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Monochorionic Dizygous Twins Presenting With Blood Chimerism and Discordant Sex

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Monochorionic dizygous twins are probably more frequent than considered previously as many cases remain unrecognized, especially when the children have the same sex. Here we present a pair of dizygous, sex-discordant monochorionic twins who were conceived after artificial insemination. Histological examination of the placenta and extensive genetic studies of the healthy boy and girl clearly proved that they indeed were monochorionic dizygous twins with a fully joined blood circulation. We conclude that when counseling parents expecting monochorionic twins of discordant sex, not only a disorder of sexual differentiation in one of the twins should be addressed but also the possibility of dizygosity with a completely normal (sexual) development of both children.

■ **Keywords:** dizygous, monochorionic, twins, counseling, blood chimerism

In general, monochorionicity, when established in multiple pregnancies, is associated with monozygosity and a common placenta, whereas dizygosity connects with separate placentas (Husby et al., 1991). However, recently a limited number of case reports described monochorionicity in dizygous twins. At present three cases have been reported of monochorionic dizygotic twins who were born after a spontaneous pregnancy presenting with discordant sexes (Hackmon et al., 2009; Nylander & Osunkoya, 1970) or discordant for Down syndrome (Shalev et al., 2006), while a number of other cases appeared to be the result of assisted reproductive technology (ART; Ekelund et al., 2008; Souter et al. 2003). Here we present another pair of dizygous, sex-discordant monochorionic twins who were conceived after artificial insemination.

Materials and Methods

A 37-year-old woman, in her fourth pregnancy with one healthy child, was referred to our university hospital for evaluation of a twin pregnancy at 20 weeks of gestation after first trimester ultrasound examination had demonstrated a monochorionic diamniotic twin pregnancy. Ultrasound evaluation indeed confirmed this finding but revealed discordant sexes. Subsequent extended ultrasound showed that both twins had a normal biometry, structural development, amniotic fluid volume, and Doppler flow patterns of the umbilical artery. Because of discordance in sex, a disorder of sexual differentiation (i.e., Turner syndrome or 46,XY gonadal dysgenesis) in one of the children was anticipated and discussed with the parents. Further follow–up ultrasound revealed normal growth in combination with normal bladder and stomach filling for both twins. Amniotic fluid volume showed discrepancy from 22 weeks onwards with the male fetus having mild polyhydramnios and the female fetus mild oligohydramnios. This discrepancy resolved during the third trimester. The further course of the pregnancy was uncomplicated. At 37 weeks of gestation, healthy twins were delivered vaginally after artificial amniotomy. Twin 1 appeared as a phenotypically normal boy with a birth weight of 2,850 g and normal male external genitalia and scrotal gonads. Twin 2 was a phenotypically normal girl with a birth weight of 2,365 g and normal

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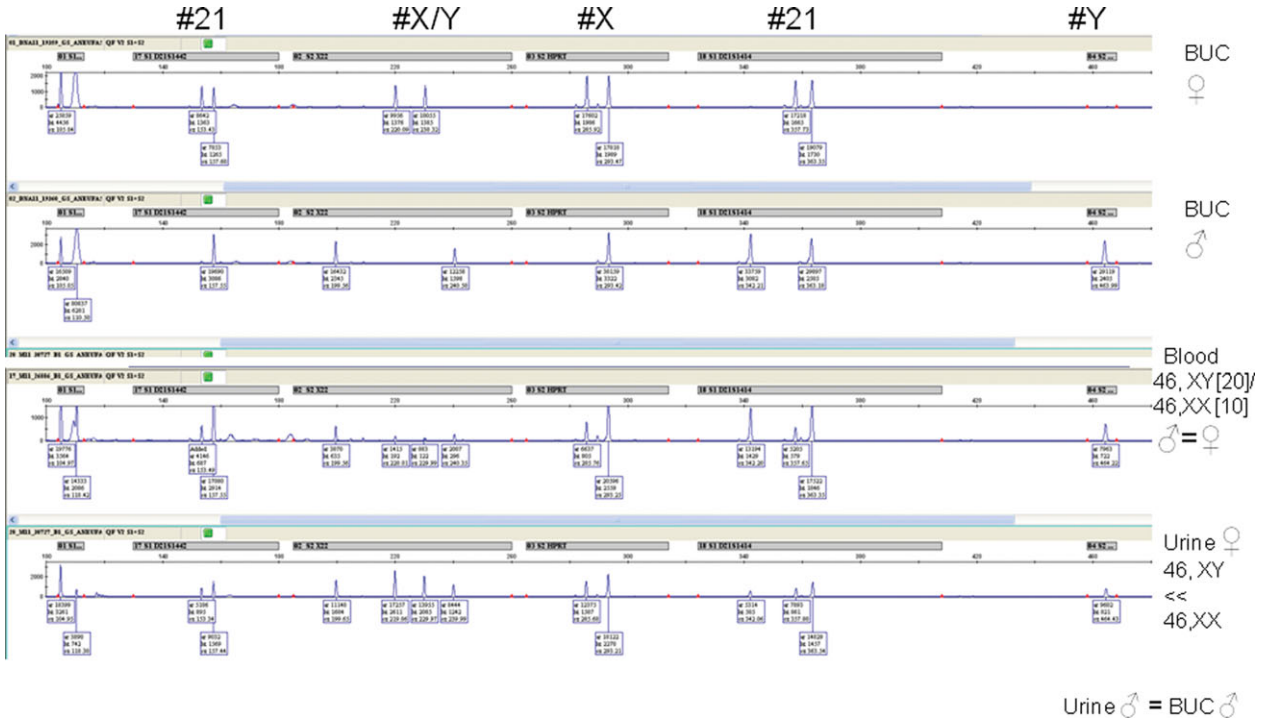

