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Clinical Report

The first Brazilian clinical report of Kleefstra syndrome, including semicircular canals agenesis as a possible phenotype expansion

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ABSTRACT

Objective: to report the first case series of Brazilian children diagnosed with Kleefstra syndrome, present a possible phenotype expansion to the syndrome and to raise physicians' awareness for this rare disease.
Results: seven patients with confirmed KS were evaluated, including 5 males and 2 females. Abnormal prenatal findings were observed in 4 patients. Most patients were born at term, with normal birth measurements. All patients had neurodevelopmental delay and 6 evolved with intellectual disability. Hearing loss was present in 57.1% of patients and 28.7% had congenital heart disease. In males, cryptorchidism was present in 75%. Despite the facial dysmorphisms, only 2 out of 7 patients had a pre-test clinical suspicion of KS. One specific patient presented bilateral agenesis of the semicircular canals, a very rare ear manifestation in Kleefstra syndrome, representing a possible phenotype expansion of the syndrome.
Conclusion: this report aims to promote awareness among physicians evaluating patients in a context of neurodevelopmental delay or congenital malformations, especially congenital heart defects. We also highlight a possible phenotype expansion of the syndrome, with a case of semicircular anomaly, not reported in this syndrome so far.

1. Introduction

Kleefstra syndrome (KS), previously known as 9q subtelomeric deletion syndrome, is a rare genetic condition with approximately 110 cases reported in the literature (Campbell et al., 2014) and no Brazilian reports up to this date. It can either be caused by chromosome 9q34.3 microdeletions, that can be detected in 75–85% of the cases, or by intragenic loss of function mutations in the *euchromatin histone methyl-transferase 1 gene* (*EHMT1*) located in the same *locus* (Kleefstra T et al., 2005). *EHMT1* encodes a histone methyltransferase responsible for the transfer of methyl groups to the lysine residues on histones, which regulates transcription and chromatin remodeling through epigenetic modification and, therefore, could have different roles in gene expression regulation (Demond H et al., 2023).

KS may present as a clinically recognizable syndrome, characterized by distinctive facial features, severe developmental delay, intellectual disability (including severely delayed or absent speech), microcephaly, hypotonia, seizures, behavioral and sleep abnormalities, vision disorders and minor genital anomalies in males (Ciaccio et al., 2018). Approximately half of the patients with KS have congenital heart defects, which are highly variable and mainly include atrial or ventricular septal defects (ASD and VSD, respectively) (Campbell et al., 2014). Congenital renal abnormalities, including vesicoureteral reflux and hydronephrosis, are also reported in 10–15% of KS patients (Campbell et al., 2014).

Here we present the first brazilian report of patients with KS, including seven individuals with the syndrome with variable clinical features. We include a possible phenotype expansion in a patient with microtia and bilateral semicircular canal agenesis, the first reported patient with vestibular involvement associated with KS.

2. Results

This is a retrospective study of patients with confirmed KS from a specialized care unit of clinical genetics in Brazil. Medical records were reviewed to collect clinical information, including prenatal and neonatal

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history, major clinical findings, imaging, molecular testing and other relevant data to each case. Clinical photographs were taken with informed consent. The diagnosis was confirmed with genetic analysis appropriate to each case.

Patients' clinical findings, initial clinical hypothesis are reported in Table 1. Facial dysmorphisms are shown in Fig. 1. A total of 7 patients (5 males and 2 females) with confirmed diagnosis were evaluated. Age at first evaluation ranged from 5 months to 12 years. Here we highlight some of the important features found in our patients in three sections, regarding **clinical information, atypical cases and molecular analysis.**

2.1. Clinical information analysis

Abnormal prenatal findings were present in 4 of the 7 patients. Patients 2 and 7 were diagnosed with congenital heart defect (ventricular septal defect in both cases) without intervention or further examination in the postnatal period. Patient 5 had a single fetal death in a twin pregnancy at eight weeks of gestational age (it was not possible, at the time, to confirm the chorionicity). Patient 6 had a polyhydramnios diagnosis in the third trimester. None of the patients had a prenatal suspicion of any genetic disease.

All patients were born at term, except for patient 7, who was born at 36 gestational weeks. Birth measurements were not usually altered; birth weight varied from 2420 g to 3370 g (mean of 2902g). Patient 7 had the highest birth weight among probands (60th centile), but there was also a history of gestational diabetes treated with insulin. Birth length varied from 46 cm to 50 cm (mean of 47.7 cm). Although not all head circumference measurements were available, only one of them showed initial microcephaly; others presented this feature only in later ages (57.1%, 4 out of 7 patients).

