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A fetus with 19q13.11 microdeletion presenting with intrauterine growth restriction and multiple cystic kidney[a]

Abstract

Background: Constitutional deletions of chromosome 19q were rarely reported in the literature. The array-comparative genome hybridization (CGH) helps to identify four children with 19q13 microdeletion and the microdeletion of 19q13.11 is a recent emerging syndrome. We report one fetus with 19q12q13.12 deletion diagnosed prenatally and compare with five cases in the literature.

Case: The 29 year-old woman was at the 27th week of gestation. Prenatal ultrasound revealed severe intrauterine growth restriction and left side multiple cystic kidney with normal amniotic fluid index. Cordocentesis was performed for karyotyping and array CGH.

Results: The karyotype of the fetus was considered as normal male. The BAC array CGH identified one deletion at chromosome 19q12q13.12. The oligonucleotide array CGH further characterized the size of the breakpoint (chr19:35,116,199-42,994,905). After counselling, the pregnancy was terminated at the 28th week of gestation. The aborted fetus had hypospadias and facial dysmorphisms.

Conclusions: Although a complete genotype-phenotype may not be established in these patients with 19q13 deletions, they shared some unique phenotypes and facial dysmorphisms. The clinician should keep in mind when anomalies are detected prenatally, array CGH may help to identify the etiology, which is critical for counselling.

Keywords: 19q Deletion; array-comparative genome hybridization; IUGR; MCKD; microdeletion.

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Introduction

Fewer than ten cases with constitutional deletions of chromosome 19 have been reported in the literature. Such a paucity of patients is likely related to the absence of a distinct banding pattern on this chromosome [10]. Using advanced molecular cytogenetic technology, the array-comparative genome hybridization (CGH) helped to identify four children with 19q13 microdeletion in 2009 [10, 13]. Although there was some phenotypic variability, these patients shared some common findings and the microdeletion of 19q13.11 was a recent emerging syndrome. The ever reported five cases with overlapped 19q13.11 deletion had phenotypes including intrauterine and postnatal growth retardation, microcephaly, feeding problems, ectodermal dysplasia, cutis aplasia over the posterior occiput, hypospadias, and clinodactyly of the fourth finger. We report detailed studies of one fetus with 19q12q13.12 deletion diagnosed prenatally and compare the findings with five previously reported cases in the literature [9, 10, 13].

Clinical report and methods

The 29-year-old mother, gravida 2, para 1, was Asian. Her first baby was a healthy girl. Prenatal ultrasound examination was performed at the 27th week of gestation because the fetus was small for gestational age. The biparietal distance of the fetus was 55 mm (50th percentile for 23 weeks), head circumference 210 mm (50th

percentile for 23 weeks), and femur length 45 mm (50th percentile for 25 weeks). The fetal measurements corresponded to 23 weeks of gestation with mild increased resistance of the umbilical artery (S/D ratio, 3.6; 71st percentile; pulse index, 1.52; normal waveform). Pulse index of right and left uterine arteries were 0.53 and 1.28, respectively, and no notch was noted. The ultrasound study also showed a multicystic left kidney and a normal-sized right kidney, normal amniotic fluid index (13.5 cm), and female genitalia. A fetal echocardiogram and color flow Doppler ultrasound studies showed no abnormalities. Fetal breathing movement, fetal movement, and fetal tone were normal.

Cytogenetic studies

Cordocentesis was performed at the 27th week of gestation. Standard karyotyping using G-banding analysis was performed on the lymphocytes in the cord blood and the peripheral blood of the parents.

Array CGH studies

Genomic DNA was extracted from the fetal cord blood and the parent's peripheral blood using the QIAamp DNA Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. DNA concentration was determined with NanoDrop ND 1000 spectrophotometer and software (NanoDrop Technologies, Berlin, Germany). Bacterial artificial clones (BAC) based array CGH was performed using the BAC HD Scan™ v2 microarray (CMDX, Irvine, CA, USA) with a resolution of 1 Mb according to the manufacturer's instructions. Oligonucleotide array CGH, Oligo HD Scan™ v.1 (CMDX, Irvine, CA, USA), with 105,000 oligo probes spaced at approximately 10- to 35-kb intervals across the genome, was used to further characterize the extend of the deletion.

