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CLINICAL REPORT

Aberrant splicing caused by a novel *KMT2A* variant in Wiedemann–Steiner syndrome

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Abstract

Introduction: Wiedemann–Steiner syndrome (WSS) is a rare autosomal-dominant disorder caused by *KMT2A* variants. The aim of this study was to characterize a novel *KMT2A* variant in a child with WSS and demonstrate integrated diagnostic approaches.

Methods: A 3-year-old female with developmental delay, distinctive facial features, and anal fistula underwent whole exome sequencing (WES). RNA analysis was performed to assess splicing effects caused by a novel variant.

Results: WES identified novel heterozygous *KMT2A* c.5664+6T>C variant initially classified as a variant of uncertain significance. RNA analysis provided evidence of aberrant splicing (exon 20 skipping), allowing reclassification to likely pathogenic. The patient exhibited typical WSS features along with a potential novel finding of anal fistula.

Conclusion: This report describes a novel non-canonical splice site variant in *KMT2A* associated with WSS. RNA analysis was critical for variant reclassification. Detailed phenotypic evaluation revealed common and expanded WSS manifestations. This case highlights the importance of combining clinical assessment, DNA testing, and RNA functional assays for the diagnosis of rare genetic disorders.

KEYWORDS
KMT2A, RNA analysis, whole exome sequencing, Wiedemann–Steiner syndrome

1 | INTRODUCTION

Wiedemann–Steiner syndrome (WSS, OMIM #605130), initially reported by Wiedemann and Steiner in 1989 and 2000, is a rare and complex genetic disorder. It is characterized by a diverse array of clinical features affecting multiple systems (Jones et al., 2012; Wiedemann et al., 1989). Individuals with WSS typically present with growth deficiency and developmental delay, including delayed milestones such as walking and talking (Steiner & Marques, 2000; Wiedemann et al., 1989; Yu et al., 2022). Commonly observed dysmorphic facial features in these patients include an increased interpupillary distance, a flattened nasal bridge, and ptosis affecting the upper eyelids (Miyake et al., 2016). A distinctive hallmark of WSS is the presence of elongated eyelashes, notably

prominent along the down-slanted palpebral fissures, accompanied by hypertrichosis. This hypertrichosis becomes increasingly evident with age, particularly manifesting on the limbs, most notably around the elbows and back (Fontana et al., 2020; Steiner & Marques, 2000; Wiedemann et al., 1989). Additional clinical features comprise sleeping difficulties, feeding and digestion complexities, skeletal anomalies, and psychiatric disorders (Cherniske et al., 2004; Mervis & Becerra, 2007; Morris & Mervis, 2000).

Haploinsufficiency of *KMT2A* (GenBank ID:4297) gene is implicated in the pathogenesis of WSS, thereby providing strong evidence for its autosomal-dominant inheritance pattern (Jones et al., 2012). As such, the diagnosis of WSS is primarily established on the typical clinical features and further confirmed by genetic testing (Jones et al., 2012; Miyake et al., 2016).

Over 373 variants in *KMT2A* gene have been included in the Human Gene Mutation Database. Among open-access patients with the 115 variants of *KMT2A* in DECIPHER (https://www.deciphergenomics.org/), the top 5 associated phenotypes are Global Developmental Delay (44 cases), Short Stature (29 cases), Hypertelorism (24 cases), Epicanthus (21 cases), and Delayed Speech and Language Development (20 cases).

In this study, we present the case of a 3-year-old girl diagnosed with WSS, who has a novel de novo *KMT2A* variant. The variant pathogenicity was evaluated based upon RNA splicing analysis and pedigree analysis, ultimately leading to a "Likely pathogenic" classification of the variant.

2 | MATERIALS AND METHODS

2.1 | Patient and ethical statement

We described the case of a 3-year-old girl diagnosed with WSS, who was the second child of a couple without family history of genetic disorder (Figure 1). The couple sought genetic risk assessment for their future child at our Reproductive Genetics Clinic. With prior written informed consent, prenatal records and clinical data of the affected child were collected. Peripheral blood samples of family members were also obtained.

This study was approved by the Ethics Committee of the Shanghai First Maternity and Infant Hospital.