At birth, 28.5% (2 out of 7) presented some kind of complication. Patient 1 was born by cesarean section at 41 weeks and presented acute respiratory distress, which was resolved within the first 5 minutes postpartum. Patient 7 had a poor perinatal outcome; he did not present spontaneous breathing, needing ventilation support and orotracheal intubation for 5 days.

Many patients had a hold up of the first clinical evaluation by a geneticist due to a delayed clinical reference from the primary care provider. Most of the time, the reference was based on neurodevelopmental delay. The mean age at the first evaluation in the Genetics Unit was 4.4 years; the earliest being 5 months of age (patient 2) and the latest at 12 years of age only (patient 1).

At the first evaluation, typical facial dysmorphisms were not always recognized. Only 28.5% (2/7) showed brachycephaly, 57.1% (4/7) arched eyebrows, 57.1% (4/7) midface retrusion, 42.8% had a short nose with anteverted nares, 3 patients showed everted vermilion of the lower lip and 2 of them had protruding tongue. None showed prognathism. Only 2 patients had a clinical suspicion of KS, highlighting that the dysmorphisms may be mild and variable, thus not clinically recognizable. The initial clinical hypothesis included Beckwith-Wiedemann in patient 3, Cornelia de Lange syndrome in patient 4, Mowat-Wilson in patient 5 and CHARGE syndrome in patient 7.

Ocular alterations were noted in 42.8% (patients 1, 2 and 7). Patient 1 had myopia, astigmatism and strabismus. Patient 2 had hyperopia, astigmatism and alacrimia. Patient 7 had hyperopia. Hearing loss was

Table 1
Clinical information.

Table 1	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Sex	Male	Female	Male	Female	Male	Male	Male
Age of first evaluation	12 y	5 mo	3 y	5 y	2 y	6 y	3 y
Birth conditions							
Birth-weight (centile)	2985 g (12th centile)	2935 g (15th centile)	2800 g (21st centile)	2420 g (17th centile)	2830 g (27th centile)	3370 g (28th centile)	2980 g (60th centile)
Birth-length (centile/SD)	49 cm	49 cm	44.5 cm (−2.84 SD)	48 cm	46 cm (−1.7 SD)	50 cm	48,5 cm
Head circumference	33 cm (4th centile)	NA	34 cm (36th centile)	NA	34 cm (39th centile)	36 cm (62nd centile)	NA
Developmental/neurological							
Microcephaly	absent	present	present	present	present	absent	present
Motor delay	present	present	present	present	present	absent	present
Hypotonia	present	present	absent	present	present	absent	present
Speech delay	present	present	absent	present	present	present	present
Behavioral deviation	Anxiety Disorder	absent	Aggressive Behavior; Obsessive Compulsive Behavior	ASD, anxiety disorder, ADHD and aggressive Behavior	absent	absent	absent
Seizures	absent	absent	absent	absent	Febrile seizure	absent	absent
Intellectual disability	present	present	absent[a]	present	present	present	present
Ophthalmologic pathologies	Myopia, Astigmatism, Strabismus	Hyperopia; Astigmatism; Alacrimia;	absent	absent	absent	absent	Hyperopia
Hearing Loss	absent	mild	absent	mild	mild	absent	severe
Congenital Heart Defects	ASD	VSD	ASD	absent	absent	absent	VSD, pulmonary valve stenosis, tricuspid regurgitation
Genitourinary abnormalities	Cryptorchidism, micropenis	absent	absent	absent	Vesicoureteral reflux, cryptorchidism	absent	cryptorchidism
Initial clinical diagnosis hypothesis	**Kleefstra syndrome**	No clear hypothesis	Beckwith-Wiedemann spectrum/Simpson-Golabi syndrome	Cornelia de Lange syndrome	Mowat-Wilson syndrome	**Kleefstra syndrome**	CHARGE Syndrome

*NA = not available.
[a] Patient is less than 5 years old, but presents developmental delay.

Fig. 1. Facial dysmorphisms. A–B: patient 1 at 12 years; C–D: patient 2 at 1 year; E–F: patient 3 at 3 years; G: patient 4 at 5 years; H: patient 5 at 6 years; I–J: patient 6 at and 6 and 12 years; K–L: patient 7 at 4 years.

shown in 57.1% of the patients (2, 3, 4 and especially patient 7). Patient 7 presented an atypical novel finding (bilateral agenesis of the semicircular canals) that will be described further on.

