

Case Report

Monozygotic twins with trisomy 18 of paternal origin: prenatal diagnosis and molecular cytogenetic characterization in a pregnancy with one structurally abnormal living fetus and one intrauterine fetal demise

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Abstract

Objective: To present prenatal diagnosis and molecular cytogenetic characterization of trisomy 18 in a monozygotic twin pregnancy, with one structurally abnormal living fetus and one intrauterine fetal demise.

Case Report: A 38-year-old woman was referred for amniocentesis at 16 weeks of gestation because of advanced maternal age. Prenatal ultrasound revealed a monozygotic twin pregnancy, with one structurally abnormal living fetus, and one fetal demise. The body structure details of the dead fetus could not be identified, whereas holoprosencephaly and omphalocele were identified in the living fetus on prenatal ultrasound. Quantitative fluorescent polymerase chain reaction assays using polymorphic DNA markers specific for chromosome 21 and chromosome 18, were applied to the uncultured amniocytes in the amniotic cavity of the living fetus and the cultured amniocytes in the amniotic cavity of the fetus with intrauterine fetal demise. The specimen showed a dosage ratio of 2:1 (paternal:maternal) for chromosome 18-specific markers in both twins. The result was consistent with monozygosity and trisomy 18, and the trisomy 18 was possibly caused by a paternal second meiotic division non-disjunction error or a postzygotic mitotic error. Conventional cytogenetic analysis revealed a karyotype of 47,XY,+18 in both twins. The pregnancy was terminated at 19 weeks of gestation, and a 2 g small-for-date macerated twin A and a 166 g malformed twin B were delivered. Twin A manifested cebocephaly and omphalocele, and twin B manifested premaxillary agenesis and omphalocele.

Conclusion: The present case provides evidence that fetal wastage may occur in one of the co-twins in monozygotic twins associated with trisomy 18, and this may in part explain the very rare occurrence of living monozygotic twins with trisomy 18.

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Keywords: intrauterine fetal death; monozygotic twins; prenatal diagnosis; rapid aneuploidy diagnosis; trisomy 18

Introduction

Trisomy 18 in monozygotic twins is very rare, with a predicted incidence of 1 in 1,000,000 births based on the calculation of 0.3 per 1000 newborn babies in trisomy 18 and 3.5–4 per 1000 births in monozygotic twins [1]. To date, only 6 cases

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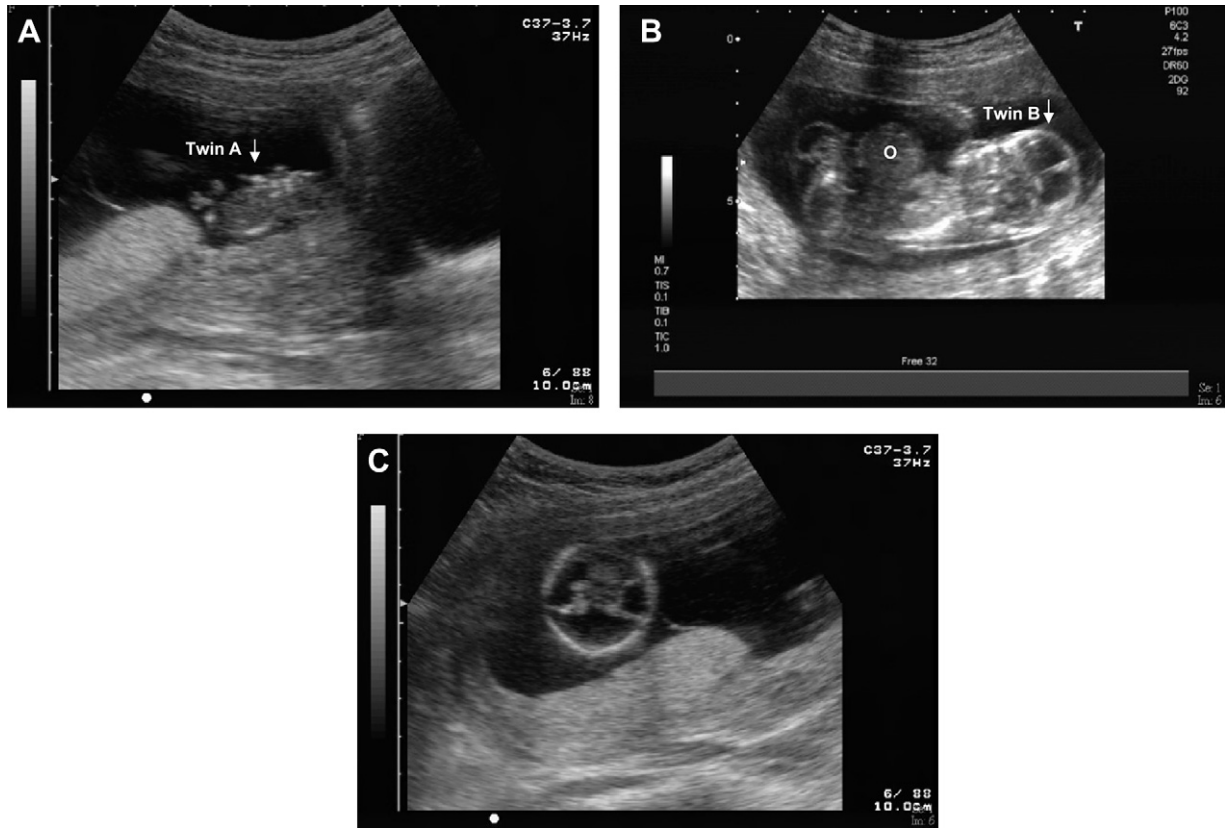


