMT2A* variants may occasionally yield subclinical phenotypes or go unrecognized in adults. A comparable canonical splice-site substitution (c.11322-1G > A) was functionally validated by Lebrun et al.,[19] confirming a LoF effect through frameshift and the loss of the C-terminal SET domain. By analogy, the c.10901-1G > T variant may disrupt normal splicing and cause haploinsufficiency, which is consistent with the established pathogenic mechanism in WDSTS. Considering its canonical location and strong *in silico* predictions, functional RNA analysis would be valuable to confirm the predicted splicing defect and strengthen variant interpretation in future studies.

In our combined analysis of truncating and non-truncating variants across all available cohorts.[4,6,14,16] Fisher's exact test revealed no statistically significant genotype-phenotype correlations (all $p > 0.05$). Nevertheless, the descriptive patterns were evident. Truncating variants were consistently associated

with a higher frequency of neurodevelopmental features, such as language delay (80% vs. 60%), behavioral problems (47% vs. 35%), and hypertrichosis cubiti (52% vs. 33%). Intellectual disability was almost universal in both groups (> 90%). The short stature and developmental delay were also more frequent in both categories, with no meaningful differences detected. Other features, including microcephaly, ocular abnormalities, hypotonia, seizures, cardiovascular anomalies, and urogenital findings, revealed no clear separation between the groups. These findings suggest that truncating variants may predispose individuals to a more pronounced neurodevelopmental and dysmorphic phenotype. However, the limited number of non-truncating cases precludes robust statistical confirmation. Similar limitations have been recorded in previous cohort studies, where non-truncating variants were reported less frequently. This reduced the statistical power for direct comparisons. Overall, our results support that truncating alleles in *KMT2A* lead to more severe expressivity, emphasizing the phenotypic overlap between the variant classes. Further research involving larger international cohorts and meta-analyses is thus essential to determine whether these descriptive trends reflect genuine biological differences or sampling biases inherent to rare disease cohorts.

Neurodevelopmental involvement was near-universal, with frequent developmental (69%) and speech delay (56%), and behavioral comorbidities spanning ASD (67%), ADHD (53%), and anxiety/other behavioral disturbances (60%) in the current study.[20,21] The proportion of patients exhibiting these behavioral disturbances was higher than in the corresponding cohorts. This distinctive behavioral profile in our Turkish cohort suggests a higher burden of neuropsychiatric manifestations compared with previously reported populations, possibly reflecting the underlying genetic, cultural, or ascertainment differences. These findings further support the importance of systematic neuropsychiatric assessment and early behavioral interventions in WDSTS.

Seizure-related manifestations were observed in 19% of individuals, which is consistent with prior studies.[1,3,5] Although some authors have proposed higher seizure risk among missense variants in specific functional domains, our findings reaffirm that epilepsy in WDSTS is not limited to any particular variant class and should prompt phenotype-based rather than variant-restricted neurological surveillance.[7,14,22] Neuroimaging abnormalities, including hypoplastic corpus callosum, white matter changes, and mega cisterna magna, were present in 38% of patients, reflecting disrupted neurodevelopment consistent with chromatin regulatory dysfunction.

The prenatal window in WDSTS remains under-documented in large cohorts. Here, we retrospectively collated heterogeneous antenatal findings, including increased miscarriage risk, IUGR, and oligohydramnios on ultrasound. Half of the cohort was born preterm (median 36.5 weeks), suggesting the need to sensitize obstetric teams to fetal growth/liquor anomalies when dysmorphology or developmental concerns later raise suspicion for WDSTS. Perinatal anthropometry was largely within reference ranges, albeit growth at the last examination revealed a short stature in 53% and microcephaly in 50%, with delayed bone age in 30% of the assessed individuals. Prenatal and perinatal findings were heterogeneous. However, their recurrence across multiple cases suggests that these complications may represent early developmental manifestations of WDSTS. This observation supports the need for systematic obstetric monitoring in suspected cases.

Endocrine comorbidities extended beyond GH deficiency to include hypothyroidism, pubertal disturbances, and insulin resistance. These findings support a structured endocrine follow-up, including auxology with standard deviation score (SDS) tracking, thyroid function, pubertal assessment, and targeted metabolic screening.[5,23,24] In our cohort, GH deficiency was identified in 5 out of 10 patients who underwent biochemical screening. Importantly, these evaluations were performed in all patients at the time of diagnosis, without any prior clinical selection. The observed rate was found to be consistent with the findings of Baer et al.,[6] who reported GH deficiency in 6 of 12 patients. However, marked differences are recorded when compared with other cohorts. For instance, Li et al.[14] restricted GH screening to three patients presenting with short stature and reported GH deficiency in all of them. Similarly, Lin et al.[4] tested only those patients who had a short stature with GH deficiency in 33% of cases. In contrast, the largest series by Sheppard et al.[5] reported endocrine evaluations in only 34% of the patients, while Yeter et al.[16] described GH screening in a single patient. These discrepancies likely reflect that GH testing in most published cohorts has been limited to patients with overt endocrinological manifestations, restricting the ability to determine the true prevalence of GH deficiency in WDSTS. A further strength of our cohort is the longitudinal evaluation of GH deficiency and treatment outcomes. Among the five individuals with biochemically confirmed GH deficiency, four received GH therapy. Two of these reached final height, with SDS values of –2.1 and –1.3, respectively. Notably, our adult patient was diagnosed with GH deficiency despite having a height within the population-normal range (166 cm; –1.65 SDS for Türkiye). Based on parental statures (father: 173 cm; mother: 163 cm), his estimated target height was approximately 174.5 cm, indicating that his achieved height falls below his genetic potential. This highlights that endocrine dysfunction may be present even in the absence of overt short stature when assessed solely using population-based SDS norms. Conversely, some patients without biochemical GH deficiency exhibited decreased height SDS or remained within normal ranges, illustrating the complex and non-linear relationship between GH status and linear growth. Taken together, these findings support the importance of incorporating parental height when evaluating growth and advocate for systematic GH testing in all individuals with WDSTS, irrespective of auxological presentation. This suggests that endocrine-growth relationships in WDSTS may be more intricate than previously appreciated.