Only 28.7% had a significant heart defect (ventricular septal defect). Two patients had atrial septal defect. Patient 7 also had pulmonary valve stenosis and moderate tricuspid regurgitation. Renal defects were infrequent in our patients; only one had mild vesicoureteral reflux (patient 5). Regarding genital abnormalities, 75% of the males presented cryptorchidism.

As previously mentioned, all patients had neurodevelopmental delay. Motor skills were delayed in all patients, including head support. Mostly, the ability to walk was reached after 2 years of age, while patient 5 walked at 5 years of age. The ability to speak was even more affected than motor skills; all patients who reached speech abilities had their first words after 2.5 years of age and at many times only after 5 years. Patient 1 started speech at 36 months, patient 5 at 8 years of age, patient 6 at 5 years and patient 7 never reached this ability. Six out of seven patients in our study evolved with clinically evident intellectual disability although we were not able to perform neuropsychological evaluations to measure IQ levels in all patients.

Epilepsy, although previously associated with KS, was not present in any of our probands so far, only patient 5 presented some sort of seizure in their clinical follow up when he had 3 episodes of febrile seizures at age 9 months that did not maintain through his life.

Behavioral issues were frequently found, present in 42.8%. Generalized anxiety disorder was present in patient 1. Aggressive behavior and obsessive-compulsive behavior was present in patient 3. Autism, generalized anxiety disorder and attention deficit was present in patient 4.

2.2. *Atypical cases*

One of the explanations why there was no initial clinical suspicion of the KS is that some of the patients presented atypical clinical findings, especially patients 6 and 7, who will now be further described.

Patient 6 is a male that in the first year of life showed sleep disturbance, macroglossia and adenoid hypertrophy. He also showed a neurodevelopmental delay, mainly at speech (first words at age 5 years). Brain magnetic resonance imaging (MRI) was indicated by the pediatrician, showing a large congenital arachnoid cyst in the right frontal region, posterior displacement of the temporal lobe and superior displacement of the base of the frontal lobe to the right, as well as slight compression on the body of the homolateral lateral ventricle and contralateral deviation of the midline structures of about 0.4 mm measured at the level of the septum pellucidum. The child was then evaluated by neurosurgery and surgical removal of the cyst was performed as treatment.

Patient 7 showed significant neurodevelopmental delay (could only walk at 24 months) and never reached speech. At birth, microtia (Fig. 1K-L), clubfoot and bilateral facial palsy were also noted. Inner ear and temporal bone MRI was performed showing complete outer ear conduct atresia at the left side and incomplete atresia at the right side.

Other findings included semicircular canals atresia and cranial nerves (VII and VIII) hypoplasia at the right side. On the left side, only nerve VII was not visualized. Some of the radiologic features are highlighted in Fig. 2. Congenital heart disease was confirmed with echocardiogram, showing the ventricular septal defect seen in the prenatal exam, but also pulmonary valve stenosis and moderate tricuspid regurgitation. The patient had a surgery for correction of congenital clubfoot at age 8 months. He also had a surgery for cochlear implant at age 2 years and 4 months for his hearing loss. At 2 years and 10 months, the patient was diagnosed with acute lymphoblastic leukemia and is currently under treatment with chemotherapy.

2.3. Molecular analysis

All patients were tested for the investigation of their clinical hypothesis. Five out of seven patients (71.4%) had copy number variations including the deletion of *EHMT1* as a mechanism of KS, no other genes were deleted in any cases. The other two patients had loss of function variants in the *EHMT1* gene. Molecular testing differed according to the clinical suspicion and main molecular mechanism for evaluation. Regarding patient 7, we also screened thoroughly for rare SNV's of CNV's in his WGS to explain the audiological abnormalities but no variants of interest were found. The results are further described in Table 2.

3. Discussion

KS is a clinically recognizable genetic condition that may be suspected by any physician and there is a need for clinical awareness and early investigation. All of the patients had a late reference to a clinical genetics unit, resulting in a delay in diagnosis and management.

When first evaluated by a physician, prenatal findings may not always be relevant and can include findings such as congenital heart defects, as seen in two of our cases with ventricular septal defects. At birth, anthropometric parameters are also not usually altered (Willemsen MH et al., 2012) as observed in most of our cases. Microcephaly is one of the typical features of KS as it is observed in nearly half of the patients (Ciaccio et al., 2018) and although some of the patients did not have an available head circumference measurement at birth, only one of them showed initial microcephaly and others presented this feature only in later ages (57.1%). These findings highlight that in some cases there won't be red flags for a genetic syndrome when the newborn is first evaluated.