Fig. 1. Prenatal ultrasound at 16 weeks of gestation shows: (A) twin A with intrauterine fetal death; (B) twin B with omphalocele (O) and holoprosencephaly; and (C) holoprosencephaly in twin B.

of trisomy 18 in monozygotic twins have been reported [1–6]. Here, we present our experience of prenatal diagnosis and molecular cytogenetic characterization of trisomy 18 in a monozygotic twin pregnancy with one structurally abnormal living fetus and one intrauterine fetal demise. To our knowledge, such a case has not been previously described.

Case report

A 38-year-old, gravida 5 para 2, woman was referred for amniocentesis at 16 weeks of gestation because of advanced maternal age. The woman had not undergone any assisted reproductive technology and did not have diabetes mellitus. Her husband was 40 years old. She and her husband were healthy and non-consanguineous. There was no family history of congenital malformation. Prenatal ultrasound revealed a monozygotic twin pregnancy with one structurally abnormal living fetus and one fetal demise. The dead co-twin had a crown-rump length (CRL) of 3.4 cm equivalent to 10.1 weeks, and the living co-twin had a CRL of 7.5 cm and a biparietal diameter of 2.6 cm equivalent to 13.2 weeks. The amniotic sac was monochorionic and diamniotic. The amniotic fluid amounts in both amniotic cavities were normal. The body structure details of the dead fetus could not be identified, whereas holoprosencephaly (HPE) and omphalocele were identified in the living fetus on prenatal ultrasound (Fig. 1). The woman underwent amniocentesis at 16 weeks of gestation. Interphase fluorescence *in situ* hybridization

(FISH) using chromosomes 13 and 18 specific probes on uncultured amniocytes showed trisomy 18 (Fig. 2). Quantitative fluorescent polymerase chain reaction (QF-PCR) assays using polymorphic DNA markers specific for chromosome 21 and

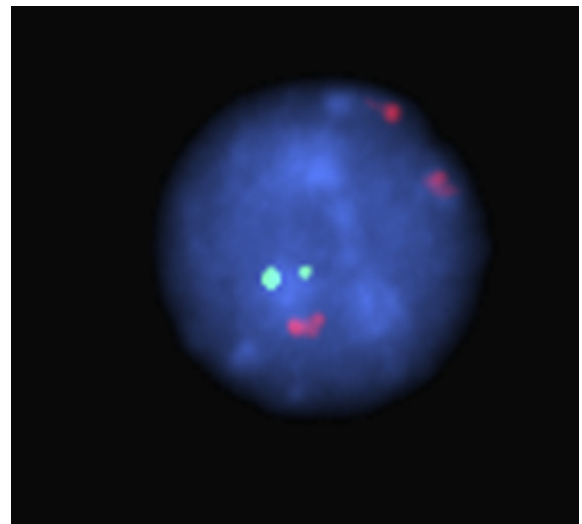


Fig. 2. Interphase fluorescence *in situ* hybridization using a chromosomes 18-specific probe (spectrum red) (CEP 18, Vysis) and a chromosomes 13-specific probe (spectrum green) (RP11-408E5, 13q12.11) shows three red signals and two green signals, consistent with the diagnosis of trisomy 18 and disomy 13.