Multiplex PCR

Multiplex PCR assays were performed to identify the genetic dosage of six genes near the breakpoint (Figure 1). The sequences of primers were listed in Supplementary Table 1. The multiplex PCR was performed in a total volume of 25 μL containing 100 ng of genomic DNA, 0.04 – 0.4 μM of each primer, 200 μM dNTPs, 0.1 units of AmpliTaq GoldTM enzyme (PE Applied Biosystems), and 2.5 μL of GeneAmp 10× buffer II (10 mM Tris-HCl, pH 8.3, 50 mM KCl), in 2 mM $MgCl_2$ as provided by the manufacturer.

The multiplex PCR products were analyzed by the high-performance DNA analysis capillary electrophoresis system with the GCK-500 cartridge kit (eGene, Irvine, CA, USA). The quantification of DNA fragments was based on the integrated peak area. The target gene copy numbers were calculated by adjusting the relative known dosage of the *KRIT1* gene. We used the following equation to

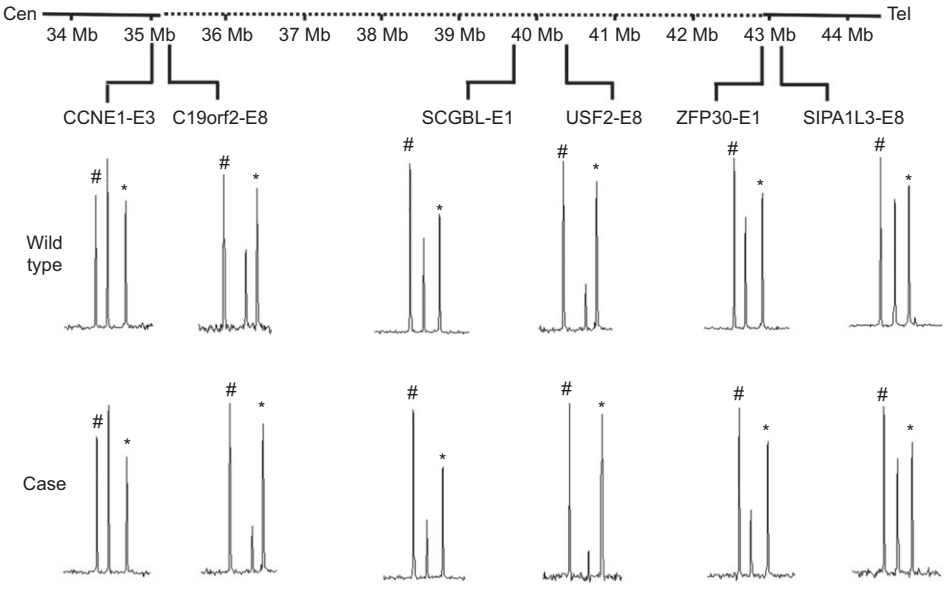

Figure 1 Multiplex PCR revealed one allelic deletion in the *C19orf2* gene, *SCGBL* gene, *USEF2* gene, and *ZFP30* genes but not in the *CCNE1* gene or the *SIPA1L3* gene.
#*FGFR2* gene; **KRIT* gene.

calculate the copy number of the target gene in the case (C) as compared with the mean of the wild type (W):

$$\frac{\text{Peak area of the target gene (C)/Peak area of } \textit{KRIT1} \text{ (C)}}{\text{Peak area of the target gene (W)/Peak area of } \textit{KRIT1} \text{ (W)}} \times 2$$

Results

The karyotype of the fetus was considered as normal male by standard G-banding method (Figure 2A). With the BAC array CGH, nevertheless, we identified one 5.84-Mb deletion at the long arm of chromosome 19 in the fetus. A total of 13 BACs (from RP11-13D7 to RP11-587I9) spanning a region from 35.45 to 41.29 Mb in 19q12q13.12 had a significantly low test/reference log2 intensity ratio (Figure 2B). The oligonucleotide array CGH further characterized the size of the deletion and the breakpoint (chr19:35,116,199–42,994,905) (Figure 2C). Multiplex PCR revealed one allelic deletion in the *C19orf2* gene, *SCGBL* gene, *USEF2* gene, and *ZFP30* genes but not in the *CCNE1* gene or the *SIPA1L3* gene in this case (Figure 1). The BAC array CGH analyses of the parental samples demonstrated that the deletion occurred *de novo*.

