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Case Report

Clinical findings and molecular cytogenetic study of *de novo* pure chromosome 9p deletion: Pre- and postnatal diagnosis

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ABSTRACT

Objective: The aim of this report is to describe the phenotype-genotype correlation of chromosome 9p deletion syndrome cases, particularly the prenatal cases.
Materials and methods: A 30-year-old woman was referred to a hospital at 19^{+1} weeks of gestation because of omphalocele detected in the fetus. The conventional karyotyping analysis and array comparative genomic hybridization (aCGH) were utilized for the prenatal diagnosis and genetic counseling in the fetus. The prenatal abnormality and cytogenetic findings in the fetus were compared with other patients with 9p deletion.
Results: Karyotype analysis of the fetus cell showed a karyotype of 46,XX,del(9)(p22). aCGH analysis detected a deletion as arr[hg19] 9p24.2p22.2(226,7812–1,7466,907) × 1. Individuals with 9p deletions tend to have features with widely variable expressivity. The common clinical manifestations of the 9p deletion include development delay, learning difficulties, hypotonia and trigonocephaly.
Conclusion: Phenotypes of 9p deletion cases are broadly in line. The prenatal diagnosis of the omphalocele provides evidence for a correlation with distal 9q deletion.

Introduction

Chromosome 9p deletion syndrome (OMIM #158170) is a well-recognized chromosome portion monosomy. The most commonly involved clinical features include mental retardation, developmental and psychomotor delay, dysmorphic facial features (trigonocephaly, upward-slanting palpebral fissures, and midface hypoplasia), hypotonia, and impaired gonadal development in some XY individuals [1–4]. However, few cases of 9p deletion syndrome are detected prenatally and little is known about the prenatal cases [1,2].

Monosomy 9p may result from parental reciprocal translocations, parental pericentric inversions, or *de novo* deletions [1,2]. Most *de novo* monosomy 9p are pure 9p distal deletions, with breakpoints that occur in bands 9p22–9p24 [3]. In recent years, with the availability of fluorescence *in situ* hybridization and the array comparative genomic hybridization (aCGH), the critical region responsible for the pure 9p deletion cardinal features has been narrowed down but with no consensus in the results [2,4]. In this study, we present the molecular cytogenetic character and clinical findings of one case of prenatal and two cases of postnatal *de novo* monosomy 9p syndrome.

Case Report

Prenatal diagnosis of 9p24.2-22.2 deletion

A 30-year-old, gravida 2, para 1, woman underwent amniocentesis at 19^{+1} weeks of gestation because a fetal omphalocele (8×6 mm^2) had been detected on routine ultrasound examination at 17^{+4} weeks of gestation age. Down syndrome screening using maternal serum biochemistry was negative. At 19 weeks, amniocentesis was performed and chromosomal analysis was subsequently performed on the amniotic fluid cells. Conventional G-banding at 400–450 band level was applied, which revealed a karyotype of 46,XX,del(9)(p22). The parental karyotypes were normal. Whole genome aCGH analysis on the DNA extracted from the cultured amniocytes detected a 15.19 Mb deletion at 9p24.2-22.2 arr 9p24.2p22.2 (2,267,812–17,466,907 bp) × 1 encompassing

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49 genes including *VLDLR*, *TYRP1*, and *CER1*, adjacent to genes *DMRT1* and *DMRT3* (Figure 1A). The pregnancy was subsequently terminated. A female nonviable fetus was delivered with an omphalocele, low-set ears, and flat nose. Postnatal cytogenetic analysis of the fetus skin tissue confirmed the prenatal diagnosis. Written informed consent was obtained from the parents for publication of this case report and accompanying images. The consent form was approved by the ethical committee of Zhengzhou University, China.

