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SHORT COMMUNICATION

'Identical' Twins with Discordant Karyotypes

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A chromosomal abnormality in one of the fetuses of a monozygotic twin pregnancy is a rare phenomenon. In the prenatal unit of our cytogenetics laboratory we have recently come across two such heterokaryotypic twin pregnancies. In both cases ultrasound abnormalities were detected in one fetus of each twin pair. Chromosomal analysis showed that one twin pregnancy was discordant for trisomy 21 and the other for 45,X. Ultrasonographic examination suggested a monochorionic twin pregnancy in each case and DNA studies confirmed that both sets of twins were monozygotic. Both pregnancies were terminated. Biopsies taken from different sites of the placentas showed chromosomal mosaicism in both cases. There was no clear correlation between the karyotype found close to the site of the umbilical cord insertion in the placenta and the karyotype of the fetus. Sampling of amniotic fluid from both sacs is recommended in diamniotic twin pregnancies if one (or both) of the fetuses has ultrasound abnormalities, even if the twins are apparently monochorionic. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS: prenatal diagnosis; monozygotic twins; heterokaryotypic twins; trisomy 21; 45,X

INTRODUCTION

Although monozygotic twins are usually referred to as 'identical twins', many monozygotic twin pairs have been described that were discordant in size and varying congenital abnormalities (Schinzel *et al*., 1979). In fact, according to Keith and Machin (1997) monozygotic twins are seldom identical. These authors have reviewed the various causes of phenotypic and genotypic discordance in monozygotic twin pairs. Genotypic discordance is caused by karyotypic differences, skewed X-inactivation in females, differential gene imprinting and small-scale mutations (Machin, 1996).

Since the first reports on discordance for 45,X (Turpin *et al*., 1961) and for trisomy 21 (De Wolff *et al*., 1962) there have been many reports on discordance for these two aneuploidies. Machin (1996) has given an overview of published cases with discordance for numerical and structural chromosome abnormalities.

The prenatal finding of such heterokaryotypic twin pairs poses serious problems to obstetricians and genetic councellors. If the twins are monochorionic, vascular anastomoses in the shared placenta are likely to be present. This may result in a so-called twin-to-twin transfusion syndrome. In the presence of anastomoses spontaneous death or selective feticide of one of the twins may cause severe neurological damage or even death of the remaining co-twin (Weeks *et al*., 1996).

In this paper we present the cytogenetic and clinical details of two twin pregnancies that were discordant both at ultrasonographic examination and cytogenetic analysis, and that were shown to be monozygotic by DNA studies.

CASE REPORTS

Case A

A 38-year-old woman pregnant of twins was referred to our hospital for prenatal diagnosis because of advanced maternal age. A nuchal translucency of >3 mm was detected in one of the fetuses. At a gestational age of 14 weeks amniotic fluid was sampled from both amniotic cavities using a single-needle approach. The results of ultrasonographic examination and chromosome analysis of different tissues are summarized in Table 1.

Following the finding of trisomy 21 in two cultures of an amniotic fluid sample from one of the twins, selective feticide was considered. Just prior to the selective feticide chorionic villi were sampled from both fetuses for direct analysis in order to identify the chromosomally abnormal fetus. Only 46,XY cells were found in both chorionic villus samples and, therefore, selective feticide was not carried out. A monochorionic pregnancy was suspected by ultrasound and DNA studies on the amniocyte cultures remaining from the initial fluid sampling were carried out. These showed identical patterns for 10 microsatellite markers (see Table 1), indicating monozygosity. They also confirmed the presence of three chromosomes 21 in one of the samples.

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Table 1—Results of ultrasound examination, cytogenetic analysis and autopsy

	Case A	Case B
Maternal age	38 years	30 years
Indication	Maternal age, NT >3 mm in one fetus (twin II)	Cystic hygroma and fetal hydrops in one fetus (twin II)
AF karyotype	At 14 weeks' GA Twin I. 46,XY[20] Twin II. 47,XY,+21[22]	At 18 weeks' GA Twin I. 45,X[4]/46,XY[46] Twin II. 45,X[50]
CV karyotype	At 16 weeks' GA Twin I. 46,XY[30] Twin II. 46,XY[30]	—
DNA studies[a]	AF cultures: monozygotic twins, trisomy 21 confirmed in one twin	AF cultures: monozygotic twins
Confirmation of karyotypes in other tissues	At 24 weeks' GA	TOP at 23 weeks' GA
	AF from spontaneously deceased twin: 46,XY[45]	Twin I. Heart blood: 45,X[1]/46,XY[29]; skin: 46,XY[29]
	Cord blood from live fetus: 47,XY+21[1]/46,XY[84]	Twin II. Heart blood: no growth; skin: 45,X[24]
	TOP at 26 weeks' GA Skin biopsies: no growth	
	Placenta biopsies: see Fig. 1	Placenta biopsies: see Fig. 1
Autopsy	Twin I: macerated male fetus, 260 g	Twin I: macerated male fetus, 360 g, no congenital abnormalities
	Twin II: male fetus, 990 g, with bilateral transverse palmar crease, low-set thumb	Twin II: macerated female fetus, 700 g, • fetal hydrops • unilateral cleft lip and palate • ruptured hygroma • heart: VSD/persisting left superior caval vein/aberrant right subclavian artery/agenesis of the right umbilical artery • fluid-filled thoracic cavities with (secondary) lung hypoplasia

