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New Insights into Kleefstra Syndrome: Report of Two Novel Cases with Previously Unreported Features and Literature Review

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Developmental delay · *EHMT1* · Kleefstra syndrome · Olfactory bulbs · Polydactyly · 9q34.3

Abstract
Kleefstra syndrome (KS) is a rare genetic condition resulting from either 9q34.3 microdeletions or mutations in the *EHMT1* gene located in the same genomic region. To date, approximately 100 patients have been reported, thereby allowing the core phenotype of KS to be defined as developmental delay/intellectual disability, generalized hypotonia, neuropsychiatric anomalies, and a distinctive facial appearance. Here, to further expand the knowledge on genotype and phenotype of this condition, we report 2 novel cases: one patient carrying a 46-kb 9q34.3 deletion and showing macrocephaly never described in KS, and a second patient carrying a classic 9q34.3 deletion, presenting with a previously unreported skeletal feature (postaxial polydactyly of the right foot) and an unusual brain anomaly (olfactory bulb hypoplasia) observed via magnetic resonance imaging. Further, we provide a review of the current literature regarding KS and compare these 2 patients with those previously described, thereby confirming that the genotype-phenotype correlation in KS remains difficult to determine.

Kleefstra syndrome (KS), previously known as 9q subtelomeric deletion syndrome (MIM 610253), is a rare genetic condition resulting from either microdeletions in 9q34.3 or mutations in the euchromatin histone methyltransferase 1 (*EHMT1*) gene, located in the same region [Kleefstra et al., 2005, 2006]. Microdeletions typically occur de novo (90%) and account for 75–80% of KS diagnoses. They can be classified into 2 categories on the basis of size (microdeletions and cytogenetically visible abnormalities) or into 3 categories on the basis of position (50% terminal deletions, 25% interstitial deletions, and 25% complex chromosomal rearrangements) [Yatsenko et al., 2009; Willemsen et al., 2012]. *EHMT1* mutations are responsible for 20–25% of KS diagnoses [Kleefstra et al., 2015]. Missense, nonsense, frameshift, and splicing mutations have all been described [Kleefstra et al., 2006; Rump et al., 2013]. Intragenic deletions, detected by multiplex ligation-dependent probe amplification, have been estimated as the causes of approximately 4% of KS diagnoses in children with a pathognomonic phenotype but no observable cytogenetic anomalies [Kleefstra et al., 2006].

EHMT1 (MIM 607001) encodes a histone methyltransferase that, together with the protein product of its paralog *EHMT2*, forms a chromatin-remodeling com-

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plex that deposits epigenetic marks, setting the genome in a "ready to repress" mode. Thus, EHMT1 can be viewed as a dynamic regulatory histone mark instead of a generic heterochromatin mark [Benevento et al., 2016]. EMHT1 has been demonstrated to be essential for neural network activity, action potential coherency, and burst time during brain development [Bart Martens et al., 2016]: EHMT1 loss may therefore potentially lead to structural and functional anomalies in the developed brain.

Although the genetic and epigenetic mechanisms underlying the condition are currently well-known, clinically, KS remains a complex and poorly recognized syndrome. The core phenotype of the syndrome includes developmental delay/intellectual disability (DD/ID), microcephaly, generalized hypotonia, neuropsychiatric anomalies, and a distinctive facial appearance [Stewart and Kleefstra, 2007; Kleefstra et al., 2009; Willemsen et al., 2012]. However, especially in patients with larger deletions, the number of comorbidities can potentially affect any part of the body and may result in an extremely severe phenotype, caused by the concurrent presence of life-threatening congenital malformations.

Overall, despite the increasing number of reports of patients with *EHMT1* mutations and a wide variety in patient deletion sizes, phenotype-genotype correlation studies are still weak, thus providing additional support for the major role of EHMT1. Current data indicate that individuals with an intragenic *EHMT1* pathogenic variant and those with a small (<1 Mb) 9q34.3 deletion have similar clinical findings [Kleefstra et al., 2015]. Individuals with larger deletions (≥1 Mb) may have more severe clinical problems, in agreement with a contiguous gene syndrome. In particular, pulmonary infections and aspiration occur more frequently in individuals with larger 9q34 deletions than in those with smaller deletions or *EHMT1* pathogenic variants [Willemsen et al., 2012].