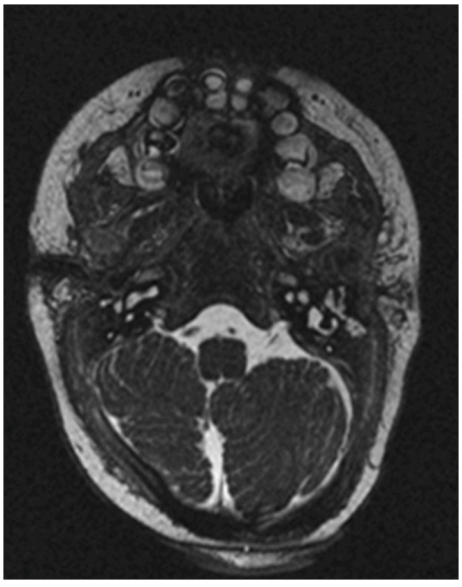

Fig. 2. MRI of ear and temporal bone (patient 7) showing signs of atresia of the external auditory canals, dysplasia of the right lateral semicircular canal and non-characterization of the facial and vestibulocochlear nerves on the right.

Differential diagnosis can also be a hard task for a clinical geneticist when investigating a patient with suspected KS due to its phenotypic variability. Main differentials include syndromes with similar dysmorphisms, developmental delay, hypotonia and abnormal behavior such as Down, Smith-Magenis and Pitt-Hopkins syndromes (Kleefstra T et al., 2010). Even though there is somewhat of a clinical *gestalt*, only 2 out of 7 patients in this study had a clinical hypothesis of KS before genetic testing, showing that the clinical variability directly impacts the clinical hypothesis.

Some of the previously reported findings were not always consistent with our patients' clinical characteristics. Classic facial dysmorphisms included in KS phenotype are highlighted in Fig. 1 and although the literature cites a clinically recognizable phenotype, we consider that the facial dysmorphisms may not always be specific to guide the clinical suspicion. Only 2 out of our 7 patients had a clinical hypothesis of KS suggesting that the syndrome does not have a clear *gestalt*. Also, with time, clinical geneticists become more familiarized with different syndromes, and KS was not so well known at the time some patients were first evaluated.

In infancy, patients with KS present a wide variety of alterations in cognitive development and functioning. Six out of seven patients in our center showed moderate to severe intellectual disability, in accordance with the previous descriptions of cognitive impairment (Vermeulen K et al., 2017). Speech delay is also a relevant neurodevelopmental feature and is usually severe, being frequently found in our patients (85%). Interestingly, seizures were not present in any of our probands so far, although expected in up to 50% of the patients (Ciaccio C, 2018). One hypothesis is that the epilepsy frequency in KS may have been overestimated in the first reports as we see more and more case reports of patients with no seizure history. Also, epilepsy may manifest in later stages of the clinical follow-up.

Hearing impairment was a frequent finding in our patients and may be underestimated in KS patients as it is currently believed to be present in only 20–30% of the patients (Okayasu T et al., 2020). In 2020, Okayasu and colleagues reported the results of an otopathology study of one KS patient demonstrating impairment of the scala communis, dysmorphia of the modiolus with a shortened Rosenthal's canal, absence of spiral ganglion cells and hair cells in the basal turn of the cochlea, displacement of approximately 30% of total spiral ganglion cells into the internal auditory canal, enlarged vestibular aqueduct, and vestibular anomalies and dysostosis of the stapes. These findings are consistent with the clinical history of conductive and neurossensorial hearing loss in KS patients. Out of our cohort, patients had both of the hearing loss mechanisms, and one of them showed novel clinical findings in KS. Patient 7 had bilateral microtia and congenital conductive and sensorineural deafness. Audiologic investigation revealed bilateral microtia as well as semicircular canals agenesis, an atypical presentation of ear malformations in KS. To this date this is one of the first clinical reports of vestibular involvement associated with KS and may correlate with the vestibular anomalies found in the model presented by Okayasu and colleagues. This result may support a phenotype expansion in the spectrum of malformations of the syndrome considering the recurrence of vestibular anomalies. We understand that the evidence is still not conclusive and hope to find more reports to further support this evidence. It is also relevant to mention the importance of molecular testing considering that this atypical finding may lead the clinical hypothesis to more specific diagnosis with previous clinical descriptions, such as CHD7 related disorders which are usually considered once bilateral semicircular agenesis is diagnosed (Green GE et al., 2014).

