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First prenatal diagnosis of a 'pure' 9q34.3 deletion (Kleefstra syndrome): A case report and literature review

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Abstract

Kleefstra syndrome (KS) is characterized by developmental delay, intellectual disability, hypotonia and distinct facial features. Additional clinical features include congenital heart defects, cerebral abnormalities, urogenital defects and weight gain. The syndrome is caused by a microdeletion in chromosomal region 9q34.3 (in 85% of cases) or by a mutation in the *EHMT1* gene coding for euchromatin histone methyltransferase 1. The prenatal phenotype has not yet been characterized. Herein, we sought to define this phenotype on the basis of a new case report and literature review.

Key words: array comparative genomic hybridization, congenital heart disease, corpus callosum, Kleefstra syndrome, prenatal diagnosis.

Introduction

Kleefstra syndrome (KS, OMIM 610253) is characterized by developmental delay, intellectual disability, hypotonia and distinct facial features (arched eyebrows, midface hypoplasia, upturned nares, full everted lower lip, Cupid's bow upper lip and prognathism). Additional clinical features include congenital heart and urogenital defects, epilepsy, behavioral and psychiatric disorders and weight gain. Various heart defects are observed in 41–43% of patients, with microcephaly in 19–50%, and abnormal features on brain imaging in 58–63%.[1,2]

Kleefstra syndrome is caused by haploinsufficiency of the *EHMT1* gene coding for euchromatin histone methyltransferase 1. The EHMT1 protein is an epigenetic regulator of gene transcription. Specific euchromatin methylation (H3K9) is a key component of the transcriptional silencing program for many genes, and is essential for early embryogenesis in murine models. In the developing mouse, EHMT1 is expressed in a broad variety of organs (including the central nervous system, kidney and heart). KS can be caused by a microdeletion in chromosomal region 9q34.3 (in 85% of cases) or by an intragenic mutation in *EHMT1*.[3] According to the literature, patients with an EHMT1 mutation and those with a 9q34.3 deletion < 3Mbp have very similar clinical features. However, the identification of increasing numbers of patients with small deletions and novel EHMT1 mutations will probably deepen our knowledge of the phenotypic and genotypic spectrum in KS.[2] Furthermore, the application of novel sequencing technologies will lead to the identification of new mutations. Almost all cases of KS are sporadic, although three familial cases (resulting from a maternal subtelomeric 9q deletion present in a mosaic pattern) were recently reported.[2]

The prenatal phenotype in KS has yet to be defined. The objective of the present study was to define this phenotype by reporting a new case and reviewing the literature.

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Case Report

At 17 weeks of gestation (WG), a 29-year-old Caucasian woman (gravida 1, para 0) was referred for amniocentesis on the basis of an abnormal test result in first trimester screening, based on risk calculation using the Fetal Medicine Foundation algorithm: estimated risk = 1 in 131 (pregnancy-associated plasma protein A at 0.77MoM and free β subunit human chorionic gonadotropin at 1.33MoM; nuchal translucency [NT] at 3.1 mm; and maternal age 29 years). The patient had no background risk for common trisomy. In the first trimester ultrasound assessment, the embryo had a normal shape and appearance, with a crown-rump length of 73.7 mm, but with NT of 3.1 mm (between the 95th and the 99th percentiles). Rapid fluorescence in situ hybridization results were normal for chromosomes 13, 18 and 21. After cell culture, a cytogenetic analysis showed that the karyotype was normal. Given that NT was below 3.5 mm, array comparative genomic hybridization (aCGH) was not initially performed.