2.2 | Whole-exome sequencing and data analysis

The genomic DNA extraction and phenotype-driven whole exome sequencing (WES) were performed as previously described (Fei et al., 2022). Genomic DNA was isolated from peripheral blood lymphocytes of the proband (II2). The DNA samples were subjected to sonication, breaking them into fragments approximately

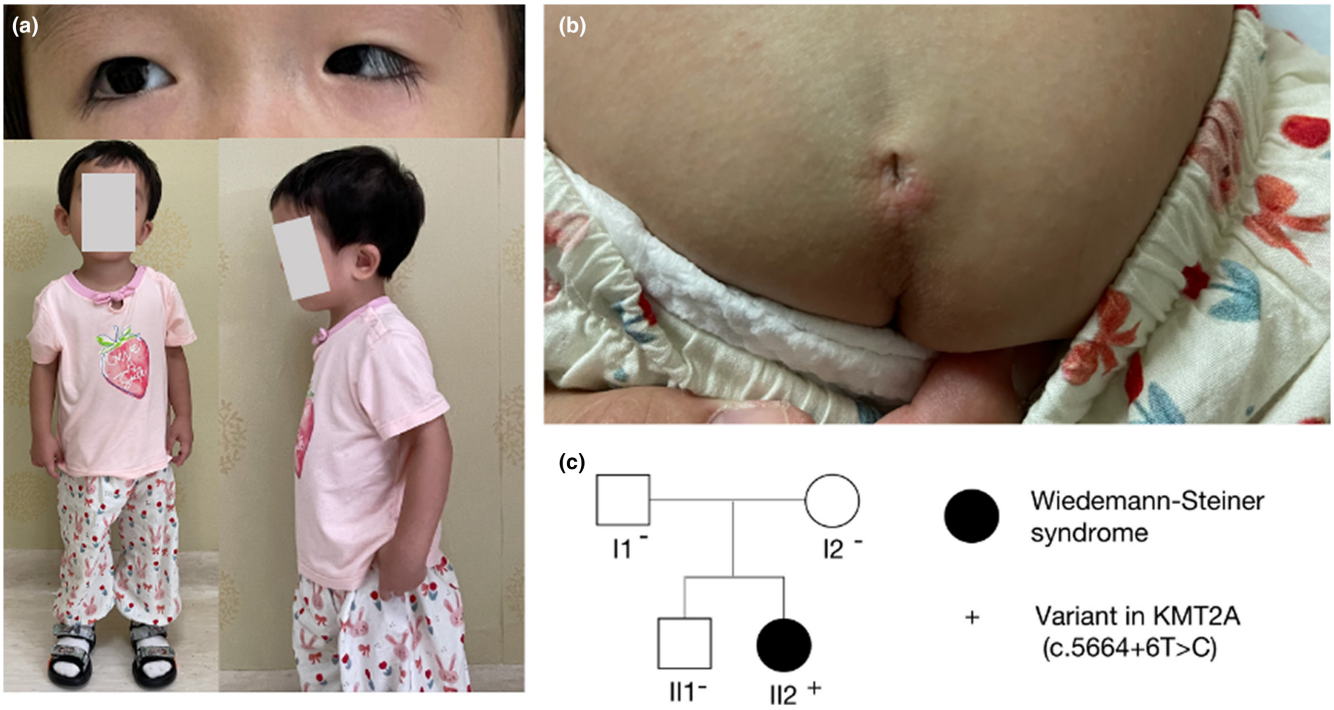

FIGURE 1 (a) Typical clinical features of the proband: wide-set eyes, flat face, upper eyelid ptosis, low-set ears, and long eyelashes. (b) Special clinical feature of the proband: anal fistula. (c) Pedigree of a Chinese family with Wiedemann–Steiner syndrome.