Most clinical findings in our sample were consistent with the literature, some findings do not agree with the previous, such as renal, genitourinary and cardiac involvement which were less frequent, this description may be expected considering the small sample size in our

Table 2
molecular analysis.

Table 2	Copy number variant	Genomic content	Point mutation	Inheritance
Patient 1	Arr[hg19]9q34.3 (140,533,290–140,716,973) x1	Exon 2 to 26 of EHMT1	–	not tested
Patient 2	Arr[hg19]9q34.3(139,830,787–141,066,491) x1	Exon 2 to 26 of EHMT1	–	not tested
Patient 3	Arr[hg19]9q34.3 (140,401,147–141,018,648) x1	Exons 2 to 25 of EHMT1	–	*de novo*
Patient 4	773Kb deletion on 9q34.3 comprising *EHMT1*	Terminal deletion, including only EHMT1[a]	–	*de novo*
Patient 5	Arr[hg19]9q34.3(139906808–141018648)x1	Exon 2 to 26 of EHMT1	–	not tested
Patient 6	–		EHMT1(NM_024757.5):c.2877_2880del:p.(Ser960Glyfs[a]7)	not tested
Patient 7	–		EHMT1(NM_024757.5):c.3072_3073del:p.Val1026Glnfs[a]150	not tested

[a] Exome sequencing does not show precise breakpoints in this case.

study. Renal defects were infrequent in our patients, only one had a mild vesicoureteral reflux. These findings, although somewhat infrequent in KS, may be valued because of the potential of complications. Regarding genital abnormalities, 75% of the males presented cryptorchidism, this finding is well established in KS with reports of up to 30% having some degree of males showing some degree of genital involvement (Kleefstra T et al., 2023).The previous estimate of a 50% rate of conotruncal heart defect does not seem to correlate with our patients (Kleefstra T et al., 2023). Only 28.7% had a significant heart defect, both of them being a ventricular septal defect, although the number of patients is limited.

Regarding the molecular mechanisms of disease, KS typically occurs as a *de novo* event and is caused by haploinsufficiency of *EHMT1* either due to deletions or intragenic pathogenic variants (Kleefstra T et al., 2010). The literature references still estimate that 75–85% of the patients have deletions of *EHMT1* and 15–25% have SNV's as a cause of KS (Campbell CL et al., 2014; Ciaccio C et al., 2018; Kleefstra T et al., 2005). Our sample had similar proportions of deletions as a disease causing mechanism considering that 71.4% (5/7) had microdeletions including *EHMT1*. We believe that this proportion may alter with the advances and accessibility to exome and genome sequencing. Some specialists already consider a 50-50% proportion of CNVs and SNVs to be more accurate (Kleefstra T et al., 2023). In our cohort, patients with pathogenic loss of function point mutations did not show milder features. On the other hand, larger deletions that involve other disease-causing genes may affect the phenotype, promoting multiple comorbidities and severe clinical conditions; in such patients the clinical features may overlap with KS (Kleefstra T et al., 2009; Willemsen MH et al., 2012). This premise, combined with a wide-range phenotypic variability, enforces the need for molecular testing when suspecting KS.

Finally, this report aims to increase awareness among physicians regarding KS as a possible diagnosis in patients with neurodevelopment delay and variable dysmorphisms. The clinical findings may not always be specific leading to difficulty in *gestalt*, our report helps to better characterize patients with the syndrome with distinct clinical findings. As molecular tests become more accessible in clinical practice around the world, the phenotype expressivity of several genetic conditions expands and novel findings start to be highlighted. Molecular testing has the potential to expand the diagnosis and to include patients who may not have been previously identified as having a specific genetic syndrome, such as the patient with bilateral semicircular canals agenesis which may be a possible phenotypic expansion. The confirmation of a diagnosis allows proper genetic counseling to the patients and their families, involving information regarding the spectrum of clinical manifestations, potential complications, natural history, existence of clinical follow-up guidelines for some syndromes and recurrence risk in the family.

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CRediT authorship contribution statement

Eduardo Da Cás: Writing – original draft, Data curation, Conceptualization. **Lucas V.L. Pires:** Writing – original draft, Methodology, Conceptualization. **Bianca D.W. Linnenkamp:** Methodology, Conceptualization. **Marcella C. Allegro:** Writing – original draft. **Rachel S. Honjo:** Writing – review & editing. **Débora R. Bertola:** Writing – review & editing. **Hiromi Aoi:** Data curation. **Naomichi Matsumoto:** Data curation. **Chong Ae Kim:** Writing – review & editing.

Declaration of competing interest

nothing to declare.

Data availability

Data will be made available on request.

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