Abbreviations: AF=amniotic fluid; CV=chorionic villi; GA=gestational age; NT=nuchal translucency; TOP=termination of pregnancy; VSD=ventricular septal defect.
[a]Microsatellite markers tested—Case A: D7S471, D7S1870, D9S7, D10S564, D10S597, D13S173, D14S271, D16S498, JW5/6 and D21S269. Probability of monozygosity: >99·9 per cent. Additional microsatellite markers on chromosome 21 tested: D21S261, D21S265, D21S1256, D21S1258, D21S270, D21S1259 and D21S1254. Case B: D4S2691, D6S89, D7S653, D10S585, D11S913, D14S262, D15S111, D17S933, D18S458, D21S269 and D22S419. Probability of monozygosity: >99·9 per cent.

At 24 weeks of gestation one of the fetuses died. An amniotic fluid sample taken from the sac of this fetus showed only 46,XY cells, indicating that it was the chromosomally normal fetus that had died. The parents then decided to have the pregnancy terminated. Cord blood taken from the surviving fetus before termination showed that only one of the 85 cells analysed was 47,XY,+21, the remaining cells having a normal male karyotype.

Skin biopsies of the fetuses taken after delivery for postnatal confirmation failed to grow. Eight biopsies were taken from different sites at the fetal side of the placenta, which was monochorionic (see Fig. 1, case A). Cultures of the placenta biopsies from the site closest to the umbilical cord insertion of the normal fetus showed only normal cells. At the site closest to the cord insertion of the abnormal fetus, however, trisomic as well as normal cells were found. For autopsy results see Table 1.

Case B

A 30-year-old woman pregnant of twins was referred for prenatal chromosomal analysis at 18 weeks' gestation because of sonographically detected cystic hygroma and fetal hydrops in one of the fetuses. The co-twin appeared to be a normal male at ultrasound examination. The results of ultrasonographic examination and of chromosome analysis of different tissues are summarized in Table 1.

Analysis of two cultures of one amniotic fluid sample showed that the karyotype of the ultrasonographically abnormal fetus was 45,X in all 50 cells. The ultrasonographically normal fetus had a mosaic karyotype with 45,X in 4 out of 50 cells examined. The remaining cells were 46,XY. DNA studies carried out with 11 microsatellite markers on 11 different chromosomes (see Table 1) indicated monozygosity. Considering the severity of the ultrasound abnormalities and the risks