Here, to further expand the knowledge on genotype and phenotype in KS, we report 2 novel cases: the first patient (patient 1) carrying a small 9q34.3 deletion (46 kb) and showing macrocephaly, and the second patient (patient 2), carrying a classic 9q34.3 deletion (448 kb), with a never reported skeletal feature (postaxial polydactyly of the right foot) and an unusual brain MRI anomaly (olfactory bulb hypoplasia). Further, we provide a review of the most recent literature on KS and compare the patients described in the present study with those reported in the literature, thus confirming that the genotype-phenotype correlation in KS remains difficult to define.

Case Reports

Patient 1

Patient 1 is a 14-month-old male child referred to our center for mild DD and minor face anomalies. He is the second child of healthy nonconsanguineous parents and was born from a dizygotic twin pregnancy. The family history was negative for ID or genetic conditions. Prenatal ultrasound (US) performed at the 12th week showed increased nuchal translucency. Amniotic cell karyotyping was therefore performed and revealed a normal male karyotype (46,XY). Fetal movements were poor compared with those of the other twin. The pregnancy was characterized by a risk of miscarriage at the 22nd week, and a caesarian section was performed at the 34th week. Auxological parameters at birth were adequate for gestational age (weight 2,195 g, 25–50th centile; length 44 cm, 25th centile; OFC 30 cm, 10–25th centile). The Apgar score at 1 and 5 min was 7/8, with minor respiratory distress. Mild patent ductus arteriosus was present at birth, but this defect spontaneously closed within the first month of life. The lingual frenulum was short, thus causing sucking difficulties. A transfontanelle US revealed hyperechogenicity of the posterior ventricle, and abdominal US was normal at birth and in the subsequent follow-up. Growth for height and weight was normal. The patient suffered from recurrent otitis media starting in the first months of life, and an audiometric evaluation showed hearing at the lower limit of normal. The patient also underwent a clinical and neurological evaluation, which revealed hypotonia, joint hypermobility, and abdominal wall diastasis with umbilical hernia.

Clinical evaluation at 14 months revealed normal auxological parameters with macrocephaly (weight 9.8 kg, 10–25th centile; length 77 cm, 25th centile; OFC 44.6 cm, >97th centile). The child showed a mildly broad forehead, synophrys, midface hypoplasia, a depressed nasal bridge, a short nose, long and flat philtrum, a tented upper lip, an everted lower lip, a protruding tongue with an open carp mouth, and low-set ears with upturned ear lobes and prominent anti-tragus. He also presented with an umbilical hernia, mild V toe clinodactyly, and a congenital melanocytic nevus on the left arm. The child showed hypotonic and psychomotor delays, was not able to walk independently, and still had not spoken his first words.

Suspecting a small cytogenetic anomaly, we performed high-resolution array-CGH, which revealed a 46-kb 9q34.3 deletion involving *EHMT1* (exons 24–27). The parents showed normal array-CGH results, thus confirming that the deletion occurred de novo.

After diagnosis, the child underwent some additional testing. Cardiac evaluation (including ECG and echocardiogram) and standard blood tests were normal, except for a low level of HDL (21 mg/dL, normal range >55 mg/dL). A brain MRI, performed at 18 months, showed a thin and mildly dysmorphic corpus callosum and some nonspecific findings that may be ascribed to prematurity (diffuse white matter reduction, mild ventricular dysmorphism, and periventricular white matter hyperintensity) (Fig. 1A–C). The last examination at 18 months still revealed the absence of both language and independent walking, although an improvement in motor and social skills was observed; a developmental assessment was performed (Griffiths scale), showing a general quotient of 10 months.

Patient 2

Patient 2, a 19-month-old male child, was referred to our center after array-CGH had been performed because of DD, which