Fetal echocardiogram performed at 20 WG showed an abnormal four-chamber view in which the left ventricle appeared to be narrower than the right ventricle. During diastolic function, the tricuspid valve diameter was 7 mm (normal value 4.5 mm), and the mitral valve diameter was 4.5 mm (normal range 4–5 mm). In the three-vessel and trachea view (Fig. 1a), the ascending aorta (AA) appeared to be narrower (diameter 2.9 mm, normal value 3.7 mm) than the main pulmonary artery (AP) (6.4 mm, normal value 4 mm). This type of dissymmetry is known to be consistent with a prenatal ultrasound diagnosis of coarctation of the aorta. Furthermore, ultrasound assessment of the brain revealed a short, thick corpus callosum (CC) (size 18 mm, CC length at the 50th percentile at 20 WG 20.4 cm) (Fig. 1b). The head circumference corresponded to the 11th percentile and the biparietal diameter to the 15th percentile. The posterior cranial fossa was normal and no morphological abnormalities were observed in other brain regions.

In view of these findings, we decided to perform array comparative genomic hybridization using an Agilent Human SurePrint G3 Human CGH array Kit 8x60k, with fetal DNA extracted from an amniotic fluid culture. Labeling and hybridization were performed according to the manufacturer's instructions. The arrays were scanned with an Agilent DNA Microarray Scanner. A deletion spanning 870 kb was found at 9q34.3 from base 140 083 938 to base 140 958 088, according to hg19 (Fig. 2). The deletion encompassed 23 genes, 15 of which are reported in the Online Mendelian Inheritance in Man database. Five of the latter (including *EHMT1*) are considered to be morbid genes. The deletion was confirmed using fluorescence in situ hybridization and a specific telomere probe for the long arm of chromosome 9.

After genetic counseling (whereby the characteristics of KS and its poor cognitive and psychiatric prognoses were explained) and in view of the abnormal ultrasound findings, the parents decided to terminate the pregnancy at 22 WG. In compliance with the parents' wishes, neither a fetal autopsy nor post-mortem magnetic resonance imaging was performed. There was no sign of facial dysmorphy at macroscopic

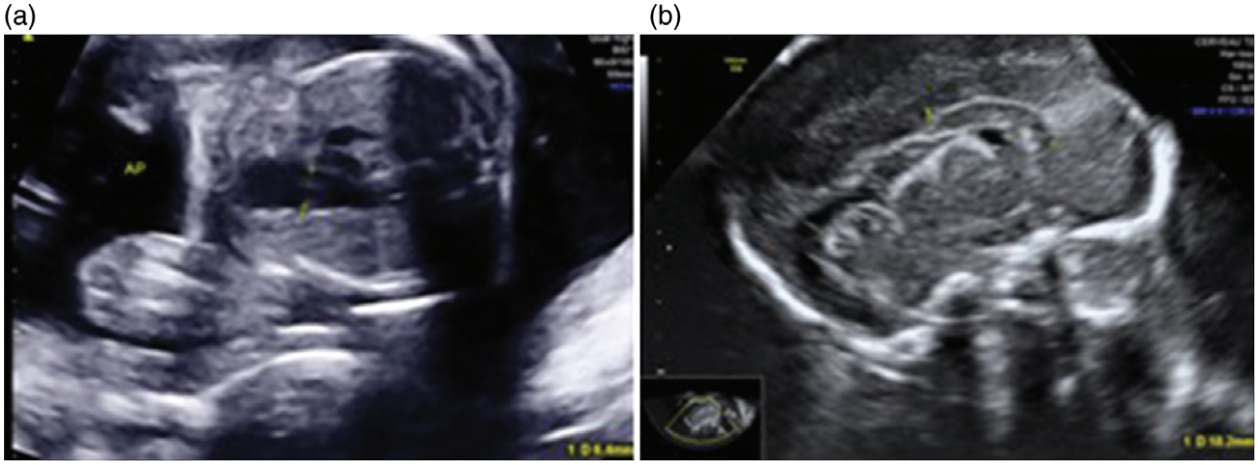

Figure 1 (a) A three-vessel view, showing dissymmetry of the arteries, (b) a sagittal view of the short, thin corpus callosum.