FIGURE 1

(Colour online) Presentation of the marker analysis after QF-PCR (Aneufast™) on various DNA samples from both twins. Only a selection of markers is shown resulting from the chromosomes 21, X, and Y. Note that in particular the markers on both X and Y chromosome (#X/Y) clearly show four different signals for the two cell lines (XX versus XY). Note: BUC: DNA derived from buccal swabs; blood: DNA extracted from white blood cells; urine: DNA extracted from urine samples.

female external genitalia. Both neonates had a good start with Apgar scores of 10 after 5 min.

Histological examination of the placenta showed a monochorionic diamniotic placenta pointing to a joined blood circulation.

Standard chromosome studies of both children were performed on umbilical cord blood samples and three weeks after birth on peripheral blood samples according to routine procedures. Approximately six weeks after birth, buccal swabs and urine samples from both children were collected. Subsequently, cells were isolated and DNA was extracted. Furthermore, DNA was extracted from peripheral blood cells from both parents and children, and on all DNAs a Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) was performed applying 16 (polymorphic) markers divided over five different chromosomes (13, 18, 21, X, and Y; Aneufast™).

Results and Discussion

Standard karyotyping of the twins revealed blood chimerism in both children with exactly the same mosaicism in the boy and the girl: two-thirds of the cells having a normal male karyotype and one-third showing a normal female pattern (46,XY[20]/46,XX[10]) in the blood samples drawn immediately after birth as well as three weeks later, which showed exactly the same mosaic pattern of XY and XX cells.

While the results of the QF-PCR of the blood samples from both children clearly showed a mix of marker signals from the XY and XX cells which could be traced back to both parents, the results from the buccal cells evidently revealed no more than two signals per marker, fitting with a single cell line showing a 46,XX karyotype in the girl and 46,XY line in the male twin. The QF-PCR results from the DNA extracted from the urine showed in the boy exactly the same signals as the buccal cells. In the urine from the girl, however, some remaining small signals could be recognized for the XY cells next to the typical XX signals (Figure 1). Probably this was due to the presence of some leucocytes derived from the blood, of which the majority had an XY karyotype in the urine. All in all, full marker analysis, as shown in part in Figure 1, clearly confirmed dizygosity of the twins.

The most likely explanation for the etiology of these monochorionic dizygous twins is that an early fusion of two separately fertilized embryos has occurred from which the outer cells subsequently formed the placenta, while the embryonic lineage remained spatially distinct as was described before (Redline, 2003; Souter et al., 2003). Our repeated

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cytogenetic as well as marker studies of the blood cells from both children showed a fully similar pattern, which suggests that the twins exchanged their entire hematopoietic stem cells repertoire during embryogenesis.

The sex discordancy in these monochorionic twins pointed toward a dizygosity, indicating that this diagnosis might be easily missed (equal sex) or not recognized during follow-up. Hence, this mechanism of twinning and subsequent (part) fusion probably occurs more frequently than expected thus far, in particular in cases of artificial reproduction, although the latter cases might be biased since in general such pregnancies will be watched over more closely. On the other hand, it seems plausible that the clinical relevance of such fusions will mostly be limited to a (transient) blood chimerism in children. However, caution should be exercised in case genetic studies are performed in these twins, as the presence of chimerism in placenta (prenatal studies) and blood (postnatal studies) might very well blur or even compromise the outcome of such investigations.

Gradually the picture emerges that blood chimerism in dizygotic twins is much more frequent than expected, in particular if sensitive measuring techniques are being used (Redline, 2003). Probably, such chimerism is due to placental blood vessel anastomoses. Indeed, there might very well exist a broad spectrum regarding dizygotic twins which ranges from truly separated placentas and blood circulation (the majority) via limited blood vessel anastomoses with a low-grade chimerism (Van Dijk et al., 1996) toward the other end of the spectrum involving a single chorion and complete blood chimerism like in the presented case. This also implies that if parents expecting a monochorionic twin of discordant sex are counseled, not only should the focus be on a disorder of sexual differentiation (e.g., Turner syndrome, ambiguous genitalia) but the possibility of dizygosity with normal sexual development and blood chimerism should also be addressed.

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