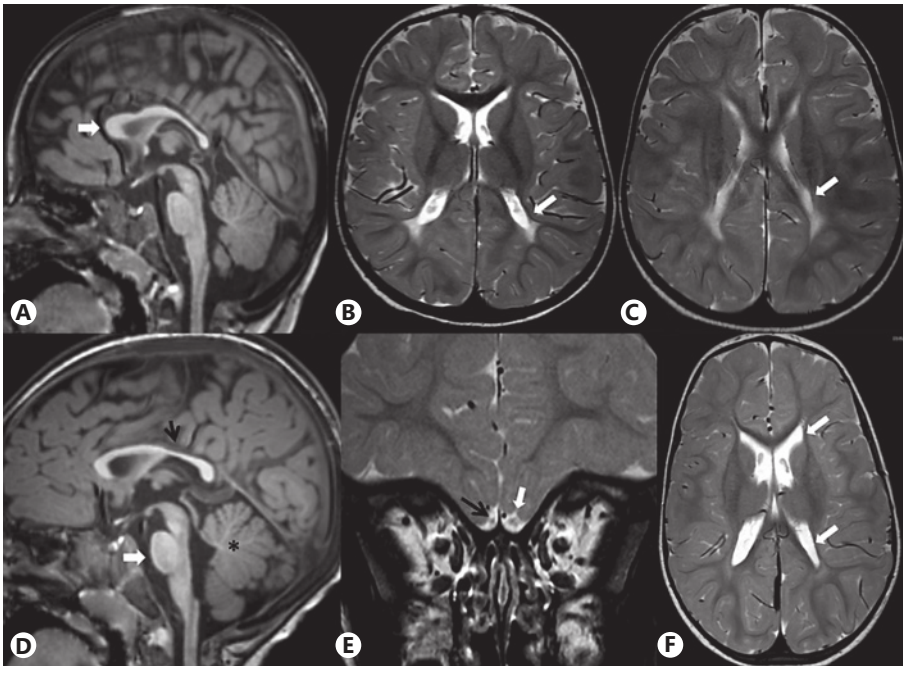

Fig. 1. Brain MRI (T2 weighted axial and coronal, T1 3D TFE sagittal images) of patients 1 (**A–C**) and 2 (**D–F**). **A** Midline T1 3D sagittal image showing mild corpus callosum dysmorphism (white arrow). **B**, **C** Axial T2 W images showing white matter hyperintensity and thinning (**B**, white arrow) and dysmorphic shape of the lateral ventricles and white matter hyperintensity (**C**, white arrow). **D** Midline T1 3D sagittal image showing brain stem minimal dysmorphic and thinning aspects (white arrow), mild vermis hypoplasia (black asterisk), and corpus callosum thinning (black arrow). **E** T2 W coronal image showing olfactory bulb hypoplasia, especially of the right one (black arrow). **F** Axial T2 W image showing white matter hyperintensity and thinning and mild dysmorphic shape of the lateral ventricles (white arrows).

showed a 448-kb deletion in the 9q34.3 region involving the *EHMT1* and *CACNA1B* genes. This patient is the first child of healthy nonconsanguineous parents and was born at term after an uneventful pregnancy. The family history was negative for ID or genetic conditions. Auxological parameters at birth were within a normal range (weight 3,860 g, 75–90th centile; length 52 cm, 75–90th centile; OFC 35.5 cm, 75–90th centile), and the Apgar score at 1 and 5 min was 6/8. The child experienced neonatal hypoglycemia and mild jaundice and was treated with phototherapy. Postaxial polydactyly of the right foot was detected with an X-ray showing 2 proximal phalanxes at the head of the V metatarsus (a dysmorphic medial one and a normal lateral one); the patient underwent surgical correction. Transfontanelle and abdominal US at birth were normal. The otoacoustic emission performed immediately after birth registered a bilateral fail, but the subsequent audiological follow-ups were normal. An EEG, performed at 6 months, was normal. At 12 months of age, the patient underwent a brain MRI, which showed diffuse white matter reduction, periventricular white matter anomalies, corpus callosum thinning, thin brain stem, mild vermian hypoplasia, and olfactory bulb hypoplasia (Fig. 1D–F). Growth for height and weight was normal; DD was present, although an improving trend of developmental stages was observed.

Clinical evaluation at 19 months was suggestive of KS, as the patient could not walk independently and still had not spoken his first words. The auxological parameters were within a normal range (weight 13.6 kg, 10–25th centile; length 85.5 cm, 75–90th centile; OFC 47.5 cm, 10–25th centile), and the patient showed the typical facial appearance for this condition, including broad forehead, widely spaced eyes, epicanthal folds, upslanting palpebral fissures, midface hypoplasia, a short nose, anteverted nares, a depressed nasal bridge, and low set ears with prominent ear lobes and a thick helix (Fig. 2). The teeth were small and spaced apart.

