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Short Communication

Prenatal diagnosis of isochromosome 20q in a fetus with vertebral anomaly and rocker-bottom feet

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ABSTRACT

Objective: Isochromosome of the long arm of chromosome 20 (i(20q)) is a rare structural abnormality in prenatal diagnosis. Thirty prenatal cases of mosaic i(20q) have been reported, among which only four are associated with fetal malformations. We describe a new prenatal case of i(20q) with fetal malformations.
Materials and methods: We also observed a discrepancy between uncultured and cultured amniotic fluid cells by using conventional cytogenetic, fluorescence *in situ* hybridization and array-SNP analysis.
Results: The short arm deletion of chromosome 20 arising from the isochromosome encompassed two candidate genes *PAX1* and *JAG1* involved in cranio-facial and vertebral development.
Conclusion: The data would allow establishing a phenotype–genotype correlation. Thus, we proposed to define a recognizable syndrome combining cranio-facial dysmorphism, vertebral bodies' anomalies, feet and cerebral malformations.

Introduction

Isochromosomes are very rare chromosomal abnormalities in prenatal diagnosis; they provide the loss of one chromosome arm and the duplication of the other.

According to the literature, isochromosome 20q (i(20q)) is usually a mosaic chromosomal abnormality associated with a normal prenatal ultrasonography [1,2]. This outcome is strongly related with the discrepancy between amniocytes and lymphocyte's newborn karyotypes. Among the thirty cases previously reported, four cases had fetal malformations [3–6]. We report a new case of i(20q) associated with fetal malformations.

Materials and methods

Clinical report

A 25-year-old woman underwent amniocentesis at 25 weeks gestational age because of isolated thoracic hemivertebraes at T6-T7 detected by prenatal ultrasound. It was the second pregnancy in a nonconsanguineous and healthy couple with no family history and a healthy daughter.

Cerebral structures were normal at ultrasound examination, whereas head circumference and biparietal diameter were below 5th percentile. The bone tomodensitometry highlighted a hypoplastic vertebra T6-T7 with a defect of mediodorsal segmentation; furthermore, a suspicion of cervical vertebral block was emitted.

On the basis of ultrasound examination and bone tomodensitometry findings and the chromosomal rearrangement, the parents opted for a medical termination of pregnancy, according to French law. Autopsy showed an eutrophic female fetus with dysmorphic features including a particular trichofacial pattern with low frontal hairlines, upslanting palpebral fissures, large ears with

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simply-folded helices, bilateral preauricular pits and prominent cheeks. A short neck and rocker-bottom feet were associated. X-ray confirmed the presence of hypoplastic vertebral bodies T6-T7, with staggered coronal clefts. Internal examination showed a thymus hypoplasia, a bilateral renal moderate hypoplasia and two secondary spleens. There was neither congenital heart defect nor cerebral malformation (Fig. 1A and B).

Array Single Nucleotide Polymorphism analysis (array-SNP)

Array Single Nucleotide Polymorphism (*array-SNP*) (Cytoscan HD, Affymetrix) was performed on DNA extracted from uncultured and cultured amniotic fluid cells. Molecular analysis used Affymetrix Cytoscan HD array, which contain 2.4 million markers including 200,000 SNPs (Affymetrix, Santa Clara, CA), according to the manufacturer's protocol. The results were analyzed with Cyto-B–N2.0.1.2 (r5919) software. Interpretation was based on Human Genome Build 32.3 (NCBI/hg19).

Conventional cytogenetic and FISH analysis

Conventional cytogenetic analysis was carried out on cultured amniotic fluid cells and on parental lymphocytes, using RHG and GTG banding according to standard protocol.

Fluorescence *in situ* hybridization (FISH) analysis was performed on uncultured amniotic fluid cells and on metaphases spread of cord blood, tendon, muscle and skin samples, using subtelomeric probes of each arm of chromosome 20 (20p/20q) (Vysis, Abbott Molecular, Des Plaines, IL, USA). Furthermore, it was carried out using a specific probe for the Alagille syndrome including JAG1 (20p11.2) (Amplitech, Cytocell, Cambridge, United Kingdom) on two cultured amniotic fluid cells: the first at the diagnosis and the second at the termination of pregnancy, according to protocol manufacturer.

Results

Array Single Nucleotide Polymorphism analysis

Array-SNP analysis did not detected imbalance genomic on uncultured amniotic fluid (arr[hg19] (1-22,X)x2). However, on cultured amniotic cells, in accordance with conventional cytogenetic, Array-SNP confirmed a complete duplication of the long arm of chromosome 20 which the minimal size was estimated to be 33.5 Mb, associated with the deletion of its short arm with a minimal size of 25.7 Mb. This result was in agreement with an isochromosome 20q. The 20p deletion encompassed two candidate genes: *PAX1* (OMIM 167411) and *JAG1* (OMIM 601920) (Fig. 1C).