Ocular, otologic, and dental findings emerged as particularly informative domains in our cohort. Ophthalmological abnormalities were present in 94% of patients, including commonly reported features such as strabismus and ptosis, as well as less frequently documented findings such as bilateral coloboma, blue sclera, and retinal arterial tortuosity. Because these latter findings are rarely emphasized in previous cohorts, our results support implementing systematic ophthalmic evaluation, including dilated fundus

examination, for all individuals with WDSTS.[5,6]

The neuro-otological manifestations were consistent with the literature; given that hearing loss is a recognized symptom of WDSTS, routine audiological surveillance remains essential.[10] Dental anomalies were identified in half of our cohort. Importantly, this study presents the first age-specific evidence of delayed tooth eruption in a 4-year-old WDSTS patient. Prior reports have more commonly described premature eruption, early exfoliation, or supernumerary/impacted teeth.[5,10] This observation broadens the phenotypic spectrum by showing that disturbances in dental timing can occur in either direction. Biologically, this is plausible given that *KMT2A* regulates developmental pathways, including HOX and WNT, integral to odontogenesis and tooth eruption.[25,26] Additionally, the higher frequency of dental anomalies in our cohort may reflect the fact that prior studies often did not classify dental findings under a separate category, leading to underreporting. Together, our results support systematic surveillance of dental morphology and careful tracking of eruption timing as part of comprehensive WDSTS management.

The extracranial somatic burden was substantial. Skeletal abnormalities were present in nearly all cases (93%), including scoliosis, pectus excavatum, and digital anomalies. Hypertrichosis was observed in 44% of patients with classic cubital involvement and more commonly on the back and lower extremities (56%). Genitourinary anomalies (31%), including cryptorchidism, hydronephrosis/horseshoe kidney, and unilateral pelviectasis, were comparable to those in previous cohorts, supporting the inclusion of baseline renal ultrasound in routine surveillance.[6,27] Gastrointestinal issues, primarily feeding difficulties and constipation (31%), further contributed to early-life morbidity and care complexity.

Cardiovascular involvement was documented in three patients, including atrial septal defect, patent ductus arteriosus, and Wolff-Parkinson-White pattern. While congenital heart defects are well recognized in WDSTS, pre-excitation syndromes have rarely been described. Our observation suggests that the cardiac phenotype may extend into the electrophysiologic domain.[5,6,25] Clinically, this supports maintaining a low threshold for baseline and interval electrocardiography, especially in the presence of palpitations, syncope, or perioperative considerations.

Immune involvement was identified in a notable proportion (40%) of our cohort, including elevated IgE levels in one patient. Although previous studies have described hypogammaglobulinemia and non-specific Ig abnormalities,[5,28] elevated IgE has not been specifically highlighted. Thus, our finding broadens the spectrum of potential immune dysregulation in WDSTS. Recent conceptual frameworks emphasize that *KMT2A* functions not only as the locus for WDSTS but as an "umbrella gene" implicated in multiple Mendelian disorders.[18,29] This broader perspective helps explain the phenotypic overlap between WDSTS and other chromatinopathies and why patients may initially receive alternative syndromic diagnoses. Our findings reinforce this view by demonstrating that WDSTS is a multisystem disorder with wide phenotypic variability, underscoring the importance of comprehensive genomic evaluation and longitudinal, multidisciplinary care.

The major strengths of this study include its multicenter design, the largest Turkish WDSTS cohort reported to date, and comprehensive molecular confirmation for all cases. The study also highlights the critical role of genetic diagnosis in rare diseases.[30] The high proportion of novel *KMT2A* variants (60%) and the systematic evaluation of underreported clinical domains, such as endocrine, ophthalmologic, dental, renal, and immunologic features, further enhance the value of our findings. Importantly, GH testing was performed uniformly in all available patients at diagnosis, minimizing the ascertainment bias that has affected earlier studies.

Nevertheless, several limitations must be acknowledged. First, the retrospective design resulted in incomplete data for certain variables (e.g., birth head circumference, bone age, and detailed cognitive assessments). Second, age heterogeneity among patients may have influenced the documentation of age-dependent features, including pubertal development and dentition. Third, the relatively small sample size (16 individuals from 15 families) limits the precision of frequency estimates and may contribute to the under- or over-ascertainment of specific clinical features. Therefore, the findings should be viewed as descriptive and hypothesis-generating rather than representative of population-level estimates. Furthermore, although the combined genotype-phenotype analysis incorporated data from five independent cohorts, the overall sample size remained modest, restricting the statistical power to detect subtle differences between truncating and non-truncating variants. Future studies using larger, harmonized multicenter datasets and deeper analyses of variant-specific effects will be essential to validate these observations.

Ethics Committee Approval: Ethical approval was obtained from the Non-Interventional Clinical Research Evaluation Committee of the Faculty of Medicine at Aydın Adnan Menderes University (protocol number: 19, date: 11/07/2025).

Informed Consent: We obtained written consent from the patient's parents for genetic testing and the disclosure of genetic and clinical data for scientific purposes.

Data Sharing Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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Supplementary 4: https://www.balkanmedicaljournal.org/img/files/supplemantry-4%281%29.pdf

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