200–300 base pairs in length. A mature Illumina paired-end sequencing library was prepared. Exome sequencing was carried out on the Illumina HiSeq2500 analyzer using the Agilent SureSelect Human All Exon V6 kit. Clean sequencing reads were aligned to the human reference genome GRCh37/hg19 using the Burrows –Wheeler Aligner (v.0.7.12). Variant calling was performed with the Genome Analysis Toolkit (v.3.8). The obtained variant sites were filtered and prioritized using Flash Analysis (https://fa.shanyint.com/home/anonymous). Additionally, Sanger sequencing was conducted to validate the variants identified through exome sequencing within the proband's family, confirming the genetic pattern and the presence of the variants. Variant pathogenicity was classified according to the ACMG/AMP guidelines and ClinGen specifications (Richards et al., 2015; Zhang et al., 2020).

2.3 | RNA splicing analysis

Total RNA extraction was performed using TRIzol reagent (TaKaRa, Shiga, Japan), followed by cDNA synthesis using Hifair 1st Strand cDNA Synthesis SuperMix (YEASEN, Shanghai, China), following the manufacturer's protocols. The PCR products generated were subsequently analyzed through 2% agarose gel electrophoresis. The RT-PCR primer sequences used in this study were as follows: KP0941-F (5′-CAAGCAACAGTGGGATGTTACC-3′) and KP0941-R (5′-GGATGTGAGACAGCAACCCA-3′).

To evaluate the splicing effect of the variant on the proband, RT-PCR products from the proband were subcloned into the pGM-T vector and were sequenced using the T7/SP6 universal primers, and splice patterns were analyzed using Sanger sequencing.

3 | RESULTS

3.1 | Clinical characteristics of a Chinese patient with Wiedemann–Steiner syndrome

A couple visited our reproductive genetics clinic seeking genetic risk assessment for their future child. There is no familial history of behavioral anomalies, intellectual abnormalities, or developmental irregularities within the family. They had previously gone through two pregnancies and gave birth to two children. The first child, a 9-year-old boy, was born naturally and showed normal physical, mental, and intellectual characteristics upon examination. The proband was born during their second pregnancy. At 10 weeks of gestation, maternal serum screening for Down syndrome indicated a high risk with odds of 1:15, but non-invasive prenatal testing did not detect any abnormalities. At 23 weeks of gestation, the fetal ultrasound showed that the fetus's size matched the gestational age (biparietal diameter at 58 mm, occipitofrontal diameter at 72 mm, head circumference at 209 mm, abdominal circumference at 195 mm, humerus length at 36 mm, femur length at 38 mm, and estimated fetal weight was approximately 582 ± 85 g). The pregnancy continued to full term, and delivered a baby girl naturally, with a birth weight of approximately 3000 g. After birth, the proband experienced recurrent unexplained infections, and characterized with delayed growth and development (height: 2nd–9th percentile, weight: 0.4th–2nd percentile, and head circumference: 9th percentile), wide-set eyes, a flat facial profile, ptosis of the upper eyelids (with the left eye being significantly more affected), low-set ears, abnormally long eyelashes (suggesting the possibility of hypertrichosis), and anal fistula (Figure 1). No behavioral abnormalities such as autism, aggressive behavior, poor sleep, or hyperactivity have been observed. The proband's score on the Wechsler Preschool and Primary School Scale of Intelligence, Fourth Edition of the Chinese Version (WPPSI-IV CN) was 82.

3.2 | Identification and initial classification of the *KMT2A* variant

The clinical manifestations suggested a high possibility of genetic etiology. Genomic DNA extraction and WES were performed as previously described. After variant filtering and prioritization, we identified a heterozygous variant in the *KMT2A* gene (NM_001197104.2: c.5664+6T>C). Subsequent orthogonal testing and pedigree analysis using Sanger sequencing showed that neither the proband's parents nor her elder brother had this variant, indicating a de novo occurrence (Figure 2).

The variant pathogenicity was classified according to ACMG/AMP/ClinGen guidelines. The NM_001197104.2: c.5664+6T>C variant was not found in Genome Aggregation Database (gnomAD) and literatures, supporting PM2_Supporting criterion. SpliceAI (https://spliceailookup.broadinstitute.org/) predicted deleterious effect of both splice donor loss (Δ score = 0.46, −6 bp of the variant) and splice donor gain (Δ score = 0.44, −44 bp of the variant), supporting PP3 criterion. Pedigree analysis demonstrated the de novo occurrence with unconfirmed parental relationship, supporting PM6 criterion. Thus, the variant was classified as variant of uncertain significance (VUS) based upon the ACMG/AMP/ClinGen criteria: PM2_Supporting, PP3, PM6.

