

Short report

Prenatal diagnosis of mosaicism for 11q terminal deletion

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Abstract

The phenotype of 11q terminal deletion also known as Jacobsen syndrome is a clinically well known entity whose diagnosis in infancy and childhood is based on clinical examination, hematological and cytogenetic findings. Hematological features in Jacobsen syndrome are very similar to those reported in Paris–Trousseau syndrome (PTS) which is also associated with 11q terminal deletion. Karyotype analysis shows a variable terminal deletion from 11q23 sub-band extending to the telomere. Most often in patients with Jacobsen syndrome, this chromosomal deletion is present in all metaphases. We report on the identification of a distal 11q deletion in mosaic (20% of deleted cells) in a fetus ascertained after amniocentesis for maternal serum screening test indicative for Down syndrome. The present case is the third prenatal diagnosis of a mosaic for a distal 11q deletion with the lowest mosaicism rate. The 2D-ultrasound examination and cord blood hematological studies were useful to estimate the prognosis at term, considering the contribution of the mosaicism rate to the phenotypic variability in Jacobsen syndrome. The identification of mosaicism for distal 11q deletion is a very rare event in prenatal diagnosis. This case

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illustrates the complexity in genetic counselling for prenatally ascertained partial monosomy 11qter in mosaic.

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1. Introduction

Jacobsen syndrome, or distal deletion of the long arm of chromosome 11 (OMIM#147791), is a rare but clinically well known entity [9,11,20] and has an incidence of approximately 1 in 100 000 births. Patients usually have visible deletions based on karyotype analysis: the breakpoint arises typically in 11q23.3 sub-band but can be located in 11q24 or 11q25 with deletions extending to the telomere [15]. Most frequently (85%), this deletion appears *de novo*. Other cases are the result of malsegregation from balanced parental rearrangements; in some patients Jacobsen syndrome is associated with a *de novo* chromosomal event as ring chromosome 11 [6]. There is a broad clinical spectrum of Jacobsen syndrome. Clinical manifestations can include developmental delay, psychomotor retardation, short stature, craniofacial features (trigonocephaly, hypertelorism, broad and flat nasal bridge, carp-shaped mouth with thin upper lip, low-set malformed ears), congenital heart defects (hypoplastic left heart syndrome, membranous ventricular septal defect, aortic arch defects), genitourinary anomalies, pyloric stenosis, ocular malformations (ptosis, colobomas, cataracts, glaucoma, strabismus, telecanthus), limb anomalies (talipes equinovarus, clino- or camptodactyly, syndactyly), recurrent infectious episodes and thrombocytopenia or pancytopenia. Hematological features in PTS include thrombocytopenia with or without pancytopenia and platelet abnormalities [2,7]. Favier et al. [8] noted clinical, hematological and cytogenetic similarities between Jacobsen syndrome and PTS. They concluded that there is clearly a clinical overlap between these two syndromes, which are both associated with 11q terminal deletion. In the PTS, hemizygous deletion of the monoallelic expressed *FLII* gene contributes to the hematopoietic defects. The majority of Jacobsen syndrome affected cases were reported in infancy and childhood, however 14 affected fetuses were described with different pregnancy outcomes [4]. Only two cases of prenatally diagnosed 11q terminal deletion mosaicism are described in the literature [3,16]. In the present study, we report on a fetus with 11q terminal deletion mosaicism. The fetus presented with minor clinical features of Jacobsen syndrome after second trimester ultrasound examination but very suggestive hematological anomalies on cord blood.

2. Patients and methods

2.1. Case report

A 37-year-old primigravid woman underwent amniocentesis at 21 weeks' gestation because of a serum screening test positive for Down syndrome (1/78 for a normal test at 1/250) without ultrasound anomalies at the first examination. At 14.4 weeks' gestation, the maternal blood screening test showed a serum alpha-fetoprotein level of 0.66 multiples of the median

(MoM) and a serum β -human chorionic gonadotrophin level of 1.52 MoM. Amniocentesis was performed at 21 weeks' gestation for foetal karyotype analysis. There was no family history of congenital malformations.

2.2. Cytogenetic and FISH analysis

Cytogenetic studies were performed on amniotic fluid and cord blood cell cultures by conventional G-banding at 400-band level. FISH analysis were performed on fetal slides with 11p spectrum orange (red) and 11q spectrum green (green) subtelomeric probes (Cytocell, Co, UK) according to the manufacturer's recommendations and with a spectrum orange (red) probe specific for *FLII* gene (BAC RP11-138K22) located in 11q24.3. Slides were analysed using Zeiss Axioplan 2 and Axioskop 2 microscopes (Zeiss, Jena, Germany) with the IKAROS and ISIS Metasystems digital imaging systems (Metasystems, Altusheim, Germany).