Table 1
Molecular results using polymorphic markers specific for chromosomes 18 and 21.^a

Markers	Father	Mother	Twin A	Twin B
D18S878	171, 179	171, 179	171, 171, 179	171, 171, 179
D18S857	93, 99	90, 96	90, 99, 99	90, 99, 99
D18S1367	173, 173	169, 181	173, 173, 181	173, 173, 181
D21S1443	247, 267	259, 259	247, 259	247, 259
D21S1413	160, 160	160, 176	160, 176	160, 176
D21S2049	120, 126	120, 132	120, 132	120, 132

Twin A: the twin with intrauterine fetal death.
^a Alleles (basepair sizes) are listed below each individual.

chromosome 18 were applied to the uncultured amniocytes in the amniotic cavity of the living fetus and the cultured amniocytes in the amniotic cavity of the fetus with intrauterine fetal demise. The specimen showed a diallelic pattern with a dosage ratio of 1:1 (paternal:maternal) for chromosome 21-specific markers, but a diallelic pattern with a dosage ratio of 2:1 (paternal:maternal) for chromosome 18-specific markers in both twins (Table 1). The twin fetuses inherited two copies of a single paternal allele in chromosome 18. The result was consistent with monozygosity and trisomy 18, and the trisomy 18 was possibly caused by a paternal second meiotic division (MII) non-disjunction error, or a postzygotic mitotic (PZM) error. Conventional cytogenetic analysis revealed a karyotype of 47,XY,+18 in both twins. The pregnancy was terminated at 19 weeks of gestation, and a 2 g small-for-date macerated twin A and a 166-g malformed twin B were delivered. The twins manifested concordant phenotype of HPE and omphalocele.

Twin A manifested cebocephaly and omphalocele, and twin B manifested premaxillary agenesis (PMA) and omphalocele (Figs. 3 and 4). Postnatal QF-PCR analysis using the umbilical cord tissues from the twins confirmed the prenatal diagnosis.

Discussion

Rapid aneuploidy diagnosis (RAD) by array comparative genomic hybridization (aCGH), using uncultured amniocytes in pregnancy with fetal structural abnormalities, has been well described [7]. In this report, we demonstrate the application of QF-PCR and FISH in RAD of trisomy 18 using uncultured amniocytes in a monozygotic twin pregnancy, with one structurally abnormal living fetus and one fetal demise. RAD refers to the applications of aCGH, multiplex ligation-dependent probe amplification (MLPA), interphase fluorescence *in situ* hybridization (FISH) and QF-PCR without the need of cell culture [8]. The aCGH has the advantage of rapid genome-wide analysis, but the disadvantages of difficulty in detecting low-level mosaicism, balanced translocation, inversion and polyploidy. MLPA, FISH and QF-PCR have the advantages of being less expensive than aCGH, and rapid detection of numerical aneuploidies of chromosomes 13, 18, 21, X and Y. Whenever prenatally detected ultrasound abnormalities raise suspicions of common aneuploidies such as trisomies 13, 18 and 21 and Turner syndrome, the application of MLPA, FISH or QF-PCR may be considered as a choice of RAD, with a less expensive cost than aCGH. In addition to RAD, QF-PCR is able to determine zygoty, parental origin of the aneuploidy, as well as meiotic or mitotic