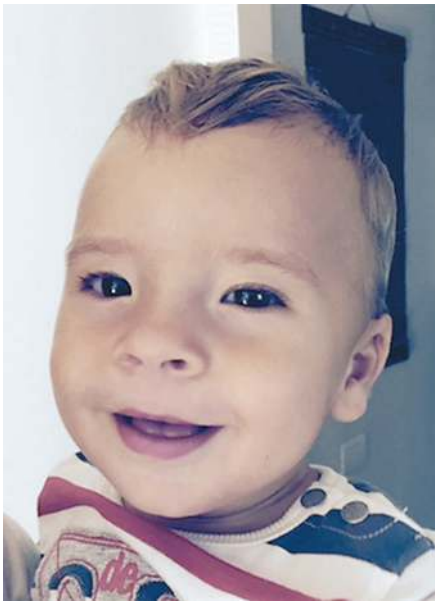

Fig. 2. Typical KS facial appearance: broad forehead, widely spaced eyes, epicanthal folds, upslanting palpebral fissures, midface hypoplasia, short nose, anteverted nares, depressed nasal bridge, and low-set ears with prominent ear lobes and a thick helix.

The child subsequently underwent additional testing: cardiac evaluation (including ECG and echocardiogram) and standard blood tests were normal, except for a mild hypertriglyceridemia (216 mg/dL, normal range <170 mg/dL); ophthalmological evalu-

ation revealed mild right eye esotropia. The parents reported some episodes of proximal myoclonus with lower limb hyperextension at the age of 20 months. A polysomnogram was therefore performed, showing good organization of the EEG pattern. A new brain MRI was performed at 24 months, confirming the previous findings and showing an improvement in myelination with a decrease in periventricular anomalies.

Discussion

KS is a relatively newly described condition, and since its first definition in 2005, the genotypic heterogeneity and phenotypic presentation of this disease have been determined by numerous and novel reports. In Table 1, we summarize the clinical presentations of the 2 patients and compare these with the clinical presentation of KS patients, thus providing an estimate of the prevalence of the most recurrent clinical problems, on the basis of the data reported in the literature to date.

Both patients described here present a very similar phenotype, although the underlying cytogenetic defect of patient 2 is approximately 10 times larger than that of patient 1. Their auxological parameters were within a normal range, except for OFC: patient 2 is relatively microcephalic, whereas patient 1 is macrocephalic. Microcephaly is considered one of the hallmarks of this syndrome, as a relative or absolute, congenital or acquired reduction of the OFC is observed in more than half of the KS patients [Stewart et al., 2004]; this report is the first to describe an OFC >97th centile in KS.

The patients share the typical facial dysmorphisms of KS, including a broad forehead (present in 55% of the subjects), synophrys (55–80%), midface hypoplasia (55–100%), a depressed nasal bridge (45–100%), a short nose (60–80%), and ear anomalies (45–80%). Peculiarly, patient 1 does not show any eye anomaly, detectable in more than two-thirds of the KS subjects, whereas patient 2 has widely spaced eyes, epicanthal folds and upslanting palpebral fissures. Hypotonia, affecting most KS patients, is also present in the current cases, and in patient 1, this effect occurs concomitantly with a tented upper lip (present in 70–75% of cases) and tongue protrusion (40–60%), as is often observed in hypotonic subjects. An increased frequency of dental anomalies, specifically neonatal teeth, retention of primary dentition, small teeth, spaced teeth, and broad alveolar margins, has been reported in this condition, with an overall prevalence of approximately 10–15% [Stewart et al., 2004; Neas et al., 2005; Willemsen et al., 2011; Kleefstra et al., 2015]. Consistently with literature data, the teeth of patient 2 are small and spaced apart.

Both patients, as almost all KS subjects, present with psychomotor delay, and in both, a slow but steady acquisition of new skills is observable; furthermore, these patients do not show any symptoms that might potentially be related to ASD or behavioral problems (affecting 30–75% and 65–70% of the subjects, respectively), and neither patient exhibited sleep problems (20–25% of KS population), reported in up to half of the children with KS [Kleefstra et al., 2005; Stewart and Kleefstra, 2007].