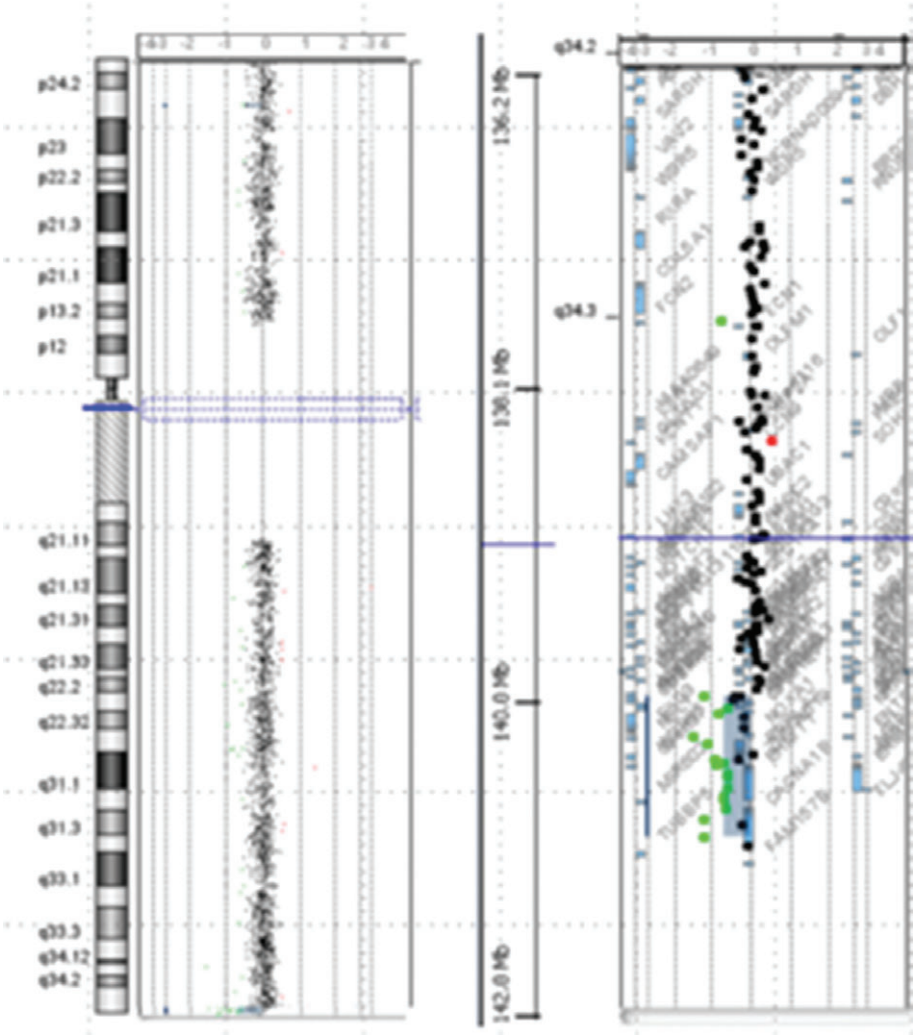

Figure 2 The array comparative genomic hybridization profile, revealing a 9q34.3 deletion.

examination. Furthermore, the parents decided not to undergo genetic testing to evaluate the mode of inheritance of the deletion.

Discussion

Herein, we report the first prenatal diagnosis of KS by identifying 9q34 microdeletion using aCGH, without associated chromosome abnormalities. Indeed, all five previously reported cases were associated with structural chromosomal abnormalities in addition to the deletion (Table 1). Cytogenetic assessment in these cases had been prompted by an abnormal maternal serum marker (MSM) assay (n = 2), elevated NT (n = 2) and abnormal ultrasound findings (n = 2). aCGH was performed in five of the six cases, following the discovery of a chromosome imbalance (n = 2), an apparently balanced de novo translocation (n = 2) or abnormal ultrasound findings during pregnancy (n = 1, the present case). aCGH was not performed in the case with a mosaic ring chromosome.