After genetic counseling, the pregnancy was terminated at the 28th week of gestation. A fetocide was performed with 6 mL potassium chloride at the fetal heart under sonographic guidance. Misoprostol 50 μg was then administered intravaginally once and then thrice orally every 6 h. The abortus was expulsed with intact amniotic bag 26 h later. The aborted fetus weighted 402 g and had ambiguous genitalia and facial dysmorphism including long face, high forehead, retrognathia, thin lips, maxillary protrusion, broad nasal root, and large posteriorly angulated and low-set ears (Figure 3). The couple refused an autopsy of the abortus.

Discussion

We described the six patients with an interstitial deletion overlapping the 19q13.11 region. The 5.84-Mb

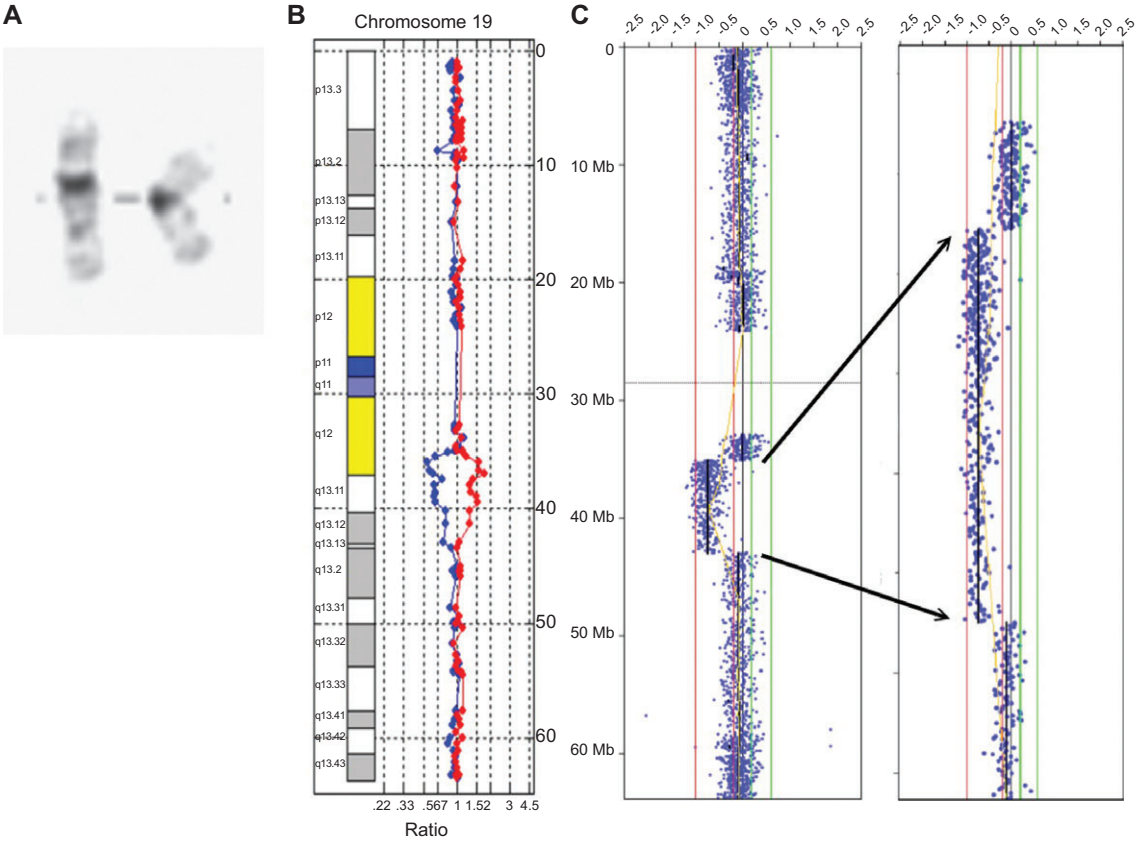

Figure 2 (A) G-banding of chromosome 19. (B) BAC array CGH revealed the deletion from 19q12 to 19q13.12. (C) Oligonucleotide array CGH revealed the deletion of chromosome 19 from 35,116,199 to 42,994,905 bp.