Diagnosis and case presentation of two postnatal cases of de novo 9p deletion syndrome

Case 1

A male patient aged 4.5 years was referred to our center for intellectual disability, speech development delay (first words spoken at 25 months, difficulty with forming complete sentences). He was the only child of healthy nonconsanguineous parents. The family history was unremarkable. All the antenatal examinations were reported to be normal. The infant was delivered at 39 weeks of gestation and had an Apgar score of 10 at 1 minute. Examination of weight, length, and occipitofrontal circumference at birth was normal. Since the first years of life, he showed feeding difficulties, and gross motor and social milestone delays. In addition, trigonocephaly, flat midface, short palpebral fissures, highly arched eyebrows, low-set ears, flat nose, thin upper lip, short neck, long fingers, and hypotonia were obviously seen. Examination of the external genitals appeared normal. Abdominal ultrasound examination and brain magnetic resonance imaging were normal. As requested by the boy's parents, aCGH was performed for the boy and his parents. The results revealed an 18.74 Mb deletion of 9p24.3-p22.1 arr 9p24.3p22.1 (1–18,743,296 bp) × 1 (Figure 1B) in the boy, but balanced chromosomal copy number in both the parents. The deleted region of the boy included OMIM genes *DOCK8*, *KANK1*, *DMRT1*, *DMRT2*, *TYRP1*, *CER1*, *DMRT3*, and *KDM4C*. Blood lymphocyte karyotyping of the parents was performed to detect the potential balanced parental reciprocal translocations or pericentric inversions, but the karyotypes were normal.

Case 2

A 9-year-old boy came for genetic counseling with intellectual disability and hyperactivity. He was the first child of healthy nonconsanguineous parents, and the family history lacked evidence of congenital diseases. The infant was delivered by cesarean section at 37 weeks of gestation due to placental insufficiency and fetal distress. His Apgar score was 8 at 1 minute. His birth weight was in the normal range. Hereditary metabolic disease test, cardiac ultrasound, and hearing test results were normal, but brain magnetic resonance imaging revealed a mild hydrocephalus. In the next few years, the boy showed muscular hypotonia, gross motor delay, hydrocephalus, intellectual disability, and hyperactivity. Physical examination at this visit showed trigonocephaly, short palpebral fissures, highly arched eyebrows, low-set ears, flat nose, and long fingers (Figure 2). Examination of the external genitalia was refused by the parents, but they claimed that it was normal. Ventriculomegaly was present, but no other brain structure malformations were found by magnetic resonance imaging. The aCGH of the boy revealed a 11.78 Mb deletion of 9p24.3-p23 arr 9p24.3p23