Conventional cytogenetic and FISH analysis

Chromosomal analysis revealed a non-mosaic isochromosome 20q on 12 colonies taken from two different *in situ* cultured of the two amniotic fluids cells. Parental karyotypes were normal, suggesting a *de novo* rearrangement. From post-mortem conventional

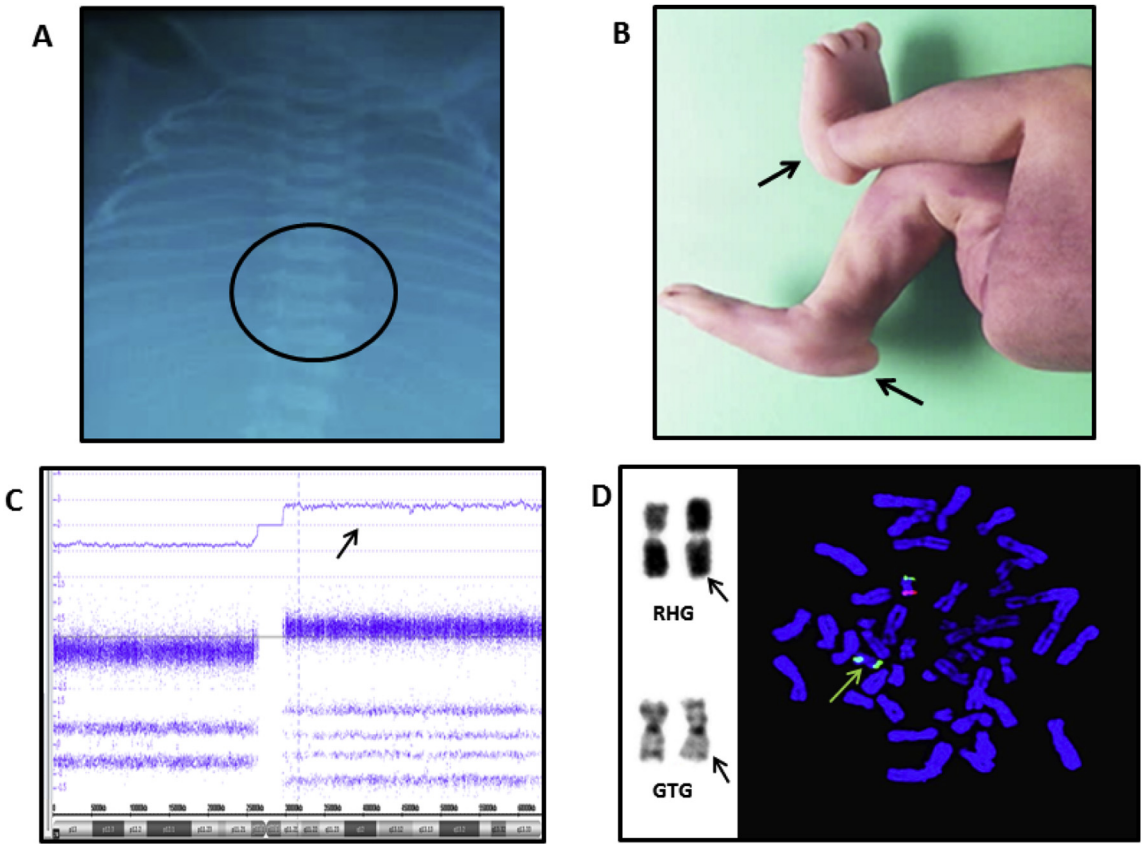

Fig. 1. A: X-ray showing hypoplastic T6-T7 vertebral bodies, with coronal clefts. B: Rocker-bottom feet. C: Array-SNP from cultured amniotic fluid show: the deletion of the short arm and the duplication of the long arm of chromosome 20 (increase of the upper line). D: Chromosome 20 in RHG and GTG banding from cultured amniotic fluid showing the isochromosome 20q. FISH analysis using Alagille probe: JAG1 (red)/20qter (green) from cultured amniotic fluid; the isochromosome 20q is revealed by two green spots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cytogenetic studies no chromosomal abnormality was identified on cord blood, tendon, muscle and skin samples.