2.3. Hematological analysis

Platelet count was assessed on Advia 2120 (Bayer) automate. Slides of peripheral blood were stained with May–Grünwald–Giemsa for light microscopy analysis ($\times 100$).

2.4. DNA analysis

The mosaicism rate for the terminal 11q deletion was assessed by semi-quantitative fluorescent PCR with 17 short tandem repeat markers (D11S1356, D11S4104, D11S4129, D11S924, D11S1299, D11S4132, D11S994, D11S4089, D11S4107, D11S1899, D11S4167, D11S1345, D11S1336, D11S488, D11S4144, D11S4167 and D11S934) located in the 11q23.3-q24.2 region (<http://genome.ucsc.edu>). PCRs were performed on total genomic DNA extracted from cultured amniotic cells with the Nucleon BACC3 kit (Amersham Life Technologies) according to the manufacturer's protocol. The PCR consisted of an initial denaturation at 95 °C for 7 min, followed by 24 cycles (95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s) and ended by a 7 min extension at 72 °C. Fluorescent PCR products were separated by capillary electrophoresis on an ABI prism 310 genetic analyser (Applied Biosystems). For heterozygous markers, peak areas were compared thanks to Genescan 3.1 and Genotyper 2.5 softwares.

3. Results

The first amniocentesis allowed the characterization of a mosaic karyotype: 46,XY[16]/46,XY,del(11)(q23)[3]. A second amniocentesis was realized at 24 week's gestation: 7 out of 36 colonies showed the same 11q terminal deletion extending from the 11q23 sub-band to the telomere (Fig. 1A). Fluorescence in situ hybridization (FISH) studies with 11q subtelomeric probe (Fig. 1B) but also with a probe specific for the *FLII* gene (RP11-138K22; Fig. 1C) confirmed the mosaic deletion with a mosaicism rate at 20%. Parental karyotypes were normal. Cytogenetic analysis (fetal karyotype and FISH) were also performed on cord blood at 24 week's gestation and showed the same mosaicism deletion rate.

2D-ultrasound examination at 25 weeks' gestation was performed to search for typical or minor signs of Jacobsen syndrome. This examination showed a light increased amount of amniotic fluid (amniotic fluid index: 280) and a single male fetus. The fetal biometry included

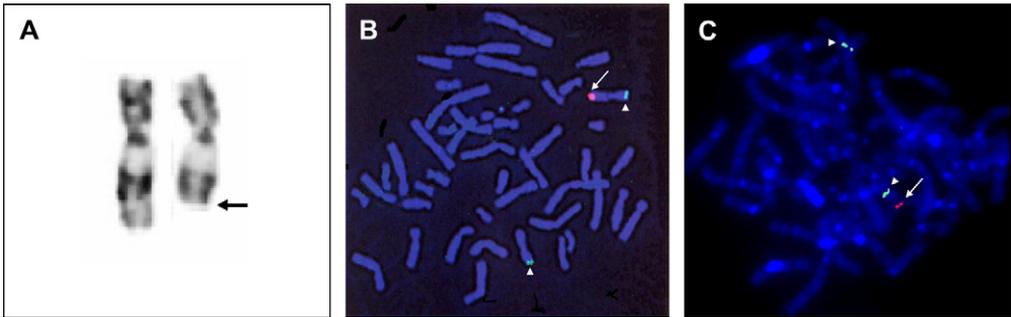


Fig. 1. Cytogenetic and fluorescence in situ hybridization analysis. (A) G-banded chromosome 11 pairs from cultured amniocytes showing the sub-band 11q23 deletion. The deleted chromosome is shown on the right. (B) FISH with subtelomeric 11q (arrow) and 11p (arrow head) probes (Cytocell) showing the deleted chromosome on amniotic cells. (C) FISH with specific probe for *FLII* (RP11-138K22, arrow). The arrow heads show control spots located in 11q14.2 (BAC RP11-589K11).

a relative macrocephaly ($PC/PA = 1,25$; > 95th centile) with normal cephalic index (0.79) and normal binocular diameter. The sonographic findings revealed prominent forehead with tight of the metopic suture, open fronto-nasal angle and parieto-frontal overlapping without trigonocephalic head shape or craniostenosis. The internal organs were unremarkable except minor bilateral pyelectasis and a relative small stomach size.

Umbilical cord blood sampling was performed simultaneously at the second amniocentesis in order to look for the chromosomal abnormality on a second tissue and to search for signs in favour of PTS, i.e. thrombocytopenia and/or typical platelets anomalies. A platelet count did not show thrombocytopenia (292 G/L) but morphological blood smear examination showed some large platelets (3–4%) (Fig. 2A) and platelets with giant alpha granule (2%) (Fig. 2B) identical to PTS. After further counselling, the parents elected to terminate the pregnancy at 31.5 week's gestation.