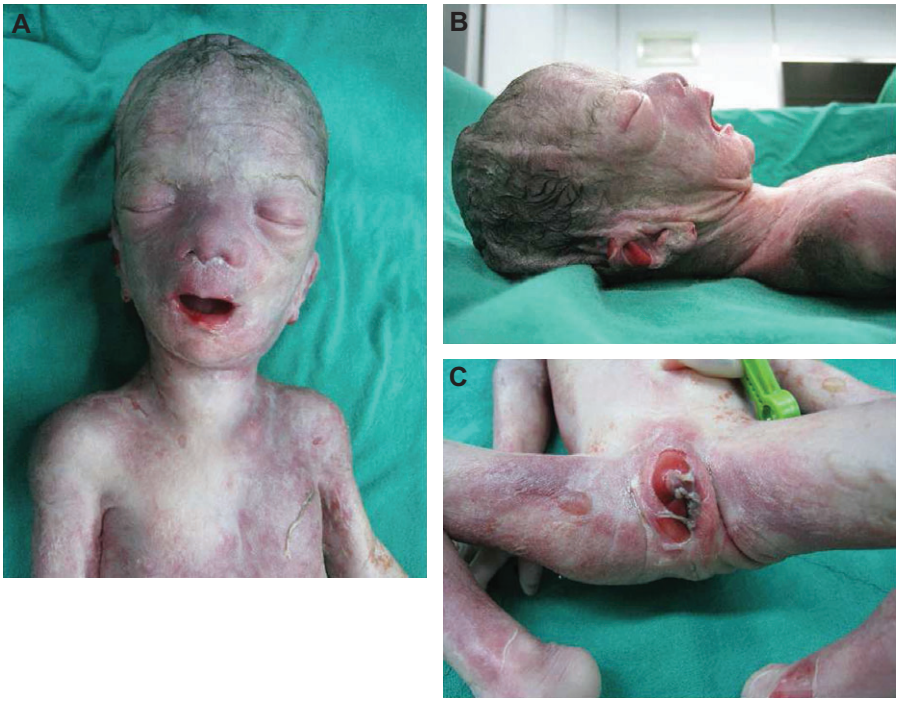

Figure 3 The aborted fetus had ambiguous genitalia and facial dysmorphism including long face, high forehead, retrognathia, thin lips, maxillary protrusion, broad nasal root, and large posteriorly angulated and low-set ears.

microdeletion was initially detected by BAC base array CGH with 1-Mb resolution. The breakpoint was extended to 7.88 Mb by the oligonucleotide array with 35-kb resolution. In addition, most array CGH results were validated by fluorescence *in situ* hybridization (FISH) technology [4, 12, 14]. We designed the multiplex PCR assays to further confirm the breakpoints. The advantage of the method is fluorescence-independent, self-designed, and less expensive. In terms of length of time, the FISH method requires 2–3 days, which involves cell culture, whereas multiplex PCR requires 3 h [8].

Because this pregnancy was terminated, we were not able to evaluate the developmental characteristics, motor functions, or the cutis aplasia in the midline of the scalp. The abortus, nevertheless, shared some overlapping phenotypes with the other five cases reported in the literature. First, all of the cases with 19q13.11 microdeletion had intrauterine growth retardation (IUGR). Second, all the male patients had hypospadias. Third, these patients had typical facial dysmorphisms, such as thin lips (6/6), micro/retrognathia (6/6), large ears (4/6), and long face (4/6). In the deleted region of our case, there were over 40 zinc finger protein genes. Whether the haploinsufficiency of these zinc finger cluster in the long arm of chromosome 19 might contribute to the phenotypes needs to be further investigated. Schuurs-Hoeijmakers et al. [13] had refined the critical region to 750 kb and identified two candidate genes, *LSM14A* and *UBA2* gene, that might be responsible for the 19q13.11 microdeletion syndrome [13].

Of the six cases with 19q deletion, only our patient and the patient of Kulharya et al. [9] had multicystic kidneys. However, the overlapping deleted regions (1 Mb) between these two cases contain mainly zinc finger protein genes and some cDNA with unknown molecular function (Figure 4). Furthermore, within the 7.8-Mb deleted region of our patient, there are several genes that may be associated with abnormal renal function. The interrupted *USF2* gene has been reported in a case with a *de novo* translocation, t(6;19)(p21;q13.1), and multicystic renal dysplasia [6]. The *UPK1A* gene encodes uroplakin protein, which may play a role in normal bladder epithelial physiology [7]. The *NPHS1* gene is primarily expressed in the renal tissue, and the mutations in this gene result in congenital nephrosis, characterized by severe proteinuria and loss of the slit diaphragm and foot processes [1, 5]. Although a complete genotype-phenotype correlation cannot be established by these six patients, the 19q13.11 microdeletion syndrome may have some recognizable facial dysmorphism and share some phenotypes.