Only three prenatally diagnosed cases (our case and two previous) featured 'pure' deletions, in which only the 9q34.3 region was involved.[4,5] The genetic diagnoses were made by whole-genome aCGH in all cases. In the two previously published cases, karyotypes showed apparently balanced reciprocal translocation. In our case, the karyotype was normal, without chromosome rearrangements. In the other three cases, the 9q34.3 deletion was accompanied by further chromosomal abnormalities: a ring chromosome 9 with deletion of the chromosome's short and long arms in two cases,[6,7] and unbalanced chromosome translocation in one case.[8]

Table 1 Summary of literature reports of prenatal KS

First author	Diagnosis	Pure deletion	Imbalance	Chromosome formula	Indication for an invasive procedure	Indication for aCGH	aCGH findings	Genome assembly	Microdeletion size	Maternal age	Prenatal ultrasound findings			Outcome
											Cardiac	Cerebral	Other features	
Present case	Prenatal	Yes	Del 9q34	46,XY	1 T MSM, risk = 1 in 131	Performed after detection of a cardiac defect	arr 9q34.3 (140 083 938–140 958 088) × 1	Hg 19	0.9 Mb	29	Coarctation of the aorta	Short, thick corpus callosum		TOP
Chen[7]	Prenatal	No	r (9)	Mos 45XY,-9 / 46,XY,r (9) (p24q34.4)	2 T screening, risk = 1 in 57	Not performed	—			41			Ambiguous genitalia	TOP
Simovich[4]	Prenatal	Yes	Del 9q34	t(2;9)(q11.2; q34.3).dn	Increased NT	An apparently balanced reciprocal translocation and ultrasound findings	No formula		2.7 Mb	42				TOP
Chen[8]	Prenatal	No	Dup 3q and del 9q	46,XX,der (9)t (3;9) (q26.31; q34.3)dn	NT = 4.7 mm and omphalocele	Characterization of a structural chromosome imbalance	arr3q26.31q29 (171 287 090–197 897 268) × 3, 9q34.3 (140 047349–141 019 600) × 1	Hg 19	1.0 Mb	35	Ventricular septal defect		Omphalocele	TOP
Penacho[6]	Prenatal	No	r (9)	46,XX,r (9) (p24q34)	1 T MSM, risk = 1 in 50 with hypoplastic nasal bone	Characterization of a structural chromosome imbalance	arr 9pterp24.2 (163 131–2 729 722)x1, 9p24(5 090 443–5 235 765) × 1, 9q34.3qter (138 523 302–141 122 055) × 1	Hg 19	2.6 Mb	31	Hypoplasic pulmonary artery	Corpus callosum agenesis	Fetal growth restriction, single umbilical artery, retrognathism, hyperechogenic bowel, subcutaneous edema	TOP
Huang[5]	Prenatal	Yes	Del 9q34	45, XX, dic (9;13)(q34; p13)	Elevated NT (4.5 mm)	An apparently balanced reciprocal translocation and ultrasound findings	arr9q34.3 (137 415 177–141 018 648) × 1	Hg 19	3.6 Mb	30				Unknown
Chen[9]	Postnatal	Yes	Del 9q34	46,XY	2 T MSM, risk = 1 in 44, no aCGH	Characteristic facial appearance of KS, intellectual disability, and developmental delay	arr 9q34.3 (140 687 823–140 695.906) × 1		8.1 kb	32				Birth
Campbell[1]	Postnatal	Yes	Del 9q34	—	—	Hypoplastic left heart syndrome and multicystic renal disease	arr9q34.3(79 085 945–90,974 367) × 1	Hg 19	2.1 Mb	23	Coarctation of the aorta		Oligohydramnios, echogenic kidneys	Birth

a CGH, array comparative genomic hybridization; KS, Kleefstra syndrome; MSM, maternal serum markers; TOP, termination of pregnancy.

In our case, unfortunately the parents refused to perform a full autopsy, a post-mortem imaging examination or genetic investigation to elucidate the mode of inheritance, and as such the impact of our present communication is limited.