Post-mortem examination showed a male fetus with normal digits and limbs who weighted 1360 g and measured 40.5 cm in length. The fetus manifested no true facial dysmorphism, a dolichocephaly (Fig. 3A) and mild bilateral dilated ureters (Fig. 3B). No abnormalities of the cardiovascular, central nervous, and gastrointestinal systems were found.

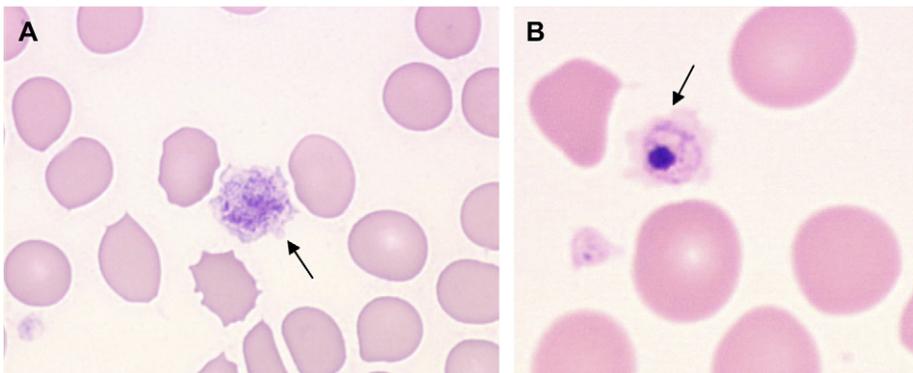


Fig. 2. Hematological analysis on MGG cord blood cells, $\times 100$. (A) Large platelet marked by an arrow. (B) Platelet with giant alpha granule marked by an arrow.

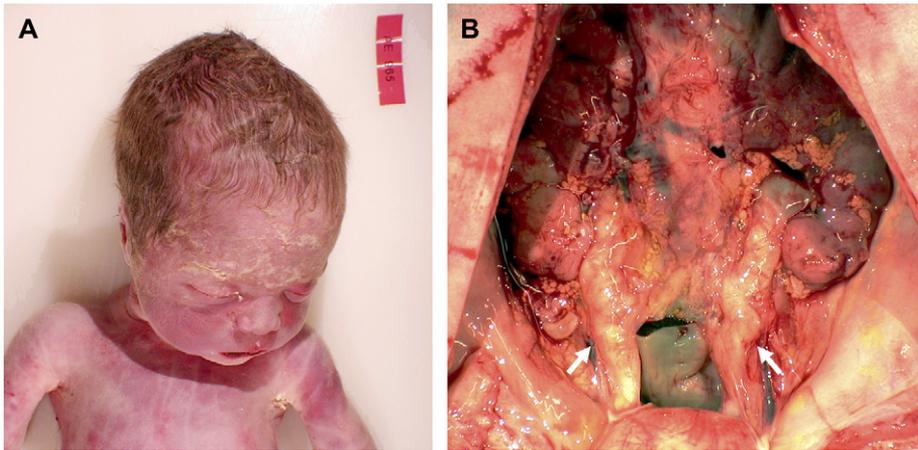


Fig. 3. Fetopathologic examination at 31.5 weeks gestation. (A) Dolichocephaly. (B) Distended ureters marked by arrows.

4. Discussion

Jacobsen syndrome is a well known contiguous gene syndrome resulting from 11q23.3-qter deletion. The human reference genome at the National Center for Biotechnology Information (NCBI build 36.2) (<http://www.ncbi.nlm.nih.gov>) reports the localization of a total of 248 genes in this chromosomal region. Clinical signs of Jacobsen syndrome can include Paris–Trousseau type of thrombocytopenia if the deletion extends to 11q23. If the diagnosis of Jacobsen syndrome is easily established in infancy and childhood, it is more difficult to recognize this pathology prenatally without any family history of chromosome 11 rearrangement. Surprisingly, the present case was identified on fetal karyotype because of a serum screening test positive for Down syndrome.

To our knowledge, only two prenatal diagnosis of mosaicism for 11qter deletion have been reported in the literature [3,16]. Bui et al. [3] described an abnormal live born infant with a web neck, a broad chest, widely spaced nipples, rocker-bottom feet, systolic murmur and sacral dimple. A mosaicism for 11q24 to 11qter deletion was detected on the fetal karyotype and confirmed in the live born tissues. The amniocentesis indication and mosaicism rate are not reported in this study. Porter et al. [16] reported absence of digits on the right hand, a pincer-like thumb, four finger nail-like structures arising from the skin over the metacarpals and overriding toes in a 20-gestational-week fetus with mosaic del(11)(q24.2). The mosaicism rate was evaluated at 76% of deleted cells and parents decided termination of pregnancy.