Brain imaging (MRI or CT scan) anomalies are reported in approximately half of the KS subjects. It is actually difficult to estimate the prevalence of such findings, because most children undergoing brain imaging have already been diagnosed with some neurologic or severe neuropsychiatric comorbidity, and the data concerning neurologically healthy KS patients are poor. When MRI/CT anomalies are present, these conditions are more frequently white matter abnormalities [Willemsen et al., 2011; Kleefstra et al., 2015; He et al., 2016] and cortical atrophy (frontal, cerebral, or cerebellar) or indirect evidence of such atrophy (prominent ventricles, increased cerebrospinal fluid spaces) [Stewart et al., 2004; Yatsenko et al., 2005; Kleefstra et al., 2009]. Both cases present with structural brain malformations; in particular, the MRI of patient 2 revealed olfactory bulb hypoplasia, a novel finding in this condition. The young age of the patient and the concurrent DD make it difficult to determine whether this abnormality correlates with a loss of or a decreased sense of smell. Epilepsy, which has been described in up to half of the KS patients, has not been observed in these patients thus far.

Overall, the children described in the present study have a mild presentation of KS, because they lack cardiovascular or renal involvement (present in 40–45% and 15–30% of KS subjects, respectively), genital anomalies (45–50% of males), skeletal malformations (30–50%), and gross ocular defects (45%). The described heart defects of KS children are primarily conotruncal malformations, such as tetralogy of Fallot or atrial septal defects/ventricular septal defects, but patent ductus arteriosus/foramen ovale, aortic coarctation, bicuspid aortic valve, aortic, mitral, or pulmonary valve stenosis, and tricuspid valve dysplasia also have moderate incidences [Stewart et al., 2004; Kleefstra et al., 2006, 2009; Willemsen et al., 2012; Hadzsiev et al., 2016]. Patient 1 presented with mild patent ductus arteriosus at birth, but this effect probably reflected prematurity, as suggested by its spontaneous remission within the first month of life.

Frequently observed renal anomalies are vesicoureteral reflux, hydronephrosis, and renal cysts [Yatsenko et al.,

Table 1. Clinical comparison of the patients in the present study and typical KS phenotypes

	Patient 1	Patient 2	Typical KS phenotype[a]
Site and size of deletions, cytogenetic/ molecular defect	arr[GRCh37] 9q34.3(140711171_140757122)×1 46 kb (*EHMT1* exons 24–27)	arr[GRCh37] 9q34.3(140560792_141008915)×1 448 kb (*EHMT1–CACNA1B*)	9q34.3 deletion *EHMT1* mutation
Centiles at evaluation			
Weight	10–25th	10–25th	50–75th
Height	25th	75–90th	25–50th
OFC	>97th	10–25th	10–25th
Microcephaly	–	+ (relative)	30–80%
Broad forehead	+	+	55%
Synophrys	+	+	55–80%
Widely spaced eyes	–	+	55–70%
Epicanthal folds	–	+	30–70%
Upslanting palpebral fissures	–	+	30–70%
Midface hypoplasia	+	+	55–100%
Depressed nasal bridge	+	+	45–100%
Short nose	+	+	60–80%
Anteverted nares	–	+	40–80%
Tented upper lip	+	–	70–75%
Everted lower lip	+	–	25–50%
Protruding tongue	+	–	40–60%
Ear anomalies	+	+	45–80%
Dental anomalies	–	Small, spaced teeth	10–15%
Hypotonia	+	+	60–80%
Psychomotor delay/intellectual disability	+	+	100%
ASD	–	–	30–75%
Behavioral problems	–	–	65–70%
Sleep disorder	–	–	20–50%
Epilepsy	–	–	20–50%
Brain imaging anomalies	+	+	50–60%
Cardiovascular anomalies	– (PDA at birth, spontaneously closed)	–	40–45%
Renal issues	–	–	15–30%
Genital anomalies	–	–	45–50% of males
Hernia	+	–	15–20%
Skeletal anomalies	–	–	30–50%
Extremities	V toe clinodactyly	Postaxial polydactyly of the right foot	70%
Hypoacusia	Lower limit of normal	–	20–30%
Ocular anomalies	–	Right eye esotropia	45%
Obesity	–	–	30–40%
Other	Decreased HDL	Increased TG	–

ASD, autism spectrum disorder; HDL, high-density lipoprotein; OFC, occipitofrontal circumference; PDA, patent ductus arteriosus; TG, thyroglobulin.
[a] Stewart et al., 2004; Yatsenko et al., 2005; Kleefstra et al., 2006, 2009; Willemsen et al., 2012; Hadzsiev et al., 2016.