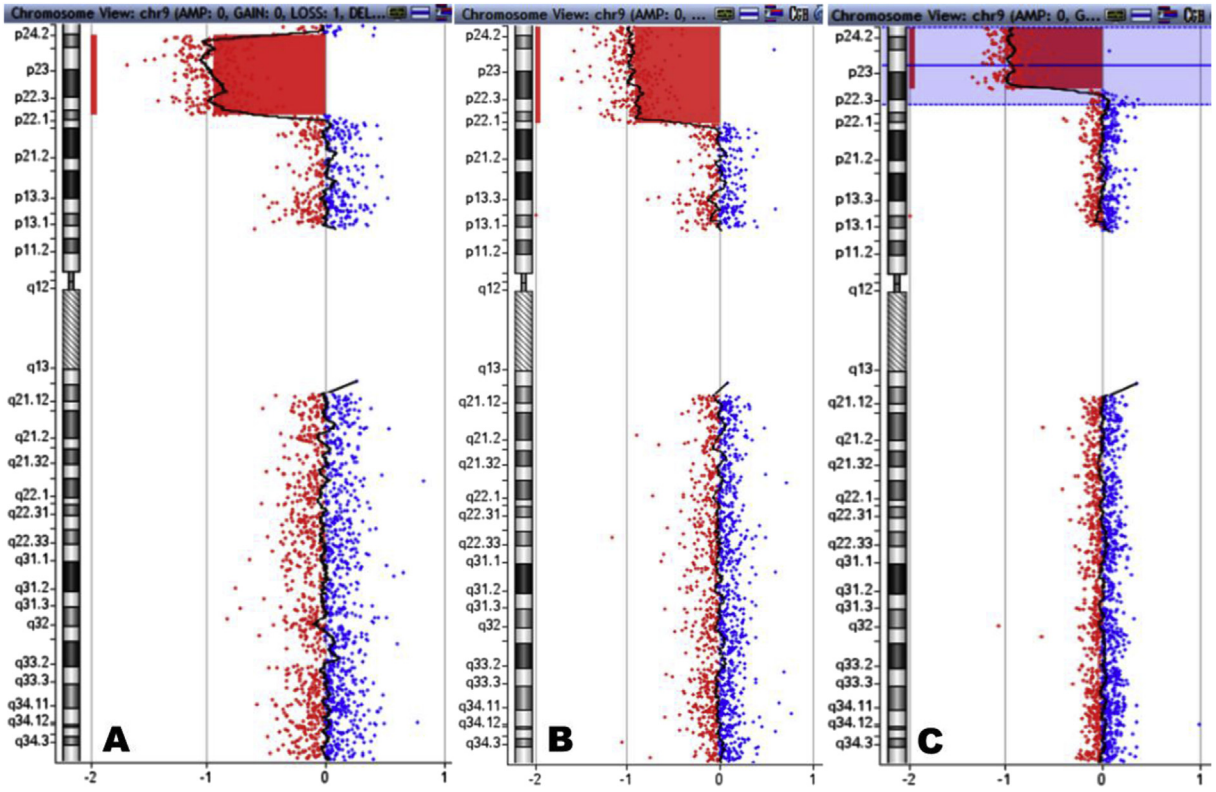

Figure 1. Whole genome aCGH detects chromosome 9p deletion of the samples. (A) Analysis by aCGH of the DNA extracted from cultured amniocytes detected a 15.19 Mb deletion at 9p24.2-22.2 [arr 9p24.2p22.2 (2,267,812–17,466,907 bp) × 1] encompassing 49 genes including *VLDLR*, *TYRP1*, *CER1*, and adjacent to genes *DMRT1* and *DMRT3*. (B) Boy 1 was detected with an 18.74 Mb deletion of 9p24.3-p22.1 [arr 9p24.3p22.1 (1–18,743,296 bp) × 1], including OMIM genes *DOCK8*, *KANK1*, *DMRT1*, *DMRT2*, *TYRP1*, *CER1*, *DMRT3*, and *KDM4C*. (C) Boy 2 was detected to have an 11.78 Mb deletion of 9p24.3-p23 arr 9p24.3p23 (271,257–12,048,612 bp) × 1, including OMIM genes *DMRT1*, *DMRT2*, *TYRP1*, *CER1*, *DMRT3*, and *KDM4C*. aCGH = array comparative genomic hybridization.

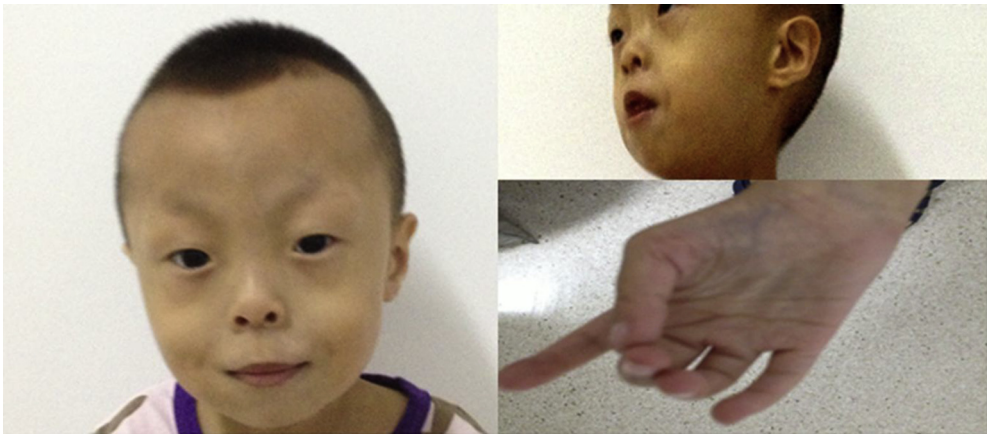

Figure 2. Postnatal Patient 2 with 18.74 Mb deletion of 9p24.3-p22.1 [arr 9p24.3p22.1 (1–18,743,296 bp) × 1] shows trigonocephaly, short palpebral fissures, highly arched eyebrows, low-set ears, flat nose, long fingers, and repetitive hand movements.