3.3 | RNA splicing analysis and variant reclassification

To assess the splicing impact of the variant on *KMT2A* mRNA, RT-PCR analysis of total RNA extracted from peripheral blood mononuclear cells of the proband (II2) and her mother (I2, as wild-type control) was performed with the primers of KP0941-F/R (Figure 3a). The distinct splicing pattern was found between the proband and wild-type control. While an appropriate 500-bp bands were detected in both the proband and wild-type control, we specifically detected an additional aberrant band in the proband

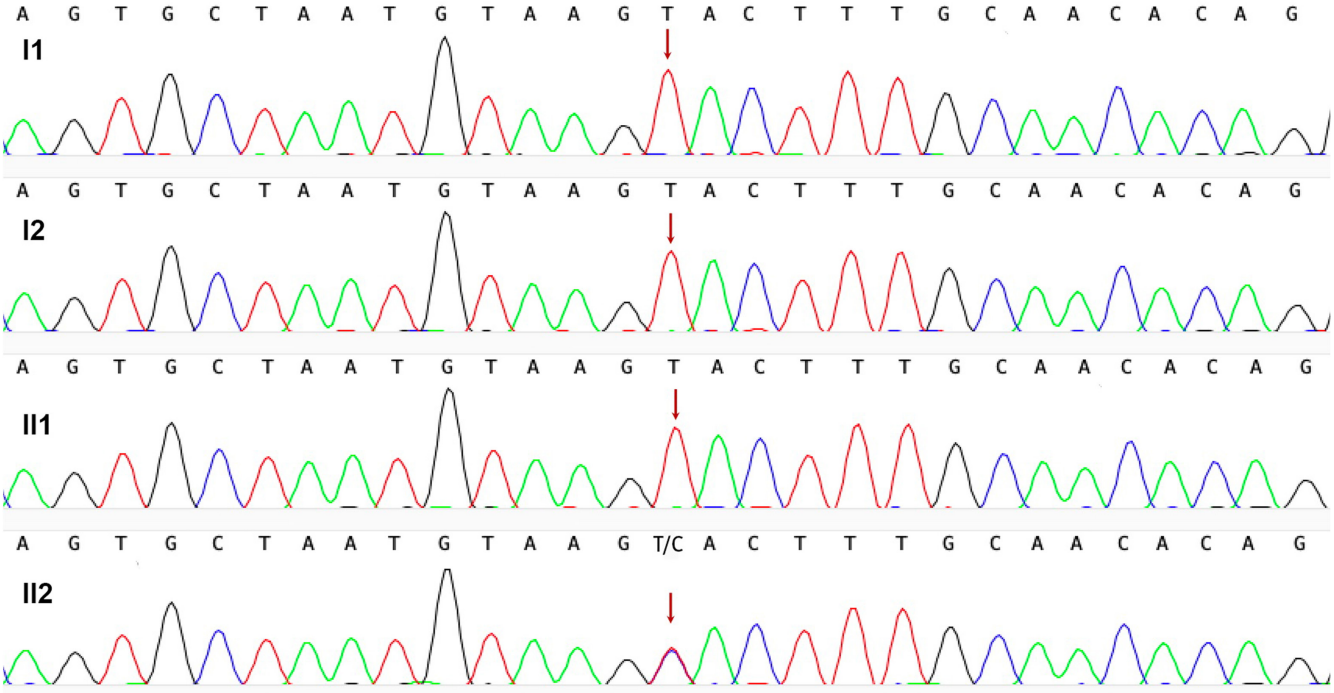

FIGURE 2 Sanger sequencing for NM_001197104.2 (*KMT2A*):c.5664+6T>C variant validation.