2005; Kleefstra et al., 2006, 2009; Willemsen et al., 2011]. Male children often present with congenital defects of the external genitalia, such as mono- or bilateral cryptorchidism, hypospadias, micropenis, and small scrotum [Stewart et al., 2004; Neas et al., 2005; Yatsenko et al., 2005; Kleefstra et al., 2009; Willemsen et al., 2011]. No genital anomalies have been reported in females, although hirsutism may be present [Stewart et al., 2004]. Umbilical and/or inguinal hernia is a common feature (15–20%) and is also present in patient 1.

Extremity anomalies are quite common in KS (70%) and also affect both patients presented here. Reported anomalies include broad toes, syndactyly, clinodactyly, brachydactyly, and single palmar crease, with the latter 2 characteristics being predominant [Stewart et al., 2004; Neas et al., 2005; Kleefstra et al., 2006]. Patient 2 exhib-

ited a previously unreported foot feature, namely, postaxial polydactyly. Polydactyly is a congenital anomaly characterized by the presence of supernumerary toes or phalanges, with or without metatarsals; this anomaly may occur as an isolated feature or in association with particular genetic conditions [Temtamy and McKusick, 1978; Turra et al., 2007]. Polydactyly has a slightly higher prevalence in males, and geographical variations have been reported: the incidence is 0.3–2.3/1,000 live births in Caucasians and 3.6–13.9/1,000 live births in Africans [Finley et al., 1994; Turra et al., 2007]. Foot polydactyly is one of the most common birth malformations worldwide, accounting for approximately 45% of all foot anomalies [Son et al., 2004], and postaxial polydactyly (an extra toe adjacent to the 5th one) has been estimated as the most frequent form of foot polydactyly, accounting for 77–86% of cases [Nakamura et al., 1988; Watanabe et al., 1992; Turra et al., 2007]. These data indicate that although extremity anomalies are quite common in KS, and it is plausible that polydactyly may be part of the KS phenotypic spectrum, confirmation of this feature by additional reports is necessary because, given the prevalence of this anomaly, polydactyly may also be a concurrent feature unrelated to this condition.

Hypoacusia affects about 20–30% of KS children, but both of the patients described herein scored in the normal limits in the hearing tests. Occasionally, the poor cooperation and/or a marked ID of these children may make it difficult to estimate the severity of the impairment. The eyes are also often involved (45%), and oculomotor problems are in part a consequence of the hypotonia and loose tendons. Eye anomalies include nystagmus, amblyopia, esotropia (present also in patient 2), exotropia, strabismus, and hypermetropia [Stewart et al., 2004; Neas et al., 2005; Stewart and Kleefstra, 2007].

Notably, both patients show fat metabolism anomalies: patient 1 has low HDL levels, whereas patient 2 has high TG levels. Obesity, a feature observed since the first description of this condition, occurs in at least one-third of cases, and it is currently the only metabolic issue reported to affect KS children [Cormier-Daire et al., 2003; Kleefstra et al., 2009]. Studies of fat metabolism in these patients are lacking, but it would be of interest to understand whether there is an underlying fat metabolism deregulation that might explain the tendency to weight gain in KS.

Although there is no specific feature of the fetus that makes KS recognizable before birth, some early alterations suggestive for this genetic condition have been reported. In particular, double artery umbilical cord, increased nuchal translucency, oligohydramnios, fetus dismaturity in the 3rd trimester, and major malformations can be detected by prenatal US [Stewart et al., 2004; Chen et al., 2013; Hadzsiev et al., 2016; Huang et al., 2017]. In effect, increased nuchal translucency was detected in patient 1. Furthermore, pregnancy with KS babies may be associated with abnormal maternal serum screen results, even in the absence of fetal US anomalies or an otherwise uneventful pregnancy [Chen et al., 2015]. In the presence of such anomalies, but a normal karyotype, fetal array-CGH analysis may be indicated.

In conclusion, KS is currently following the ordinary way to knowledge that every newly discovered rare condition has to ride. It is therefore important to report every case of previously undescribed or rarely described features, in order to expand and confirm the phenotype and readily recognize and address the potential clinical manifestations related to the condition.

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Statement of Ethics

Written informed consent for publication was obtained from the patients' parents. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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