In the majority of pediatric case reports, the pregnancy was unremarkable.[9,10] When considering fetuses with only a 9q34 deletion, our case is the first to display a cardiac defect (observed in around half of all cases of KS) and an abnormal CC (abnormal features on postnatal brain imaging are reported in more than two-thirds of cases).[10] In the other two cases, only increased NT was reported; in one case, the pregnancy was terminated before any further ultrasound assessments could be performed,[4] and no follow-up data were reported for the other case.[5]

Various abnormal ultrasound findings were described in the three cases with an additional chromosome imbalance. In the case with a mosaic ring chromosome 9 (with deletion of the chromosome's short and long arm) and a chromosome 9 monosomy, only ambiguous genitalia were described.[7] Penacho *et al.* also reported a case with homogeneous ring chromosome 9 with three separate deletions.[6] Elevated levels had prompted a prenatal diagnosis, and an ultrasound assessment at 18 WG revealed fetal growth retardation, severe bradycardia, a hypoplastic pulmonary artery, a single umbilical artery, retrognathism, a hypoplastic nasal bone, CC agenesis, hyperechogenic bowel and subcutaneous thoracic edema. The two cases of ring chromosome could not be compared because aCGH was not performed in one instance (thus the deletion may have been smaller) and because of mosaicism in the other instance. In the case with a 3q26.31–q29 duplication and a 9q34.3 microdeletion (972 kb), postnatal characteristic features of both 3q duplication syndrome and KS were observed; a fetal autopsy revealed arched eyebrows, midface hypoplasia, upturned nares, full everted lower lip, Cupid's bow upper lip, prognathism and ventricular septal defect.[8]

In the literature, Campbell *et al.* reported a case of a 23-year-old woman whose pregnancy was complicated by oligohydramnios, echogenic kidneys and coarctation of the aorta, leading to severe hypoplastic left heart syndrome and multicystic kidney in the fetus.[1] A 2.1 Mbp deletion encompassing *EHMT1* was discovered postnatally.

Finally, only one of the cases with a prenatal description was diagnosed postnatally with KS, despite the fact that elevated MSM levels had prompted prenatal karyotyping.[9] The decision to perform aCGH was indicated by the child's facial appearance, intellectual disability and developmental delay – all of which were suggestive of KS.

In the seven cases with available ultrasound findings (4 cases of a 'pure' 9q34 deletion and 3 with associated chromosome abnormalities), a cardiac defect was observed in four instances (2 'pure' cases and 2 with associated chromosome abnormalities), increased NT in three instances (2 'pure' cases and 1 'associated' case) and an abnormal CC in two instances (1 'pure' case and 1 'associated'). No antenatal cases of KS are listed in the DECIPHER database (http://decipher.sanger.ac.uk).

The congenital heart defects observed in KS are usually atrial or ventricular septal defects, although the phenotype can vary markedly. An abnormally short, thin CC has been reported postnatally, and brain abnormalities are observed in about 60% of cases of KS. Despite the presence of microcephaly, brain imaging results are normal in most cases; however, mild frontal cortical atrophy, left ventricle dilatation, generalized atrophy with marked deep white matter changes in the posterior brain, 'small brain' and delayed myelination have been reported.[10]

As reported previously by Jansen *et al.*, an aCGH assessment should be considered when a cardiac defect is found during prenatal screening.[11] It is not good practice to look for a 22q11.2 deletion (DiGeorge syndrome) alone, because 40% of these cases are associated with other chromosome abnormalities (including KS). More generally, aCGH should be performed in cases with abnormal ultrasound findings.

Next-generation sequencing will doubtless reveal new point mutations in general and mutations in the *EHMT1* gene in particular. Future studies are likely to provide us with a better understanding of the genetic aetiology of specific phenotypes of KS.

Disclosure

The authors have no conflict of interest to declare.

Author contributions

All authors have read and approved the final version of the manuscript.

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