FISH analysis using telomere probes of short and long arm of chromosome 20 on uncultured amniotic fluid and tissues from post-mortem samples were normal. Array-SNP on cultured amniotic fluid was confirmed by the lack of the specific probe JAG1 (20p11.2) on all cells from the first amniotic fluid, but on 86% of amniotic cells from the second amniocentesis performed during the medical termination of pregnancy (Fig. 1D). Based on this finding, the karyotype was: 46,XX,i(20)(q10).arr[hg19] 20p11.1p13(61,568-25,731,596)x1,20q11.21q13.33(29,421,135-62, 915,555)x3 dn.

Discussion

Thirty prenatal cases of mosaic i(20q) have been reported since 1990, among which only four cases were associated with fetal malformations [3–6]. Our case represents the fifth one.

We describe a new prenatal case of an isochromosome 20q associated with fetal malformations, detected by conventional cytogenetic on cultured amniotic fluid, showing a discrepancy on uncultured amniotic fluid diagnosed by array-SNP. This last analysis from cultured amniotic fluid cells revealed an isochromosome 20q. Only five reported cases in the literature studied both uncultured and cultured amniotic fluid cells [2,7,8]. They provide evidence for cytogenetic discrepancy between both types of these amniotic cells, which were concordant with the present case. In all five cases, FISH analysis was performed on uncultured cells and did not detect the i(20q); likewise four of them [8] had no genomic imbalance using chromosomal microarray analysis (CMA) as arrayCGH. In fifteen of the twenty six cases previously reported without fetal malformation, fetal blood karyotype was normal [5]. Present case and Goumy's one [6] showed similar fetal malformations associated with this discrepancy. Some authors considered that the particularity between uncultured and cultured amniotic fluid cells could be a tissue-limited cell culture artifact [9]. We speculate that the malformations are in correlation with the isochromosome 20q which should not be considered as a cultural artifact. Indeed, our cytogenetic results were obtained from several different *in situ* colonies cultures.

In our case, FISH analysis on cells from the first amniocentesis showed a non-mosaic i(20q); therefore, in repeated amniocentesis, we found 86% of i(20q). A decrease of abnormal cells was noted in 3/4 Chen'cases [8] when amniocentesis was repeated. This result could be related to normal fetal cells released in amniotic fluid. It is most likely that the cell line with the isochromosome was of post-zygotic origin, issued from a normal 46,XX conceptus. Furthermore, it is possible that these cells were initially a very small colony confined to specific embryonic tissue layer. Thus, they were probably only highlighted after the cultures of amniotic fluid cells [10,11].

No chromosomal anomaly was observed in fetal blood and tissues using conventional and molecular cytogenetic analyses in the present case. In 2/4 previously cases described with fetal malformations, cytogenetic analyses were performed on both fetal tissues and blood [4,5]. Pfeiffer [4] reported the first case associated with fetal malformations in which cultured amniotic cells identified 90% of i(20q) and FISH analysis showed 6%, 13.7% and 42% of the isochromosome in buccal smear, urinary sediment and cord blood respectively. In contrast, karyotypes on fetal tissues were normal in the case described by Chen [5] presenting mainly arthrogryposis, and amyoplasia. In the four previous cases with fetal malformations, the authors argued the uncertainty of the causal relationship between the chromosomal abnormality and observed malformations. However, cases of Pfeiffer [4] and Goumy [6] were substantially closed to our in terms of craniofacial dysmorphy, rocker-bottom feet anomaly and dissegmentation of thoracic spine. Thus, despite the small number of cases reported with fetal malformations and i(20q), a phenotype–genotype correlation could be proposed. The short arm of chromosome 20 contains two candidate genes: *PAX1* (OMIM 167411) whose deletion provides an oto-facio-cervico syndrome and *JAG1* (OMIM 601920) responsible for Alagille syndrome (OMIM 118450). These two genes are involved in vertebral development [12].

In some cytogenetic laboratories, the CMA as a first line tool on uncultured amniotic fluid cells tends to replace conventional fetal karyotype when fetal malformations are detected. This analysis has a better resolution and provides a quick result compared to conventional karyotype. However, the CMA remained normal in the case described by Goumy [6] and in our case. We think that a normal CMA from uncultured cells should not be sufficient in the cases of fetal malformations and that conventional karyotype has still its place in prenatal diagnosis.

We propose two strategies for prenatal genetic counseling in diagnosis of isochromosome 20q. In the absence of fetal malformation, when the isochromosome 20q is revealed after culture of amniotic fluid cells, genetic counseling could be reassuring. Otherwise, when fetal malformations are detected with normal molecular cytogenetic on uncultured amniotic fluid cells, further consideration should be given for additional karyotype analysis. Diagnosis of i(20q) with vertebral anomaly would tend to make an unfavorable prognosis for the pregnancy.

Conflicts of interest

The authors declare no conflict of interest.

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