The patient of Kulharya et al. [9] had the largest deleted region (about 11 Mb) and was identified initially by karyotyping. The other four cases reported by Malan et al. [10] and Schuurs-Hoeijmakers et al. [13] were diagnosed

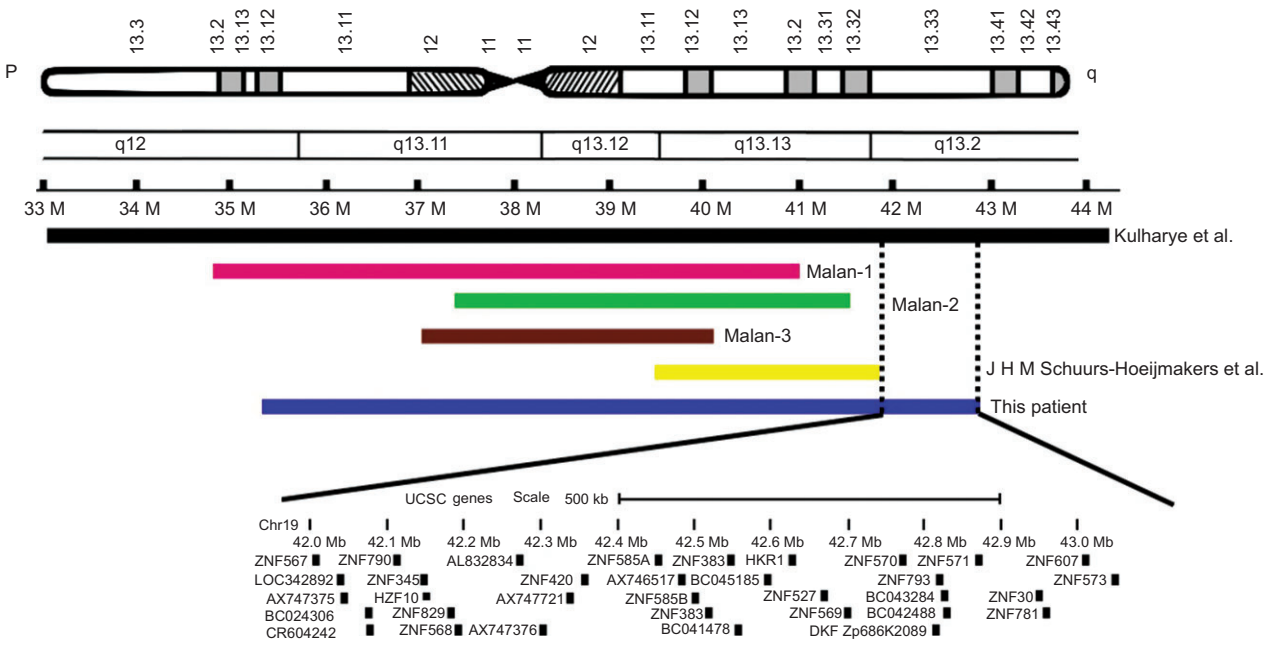

Figure 4 Six cases with 19q13.2 deletions in the literature.

postnatally by array CGH technologies due to the small deleted size and the limited resolution of standard karyotyping. In our patient, standard G-banding also failed to identify the etiology of the abnormal sonographic findings. According to the literature [3], unilateral multicystic dysplastic kidney disease with normal karyotype and adequate amniotic fluid is a reassuring finding. However, this reported case also had IUGR, which prompted us to do further workup. By applying the array CGH, we detected the microdeletion at chromosome 19q12q13.12, which involved not only the 19q13.11 but also the *SCN1B* gene and the *COX6B1* gene; the mutations in the former gene may result in generalized epilepsy with febrile seizures and arrhythmia, whereas the mutation in the latter gene are reported in patients with severe infantile encephalomyopathy [2, 11, 15, 16]. Under the situation, the information was critical for the parents to make decisions at prenatal counseling. The case demonstrated the benefit of array CGH in the prenatal application.

Conclusions

Array CGH helps to identify microdeletions and provides higher resolution than G-banding karyotype. The cases with microdeletion of 19q13.11 share several common phenotypes and microdeletion of 19q13.11 is a recent emerging syndrome.

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