(271,257–12,048,612 bp) × 1, which included OMIM genes *DMRT1*, *DMRT2*, *TYRP1*, *CER1*, *DMRT3*, and *KDM4C* (Figure 1C). The parents showed normal blood lymphocyte karyotypes.

We adopted the following methods for our investigations:

Conventional cytogenetic analysis

Conventional karyotype analysis of cultured amniocytes and peripheral blood was performed using G-banding techniques at the 400–450 bands of resolution, according to the standard cytogenetic protocol.

Chromosomal microarray analyses

0.Ggenomic DNA (5 μg) was used to perform the genome-wide aCGH using the SurePrint G3 Human CGH Microarray Kit, 8x60K G4449A (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol. Analyses of the data were conducted with Agilent CytoGenomics software.

Discussion

Prenatal diagnosis and literature review

Although the symptoms of 9p deletion syndrome are typical and a number of cases are suspected in infancy, 9p deletions are rarely detected as a prenatal diagnosis [5]. In our case, a female fetus demonstrated an omphalocele that was found with a pure 15.19 Mb deletion at 9p24.2-22.2. We reviewed the literature with 9p deletion and positive prenatal ultrasound findings (Table 1) including ambiguous external genitalia and sex reversal [6–9], single umbilical artery (SUA) [6,7,10], intrauterine growth restriction [5,10,11], omphalocele [9], ventriculomegaly [12], increased nuchal translucency thickness [10], and other fetal structural abnormalities [6,7,9,10]. Of these, external genitalia malformation is a relatively common feature, which concurs with the reports that there may be a potential association between 9p24.3 region (including *DMRT1*/*DMRT3* genes) and the sex development disorders in male patients. Among the five earlier reported male fetuses, two were found with ultrasound-detectable external genital abnormalities [6,8] and another two were found with sex reversal that could not be detected by ultrasound during pregnancy [7,9]. The association of SUA with fetal anomalies has been described in numerous reports. Approximately 10% of SUA fetuses were found with chromosomal defects, and the most common is the trisomy 18. In our review of 11 prenatal 9p deletion cases, three (27.27%) fetuses were found with SUA. The absence of the association between SUA and 9p deletion may be because of the low discovery rate of 9p deletion syndrome during pregnancy. Similarly, although an omphalocele is a prominent congenital deformity for chromosomal abnormalities in fetuses and newborns for a long while, it has seldom been reported in 9p deletion syndrome cases. The only report of an omphalocele in a fetus was by Hou et al [9]. They found the male fetus with an omphalocele, symbrachydactyly, missing wrist bones and metacarpals, and sex reversal. In addition, we found three cases that showed omphaloceles in a newborn baby. One of them had pure 9p deletion [karyotype 46,XX,del(9)(p22)] [13], and the other two cases involved other chromosomal structural abnormalities [2]. These cases demonstrating omphaloceles were involved in *de novo* 9p13-22 breakpoints. However, two studies [9,13] did not perform aCGH or more detailed fluorescence *in situ* hybridization to detect the precise region of the deletion and eliminate other chromosomal anomalies. Therefore, we assume that further genetic work on breakpoints in regions 9p22 might be necessary to identify the genetic causes of omphalocele in 9p deletion fetus.

Phenotypic and genotypic features

Cytogenetic studies have suggested the breakpoint sites of deletion 9p syndrome mainly in the regions 9p21-p24 [1,2,14,15]. With the availability of higher-resolution methods such as aCGH and fluorescence *in situ* hybridization, studies have delineated the critical region of 9p deletion syndrome to a more narrow region on 9p22.2-23 [14,15]; deletion of the first 2 Mb of 9p was reported to be critical for the typical facial features [4], and a 3.5 Mb region between 11.4 Mb and 14.9 Mb is the critical region of the 9p deletion syndrome. Our present male cases demonstrated *de novo* and pure deletion at 9p24.3p22.1 (1–18,743,296 bp) and 9p24.3p23 (271,257–12,048,612 bp), respectively. The deletions included the first 2 Mb and part of the region between 11.4 Mb and 14.9 Mb of 9p. The phenotypic features observed in these two children are broadly consistent with the previously reported phenotypes. They shared speech development delay, learning difficulties, hypotonia, trigonocephaly, arching eyebrows, low-set ears, and flat nose. However, both the boys apparently did not present external genitalia malformation, although the deletion region including *DMRT1* and *DMRT3* genes was localized on the sex reversal region 9p24.3 [3,6]. The boy with deletion of 9p24.3p23 showed hydrocephalus and hyperactivity that have not previously been reported in patients with *de novo* pure 9p deletion syndrome. It is assumed that

Table 1
Views of prenatal diagnosis with abnormal ultrasound findings and postnatal cases with an omphalocele (reported cases with chromosome distal 9p deletion syndrome).