In our case, the fetal karyotype showed a somatic mosaicism with normal cells and 20% of 11q23 deleted cells. Prenatal 2-D ultrasound examination revealed only mild abnormalities: craniofacial features indicative of an unusual growth of calvaria, a small gastric size, a light increased amount of amniotic fluid in favour of possible neurological insufficient swallowing, and mild bilateral pyelectasis. Autopsy showed a normal stomach, mild bilateral pyelo-ureteral distension (Fig. 3A) and filled bladder without urethral obstruction. The fetus had a normal forehead with a dolichocephaly (Fig. 3B). Furthermore, digits and limbs were strictly normal at post-mortem examination. Bilateral pyelo-ureteral distension and dolichocephaly have also been reported in association with distal 11q deletions [4,5,10,12,14,18,19]. Chen et al. [4]

described a 31-year-old primigravid woman who underwent amniocentesis at 20 weeks' gestation because of a maternal serum alpha-fetoprotein (MSAFP) level of 2.63 multiples of the median. Amniocentesis revealed a 46,XY,del(11)(q24.2) fetal karyotype; the *de novo* deletion was observed on all metaphases. Chen et al. [4] suggested that *de novo* distal 11q deletions may be associated with elevated MSAFP and abnormal sonographic findings of the digits and limbs in the second trimester. In the present case MSAFP level was decreased to 0.66 MoM, maternal serum human gonadotrophin chorionic (MShCG) was increased to 1.52 MoM. The phenotypic variability in prenatally reported cases of Jacobsen syndrome is most probably linked to the percentage of cells bearing the 11qter deletion.

Hematological features of PTS include thrombocytopenia (47%) or pancytopenia (23%) [15] and platelets morphological abnormalities, i.e. some large platelets and the presence of red giant alpha granules in a platelet sub-population. The percentage of abnormal platelets is different from one patient to another (1% to 10%) and may be variable in the same patient with time. Although these morphological abnormalities are suggestive of PTS, there have been described in patients with myeloproliferative and myelodysplastic syndromes, May–Hegglin abnormality, Bernard–Soulier syndrome and also in 0.04% of patients without any hematological pathology [2,13]. The ineffective platelet production in bone marrow in PTS is explained by dysmegakaryopoiesis with many micromegakaryocytes constantly observed in patients affected by this pathology [2,7]. The dysmegakaryopoiesis has been linked to hemizygous loss of *FLII* monoallelic expression in CD41⁺ CD42⁻ progenitors [17]. Up to now, hematological features of PTS syndrome have never been reported prenatally [3,16]. In cord blood of the present fetus, some large platelets (Fig. 2A) and rare platelets with giant alpha-granules (Fig. 2B) were found and help us to conclude that this fetus with only 20% deleted 11q23 cells was indeed affected by Jacobsen syndrome, the low mosaicism rate certainly explaining the attenuated phenotype. We confirmed that the *FLII* gene is included in the deletion; this finding is in agreement with hematological features of PTS in this fetus. The very high penetrance of PTS in the 11q deletion disorder and its exclusive association with this chromosomal deletion suggests that this bleeding disorder may be pathognomonic for 11q terminal deletion disorders [10].

In previous studies, the extent of 11q terminal deletions has been evaluated by quantitative real-time PCR [1] or by quantitative fluorescent PCR [4,5]. In the present case, we tried to evaluate the extent of the deletion by quantitative fluorescent PCR with seventeen microsatellite markers. The most proximal marker (D11S1356) and the most distal one (D11S934) were located 117.4 Mb and 125.6 Mb away from 11pter respectively. The results did not allow the detection of markers located within the deleted region; again this is most probably due to the low mosaicism rate (20%) for the 11q terminal deletion in our case.

The management of a mosaic for 11q terminal deletion detected in prenatal period is difficult because the clinical outcome at birth can not be correctly evaluated. We report the third prenatal diagnosis of a mosaic for a distal 11q deletion. Our case represents an interesting observation for several reasons. Initially, the first amniocentesis was performed because of a serum screening test positive for Down syndrome and surprisingly, fetal karyotype analysis revealed a moderate mosaicism rate for 11qter deleted cells. Echographic findings were not totally comforting about normal craniofacial growth and we suspected a possible neurovegetative dysregulation to explain the unusual size of the stomach in utero and the minor abnormalities of the urinary tract. We wish to emphasize that for our case cord blood analysis was crucial to establish PTS in prenatal period. These hematological data, combined with minor echographic signs, influenced parent's decision for termination of pregnancy. This decision needed several

ethical discussions with medical experts from the Centre Pluridisciplinaire de Diagnostic Prénatal (CPDP) de Lorraine before approval of parents' decision for termination of pregnancy.

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