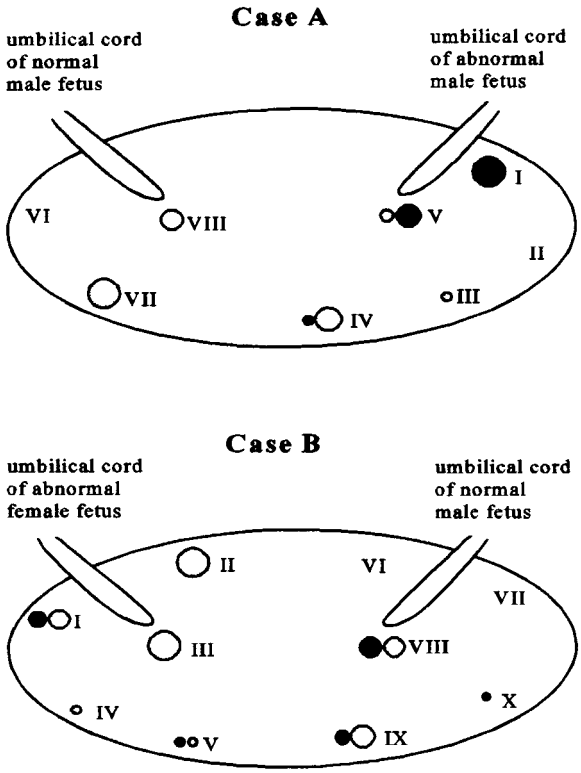

Fig. 1—Sites of biopsies taken from placentas of case A and case B. Numbers I to X indicate the approximate sites of biopsies. Open circles indicate normal, and black circles indicate abnormal cell clones. The size of the circles is roughly proportional to the size of the cell clones analysed. Results from the different biopsies and the number of cells examined are as follows: case A: I. 47,XY,+21[15]; II. no growth; III. 46,XY[2]; IV. 47,XY,+21[2]/46,XY[13]; V. 47,XY,+21[12]/46,XY[3]; VI. no growth; VII. 46,XY[15]; VIII. 46,XY[11]; case B: I. 45,X[6]/46,XY[9]; II. 46,XY[15]; III. 46,XY[15]; IV. 46,XY[2]; V. 45,X[1]/46,XY[1]; VI. no growth; VII. no growth; VIII. 45,X[7]/46,XY[7]; IX. 45,X[3]/46,XY[12]; X. 45,X[1]

associated with selective feticide, this pregnancy was terminated at the parents' request at 23 weeks of gestation.

After delivery different tissues were sampled for confirmation of the cytogenetic findings. Fibroblast cultures showed a normal male karyotype in all 29 cells examined from the male fetus and 45,X in all 24 cells examined from the female fetus. The heart blood cultures from the female fetus failed to grow, but those from the male fetus showed one 45,X cell among 30 cells examined. The rest of the cells were 46,XY.

The placenta was monochorionic. 10 biopsies were taken from different sites at the fetal side of the placenta and were cultured (see Fig. 1, case B). Both normal and 45,X karyotypes were found in four of the cultures, including the culture from the site closest to the umbilical cord insertion of the normal male fetus. In the biopsy closest to the cord insertion of the abnormal female fetus only 46,XY cells were found. For autopsy results see Table 1.

DISCUSSION

Each of the twin pairs described here originated from one zygote. Twinning must have occurred after the formation of chorion but before amniogenesis took place, resulting in monochorionic, diamniotic twin pregnancies. This suggests that the twinning events took place between four and eight days after conception (Boklage, 1981).

In case A a post-zygotic error must have occurred in a normal or trisomic embryo, at, or just after, the splitting of the embryo into two individuals. The question as to whether the original zygote was normal or trisomic was addressed by testing seven different DNA markers along the length of chromosome 21 (see Table 1). As none of these markers showed three different alleles in the cells from the trisomic fetus, a meiosis-I error (leading to a trisomic zygote with subsequent trisomy rescue) is highly unlikely. There was no evidence for uniparental disomy in the cells from the normal fetus. The question whether a meiosis-II or a mitotic error had occurred, could not be answered. As to the question of parent of origin of the extra chromosome, only one of the markers on chromosome 21 was informative. This showed two different alleles, the mother's being present in two-fold. Further DNA studies could not be pursued because of insufficient numbers of fetal cells.

In case B there can be no doubt that the error occurred post-zygotically, since the Y chromosome cannot have been acquired. The mosaicism found in both amniotic fluid and in blood cultures from the male fetus may reflect true mosaicism, but it could also have resulted from either twin-to-twin transfusion or contamination of cells from the co-twin. The abnormal female fetus did not appear to be a mosaic, as only 45,X cells were found in the amniotic fluid and skin cultures. Twin pairs similar to our case, i.e., discordant for Turner syndrome or for gonadal dysgenesis, are the most frequently reported of all heterokaryotypic monozygotic twin pairs, with a high incidence of mosaicism in one or both of the twins (Dallapiccola *et al.*, 1985; Perlman *et al.*, 1990).

There was no clear correlation between the karyotypes of the fetuses and the karyotypes found in the placenta, not even at the sites of the umbilical cord insertions (Fig. 1). Although both normal and abnormal cell lines were found in the placenta cultures from some of the sites, mosaicism could not be ruled out in cultures from all the sites because of the limited number of cells available. The apparently discrepant findings in the placenta in both cases, together with the finding of only 46,XY cells in the chorionic villi from both fetuses in case A, clearly show that chorionic villi are not the tissue of choice for chromosomal investigations in monochorionic twin pregnancies. The fact that only one trisomic cell was found in the cord blood sample of the abnormal fetus in case A also stresses the fact that, in the case of monochorionic twins, the results from fetal blood cells should be interpreted with caution (Gonsoulin *et al.*, 1990). Fetal blood is particularly unsuitable for karyotyping of monozygotic twin pairs with twin-reversed arterial perfusion (TRAP) in one of the fetuses. The TRAP sequence occurs as a consequence of umbilical artery-to-artery anastomoses (Van Allen *et al.*, 1983; Moore *et al.*, 1990; Fisk *et al.*,

1996) and may be considered an extreme form of twin-to-twin transfusion. In about 50 per cent of these TRAP twins the fetus in which the blood flow is reversed, the so-called acardiac monster, is chromosomally abnormal, while the apparently normal co-twin is chromosomally normal (Scott and Ferguson-Smith, 1973; Moore *et al.*, 1987), but blood cells from both fetuses are normal.