Author	Prenatal ultrasound findings	Prenatal diagnosis and karyotype	FISH/aCGH detected minimal deletion
Vialard et al [6]			
Fetus 1	24 wk: ambiguous genitalia with clitoral hypertrophy, normal growth	Amniocentesis: 46,XY,del(9)(p22)	NA
Fetus 2	Hypoplastic left heart, single umbilical artery, normal growth	Amniocentesis: 46,XX,del(9)(p22)	NA
Chen et al [5]	25 wk: IUGR	Amniocentesis: 46,XX,del(9)(p24.1p24.3)	aCGH: arr 9p24.3p24.1 (198,350–6,256,729) × 1
Witters et al [7]	12 wk: micrognathia, an enlarged posterior fossa, bilateral pes equinovarus, single umbilical artery, a thickened umbilical cord, sex reversal	CVS: 46,XY,der(9)t(3;9)(p14.2;p24)	NA
Brisset et al [10]	12 wk: NT = 4.4 mm; 22 wk: NT = 9.5 mm; single umbilical artery, partial agenesis of the cerebellar vermis, bilateral cystic choroid plexus, facial dysmorphisms, IUGR	Amniocentesis: 46,XX,add(9)(p24.3)	FISH and aCGH: 46,XX,der(9)t(9;17) (p24.3;q24.3), 2.4 Mb 9p loss and 17q24.3-qter gain
Chen et al [8]	15 wk: Down syndrome risk of 1/57 21 wk: ambiguous external genitalia	*In situ* cultured amniocytes: 45,XY,−9[2]/46,XY,r(9)(p24q34.3)[9]	NA
Chen et al [12]	21 wk: ventriculomegaly, normal male external genitalia	Amniocentesis: 46,XY,inv dup del(9) (:p22.1 → p24.3::p24.3 → qter)	FISH, aCGH, microsatellites
Penacho et al [11]	25 wk: IUGR	Amniocentesis: 46,XX,r(9)(p24q34)	2.57 Mb deletion at 9p24.2, 2.60 Mb deletion at 9q34.3qter, and 0.15 Mb deletion at 9p24.1
Hou et al [9]	17 wk: omphalocele, symbrachydactyly, wrist bones and metacarpals missing, sex reversal, relative risk of Down syndrome 1/335	Amniocentesis: 46,XY,del(9)(p13)	NA
Our case	17 wk: omphalocele	Amniocentesis: 46,XX,del(9)(p22)	aCGH: arr 9p24.2p22.2 (2,267,812–17,466,907 bp) × 1]
Nagy et al [13]	Postnatal: omphalocele and typical 9p deletion syndrome phenotype	Postnatal: 46,XX,del(9)(p22)	NA
Swinkels et al [2]	Postnatal: omphalocele and typical 9p deletion syndrome phenotype	46,XY,der(9)t(9;16)(p22;q24) 46,XX,der(9)inv(9)(p24.3p22)	p22.3–p22.3 and pter pter-p22.2

aCGH = array comparative genomic hybridization; CVS = chorionic villus sampling; FISH = fluorescence *in situ* hybridization; IUGR = intrauterine growth restriction; NA = not available.

those features may be associated with the disruption of unknown genes in this region.

In conclusion, we have presented one case of prenatal and two cases of postnatal *de novo* monosomy 9p syndrome. The present case provides evidence for a correlation between omphalocele and distal 9q deletion. Besides, the literature review of the association of uncommon prenatal ultrasound findings with 9q deletion syndrome demonstrates that external genitalia malformation, SUA, and intrauterine growth restriction are relatively common in 9p deletion prenatal cases.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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