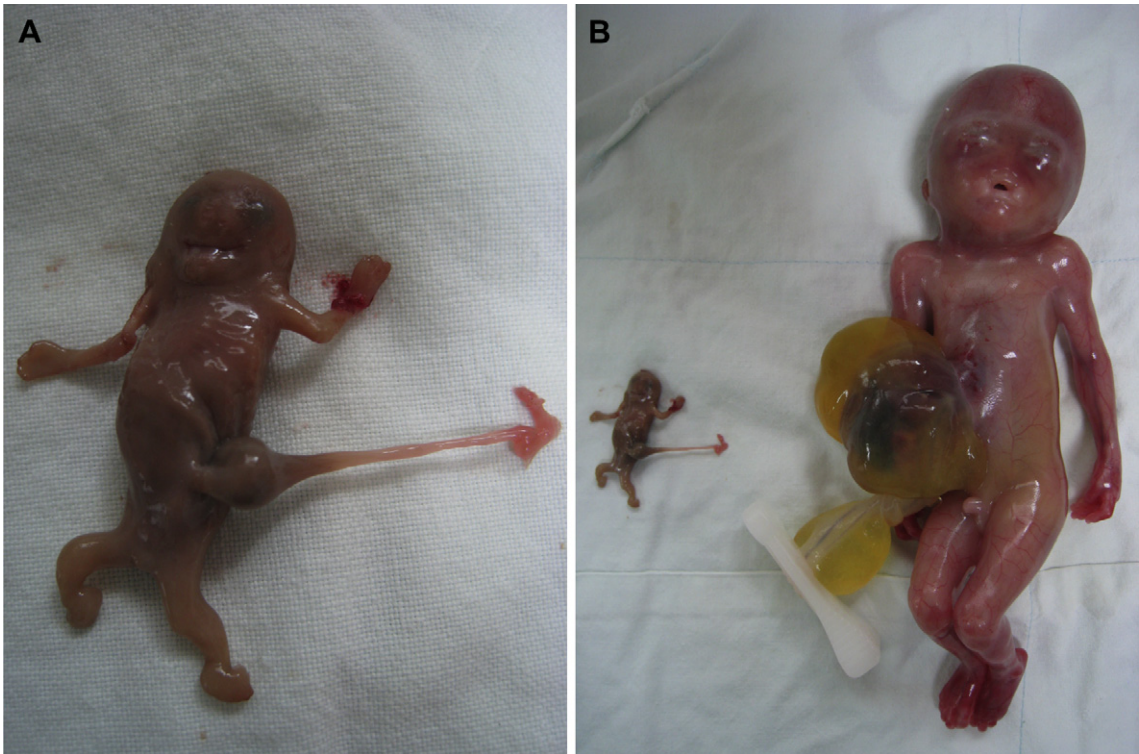


Fig. 3. (A) amplified image of twin A with intrauterine fetal demise; and (B) twin A and twin B at birth.

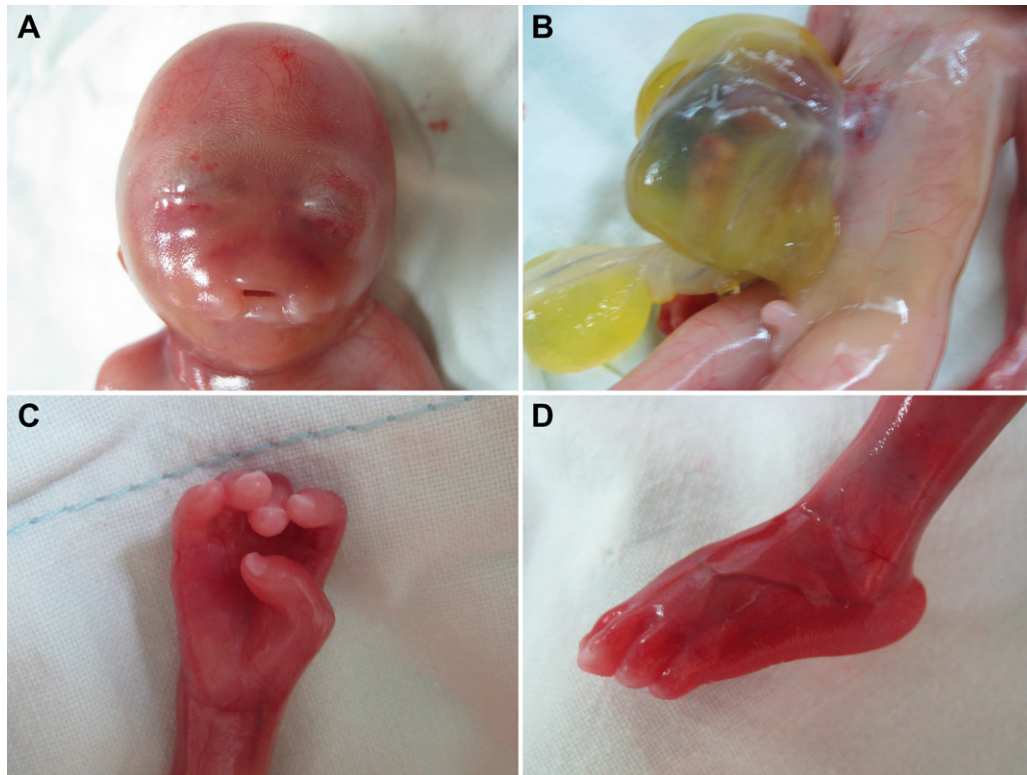


Fig. 4. The phenotypes of twin B: (A) holoprosencephaly and premaxillary agenesis; (B) omphalocele; (C) clenched hand; and (D) rocker-bottom foot.

nondisjunction. The information acquired by QF-PCR is very useful in genetic counseling of prenatally detected chromosome abnormalities in twins.