Vascular anastomoses occur in the placenta of at least 70 per cent of monochorionic twins (Van Dijk *et al.*, 1996) and are virtually absent in dichorionic placentas (Machin and Still, 1995). It is, therefore, important to determine the chorionicity of twins early in pregnancy in order to anticipate the consequences of twin-to-twin transfusion and of selective feticide in case one of the fetuses is abnormal. Using modern ultrasonographic technology the chorionic status can easily be determined in most twin pregnancies in the first trimester. However, although dichorionic placentas can be entirely separate organs, they are fused in about 40 per cent of cases (Van Dijk *et al.*, 1996). If the placentas are fused, some doubt may still remain as to whether the placenta is mono- or dichorionic. Although the ultrasound parameter of the thickness of the dividing membrane holds in most cases, in some 10 per cent of dichorionic pregnancies the dividing membrane is thin, in which case the twin pregnancy may incorrectly be assumed to be monochorionic. As there is no reliable way of assessing beyond doubt if a pregnancy is monochorionic (Wapner, 1995) DNA studies are recommended in these cases. DNA 'fingerprint' analysis may indicate a dizygotic pregnancy, and thus exclude monochorionicity (Appelman *et al.*, 1994). Depending upon the duration of pregnancy, selective termination of the affected fetus can be carried out safely if the twins are found to be dizygotic.

Both sets of twins described here were monochorionic. The difference in weight between the twins in each pair and the mosaic karyotypes found in the fetal blood samples suggest that some degree of twin-to-twin transfusion may have occurred in both sets of twins. According to the autopsy findings this may have been the cause of death of the normal fetus in case A. In both cases the chromosomally normal fetus was the smaller of the pair (donor) and the abnormal fetus was the larger (recipient). It is difficult to decide whether, in this case, this is due to the chromosomal abnormality or to chance, but in many TRAP twins the abnormal acardiac fetus is larger than the normal co-twin (Moore *et al.*, 1990).

The two events, mitotic non-disjunction and monozygotic twinning, may not be unrelated (Edwards *et al.*, 1966; Perlman *et al.*, 1990). Machin (1996) has suggested that, because there are significant numbers of monozygotic twin pairs that are neither genotypically nor phenotypically 'identical', the post-zygotic events not only precede the twinning event, but may actually trigger it. If two different cell clones exist within one early zygote, the differences between the clones may be sufficient to cause mutual 'recognition and repulsion', resulting in the 'splitting' into two embryos. If, indeed, post-zygotic non-disjunction and monozygotic twinning, which occurs in about 1 in 250 to 300 pregnancies, are related processes, the prevalence of heterokaryotypic monozygotic twinning is likely to be much higher than the product of the incidence of non-disjunction and the incidence of monozygotic twinning. The issue is complicated by the fact that, although information is available on the incidence of aneuploidy in newborns (Nielsen and Sillesen, 1975), there is very little information on the incidence of post-zygotic non-disjunction in early pregnancy. Another approach is to consider the frequency of TRAP twins, 50 per cent of which are heterokaryotypic. TRAP twins are estimated to occur in 1 out of 100 monozygotic twin pairs (Napolitani and Schreiber, 1960). This is an underestimate because of early loss of monozygotic twins and TRAP twins in particular (Van Allen *et al.*, 1983). In any case, this gives some indication of the incidence of heterokaryotypic monozygotic twins.

In the absence of more information further speculation on the frequency of karyotypic discordance in monozygotic twins must await multicentre, multidisciplinary studies on large cohorts of twins (Machin, 1996).

CONCLUSIONS

From these case reports the following conclusions can be drawn.

- The finding of two different karyotypes in twin gestations does *not necessarily* indicate dizygotic twins.
- DNA studies are recommended to exclude monochorionicity whenever there is doubt about the chorionic status of twins. This is in order to be prepared for possible consequences of twin-to-twin transfusion or of selective feticide in case phenotypic or karyotypic abnormalities are found in one of the fetuses.
- Amniotic fluid should be sampled from both sacs in diamniotic twin pregnancies if one (or both) of the fetuses has an ultrasound abnormality, even if the twins are apparently monochorionic.
- Chorionic villi are not the tissue of choice in monochorionic twin pregnancies, as even samples taken from sites near the insertion of the umbilical cord may not reflect the karyotype of the fetus attached to it. Rapid identification of the abnormal fetus before selective termination may be done by interphase FISH on uncultured amniocytes.
- The term 'monozygotic twins' is to be preferred to 'identical twins'.

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