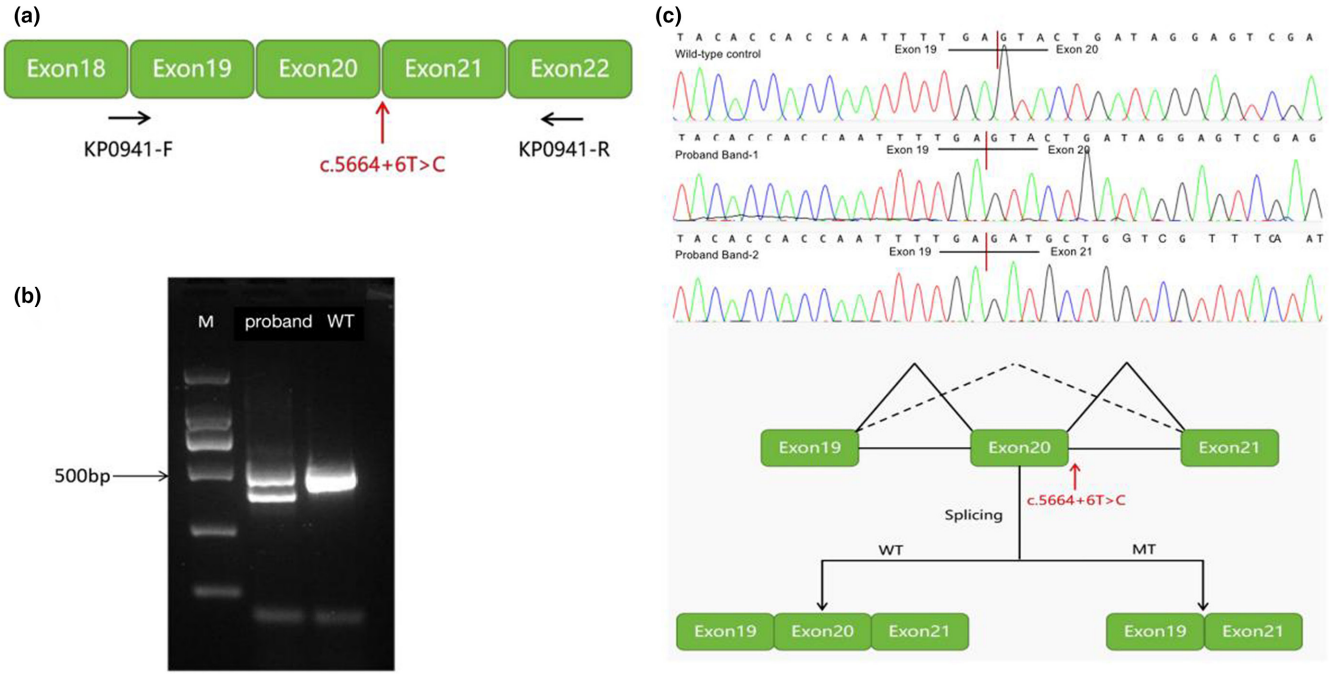

FIGURE 3 (a) Schematic representation of the variant and RT-PCR primers. (b) Gel electrophoresis image of the amplified products: M = marker, Proband, WT = wild-type control. (c) Sanger sequencing of RT-PCR amplified products. Top: Sanger sequencing results. Bottom: Schematic representation of aberrant splicing pattern: NM_001197104.2:c.5664+6T>C variant causes *KMT2A* exon 20 skipping.

(Figure 3b). Subsequent sequencing of the RT-PCR products from the wild-type control confirmed the 500-bp bands as wild-type transcript with exon 20. The RT-PCR products from the proband were subcloned into pGM-T vector. Totally, 24 clones were sequenced using T7/SP6 universal primer. Sequence analysis showed an approximately 1:1 ratio (11:13) of transcripts with exon 20 and those skipping exon 20 (Figure 3c), suggesting escaping from nonsense mediated mRNA decay (NMD). These results indicated that the c.5664+6T>C variant caused skipping of exon 20 (r.5558_5664del), which would introduce a premature termination codon (p.Ser1853Argfs*12) and escape the NMD.

Based on this splicing event, we replace the PP3 criterion originally assigned to the variant with PVS1_Strong, as recommended by ClinGen splicing subgroup (Walker et al., 2023). Consequently, the *KMT2A* NM_001197104.2:c.5664+6T>C variant was reclassified as "Likely pathogenic" with the following ACMG/AMP/ClinGen criteria: PM2_Supporting, PM6, PVS1_Strong.