The present case was associated with structural abnormalities of omphalocele and HPE, which are not uncommon in fetuses with trisomy 18 or trisomy 13. Therefore, we first selected trisomy 18-specific markers for RAD, in addition to the trisomy 21-specific markers. Both omphalocele and HPE can be associated with trisomies 18 and 13 [9,10]. Omphalocele is more frequently observed in fetuses with trisomy 18, but HPE is more frequently observed in fetuses with trisomy 13. Snijders et al [11] found that omphalocele was diagnosed in 31% of fetuses with trisomy 18 ($n = 137$) and in 17% of the fetuses with trisomy 13 ($n = 54$). Chen [12] found that among 1148 fetuses with prenatally detected omphalocele, 415 cases (36.1%) had chromosome abnormalities including trisomy 18 ($n = 277$) in 66.7% (277/415) of the cases and trisomy 13 ($n = 72$) in 17.3% (72/415) of the cases. Snijders et al [11] found that HPE occurred in 39% of fetuses with trisomy 13 ($n = 54$) and 3% of the fetuses with trisomy 18 ($n = 137$). They also reported that among 132 fetuses with prenatally detected HPE, 33% of the cases had chromosome abnormalities including trisomy 13 ($n = 30$) and trisomy 18 ($n = 7$). In a study of 59 fetuses with HPE, Chen et al [13] found 34 cases (57.6%) had chromosome abnormalities including trisomy 13 ($n = 19$) and trisomy 18 ($n = 4$).

The present case was associated with a paternal origin of the extra chromosome 18. Studies on the extra chromosome in trisomy 18 have shown that 91% are of maternal origin, with 60% due to a meiosis II error, 30% due to a meiosis I error and

about 8% due to a mitotic error [14–16]. Trisomy 18 has a low paternal error rate. In a study of 31 cases of fetal trisomy 18, Chen et al [17] found a result of maternal nondisjunction in 90.3% of the cases and of paternal nondisjunction in only 9.7%. Clinical reports of trisomy 18 in monozygotic twins are uncommon. Lapi et al [2] first reported a case of presumptive monozygotic trisomy 18 twins with only one live-born twin who died at 15 minutes of age. Bhatnagar et al [3] reported trisomy 18 in monozygotic twins associated with a viable co-twin and a holoacardius. Mulder et al [4] reported a case of a pair of monozygotic twins with trisomy 18 discordant for major anomalies, with a left diaphragmatic hernia and a small ventricular septal defect in one twin, and bilateral diaphragmatic hernias, hydrocephalus, radial aplasia and complex congenital heart defects in the other twin. Shah et al [5] reported prenatal diagnosis of trisomy 18 in monozygotic twins by cordocentesis in a twin pregnancy with fetal growth lag and structural abnormalities detected by ultrasound. The live-born monozygotic twins differed in phenotype, with omphalocele in one twin and clitoromegaly in the other twin. Schlessel et al [1] reported a case of trisomy 18 in live-born monozygotic twins with prenatal ultrasound findings of intrauterine growth restriction and structural malformations in one of the twins. Phenotypic discordance in the trisomic twins included complex congenital heart defects in one twin and myelomeningocele, hydrocephalus and omphalocele in the other twin. Lee et al [6] reported trisomy 18 in a pair of live-born monozygotic twins with discordant phenotypes. Both twins had esophageal atresia with a tracheoesophageal fistula, but were discordant for types of congenital heart defects, cleft lip and choanal atresia.

In conclusion, we have presented prenatal diagnosis and molecular cytogenetic characterization of trisomy 18 in monozygotic twins in a pregnancy with one structural abnormal living fetus and one intrauterine fetal demise. Our presentation provides evidence that fetal wastage may occur in one of the co-twins in monozygotic twins associated with trisomy 18, and this may in part explain the very rare occurrence of living monozygotic twins with trisomy 18.

Acknowledgments

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