4 | DISCUSSION

In this study, we identified a novel de novo heterozygous *KMT2A* variant c.5664+6T>C in a 3-year-old female patient presenting with features characteristic of WSS. The variant was initially classified as a VUS based on ACMG/AMP/ClinGen criteria PM2_Supporting, PP3, and PM6. However, RNA analysis provided evidence of aberrant splicing resulting in exon 20 skipping and introduction of a premature termination codon, allowing reclassification as "Likely pathogenic" based on criteria PM2_Supporting, PM6, and PVS1_Strong. This allowed a definitive diagnosis for the patient.

Non-canonical splicing variants in *KMT2A* have been reported in other cases of WSS (Clinvar Variation ID: 423826, 435659). These variants could disrupt splicing by affecting regulatory splice site elements like the variant identified in our patient. RNA studies have been to be critical for reclassification of such VUS by directly assessing their effects on splicing (Wai et al., 2020). In our case, the SpliceAI predicted that the *KMT2A* c.5664+6T>C variant could cause both splice donor loss and splice donor gain. The algorithm predicted a Δ score of 0.46 for loss of the natural splice donor and a Δ score of 0.44 for creation of a new splice donor 44 bp upstream. However, our RNA analysis detected only exon 20 skipping and usage of the natural splice donor. No cryptic upstream splice donor usage was observed. This highlights limitations of in silico predictive tools, which cannot perfectly recapitulate the intricate biology of splicing. This also has implications for variant classification when integrating in silico predictions. Observed RNA data should be more heavily weighted compared to predictive outputs alone. Discrepancies, like the one here, further emphasize the primary value of examining RNA splicing directly.

The *KMT2A* c.5664+6T>C variant resulted in exon 20 skipping and introduced a premature stop codon, which was initially predicted to trigger NMD. However, our RNA analysis revealed approximately equal levels of mutant and wild-type transcripts, indicating an escape from NMD. This phenomenon aligns with previous studies that have shown NMD escape can occur for some PTC-introducing variants predicted to undergo NMD (Zhang et al., 2017). The abnormal transcript escaping NMD may produce a truncated protein product lacking functionally critical domains. Our findings highlight the importance of RNA studies to provide experimental evidence regarding NMD events, rather than relying solely on predictive algorithms.

The *KMT2A* gene encodes a histone methyltransferase. Histone methylation can exert activating or inhibitory effects on transcription, playing a crucial role in chromatin modification and shaping the genome's epigenetic landscape (Fontana et al., 2020). The gene's activity elucidates that WSS is a chromatinopathy, exhibiting a diverse clinical phenotype that impacts multiple anatomical regions such as the head, skeleton, skin, and nervous system. Regarding the gastrointestinal system, some cases have reported symptoms like constipation among affected children (Sheppard et al., 2021). However, as of now, there have been no documented cases of anal fistula as a related phenotype. The finding of anal fistula in the patient may represent a novel gastrointestinal manifestation, although other contributing factors cannot be ruled out. Further case reports and research are needed to determine if anal fistula is part of the WSS phenotype or an unrelated finding.

5 | CONCLUSION

In summary, we have characterized a previously unreported non-canonical *KMT2A* variant associated with WSS. Detailed clinical examination identified distinctive manifestations including a potential new finding of anal fistula. The case highlights the importance of combining DNA testing, RNA analysis, and detailed clinical phenotyping for diagnosing rare disease patient. Our study expands the spectrum of *KMT2A* variants known to cause WSS and further demonstrates the utility of RNA-based assays for classifying non-canonical splicing variants.

AUTHOR CONTRIBUTIONS

Junyu Zhang and Xiaoming Teng designed this study. Jianing Niu is responsible for sample collection and genetic testing. Jianing Niu and Junyu Zhang drafted this paper. Xiaoming Teng and Junyu Zhang revised the manuscript, and supervised all the work.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts to declare.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ETHICS STATEMENT AND CONSENT TO PARTICIPATE

The samples used in this study were collected with appropriate informed consent and approval of the Ethics Committee of the Shanghai First Maternity and Infant Hospital. The methods used in this study were carried out in accordance with the approved guidelines.

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SUPPORTING INFORMATION

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

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