

## Case Report

# Chimaeric twins: Why monochorionicity does not guarantee monozygosity

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## Introduction

Monochorionic placentation has been considered pathognomonic of monozygosity for generations of obstetricians. This rule has been considered so robust that almost all parents of twins with a single placenta diagnosed on first-trimester ultrasound have been advised that no further testing is ever required to prove their twins' monozygosity.

In recent years, there have been a number of reports of monochorionic dizygotic (MCDZ) twins, almost exclusively following treatment with assisted reproductive technologies (ART), often involving microinjection techniques.<sup>1-3</sup>

This case report of spontaneous MCDZ twins highlights a rare condition of which obstetricians should be aware and demonstrates the steps needed for confirmation of the diagnosis.

## Case Report

The patient was a 36-year-old woman who conceived spontaneously. An ultrasound at 12 weeks gestation diagnosed monochorionic diamniotic (MCDA) twins. The 19-week morphology ultrasound showed normal and equal growth of both twins, but subjectively discordant amniotic fluid volumes. The placenta was again reported as MCDA. The amniotic fluid volumes remained discordant from 22 through to 33 weeks of gestation; although growth of both twins remained equal, the bladders of both twins remained visible throughout pregnancy, and Doppler

blood flow studies were also normal for both twins on all occasions. The pregnancy was managed as a Quintero stage one Twin-Twin Transfusion Syndrome (TTTS) with no intervention deemed necessary.

Delivery was by elective caesarean section at 36 weeks of gestation after corticosteroids had been administered. Both twins were male, weighing 2490 and 2310 g, respectively. Apgar scores, haemoglobin estimations and paediatric examination of both twins were normal. After a short period in the neonatal nursery, they were both discharged home on day nine and have had no long-term problems. Histological examination of the placenta revealed MCDA placentation with no other pathological features evident.

Despite the clear evidence of monochorionicity on antenatal ultrasounds, the evidence of an early stage TTTS, and histological evidence of monochorionic placentation, the twins' parents were unable to reconcile the presumed diagnosis of monozygosity with their sons' significantly discordant facial features at 14 months of age. They arranged zygosity testing on buccal smears at a local commercial laboratory. The report was of 'nonidentical DNA indicating dizygosity'.

The parents were understandably confused and contacted the first author for clarification of the situation. After reviewing photographs, ultrasounds and the histology of the placenta, the possibility of chimaeric twinning was raised, and appropriate investigations were undertaken.

## Materials and Methods

The DVD of the 12-week ultrasound was reviewed by an obstetric sonologist who was blinded to the clinical scenario. MCDA twins were confirmed.

Peripheral blood leucocytes and buccal epithelial cells were collected, processed and DNA extracted as

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**Table 1** Zygosity results from DNA from buccal cells and white blood cells

Buccal cell DNA				Blood cell DNA			
Sample	Marker	Allele 1 size	Allele 2 size	Sample	Marker	Allele 1 size	Allele 2 size
Twin 1	D11S4151	334*	334*	Twin 1	D11S4151	334*	334*
Twin 2	D11S4151	338	340	Twin 2	D11S4151	334*	334*
Twin 1	D11S904	189	203*	Twin 1	D11S904	189	203*
Twin 2	D11S904	189	193	Twin 2	D11S904	189	203*
Twin 1	D12S345	219	219	Twin 1	D12S345	219	219
Twin 2	D12S345	215	219	Twin 2	D12S345	219	219
Twin 1	D12S78	182	194	Twin 1	D12S78	182	194
Twin 2	D12S78	Failed	Failed	Twin 2	D12S78	182	194
Twin 1	D14S283	135	141	Twin 1	D14S283	135	141
Twin 2	D14S283	135	141	Twin 2	D14S283	135	141
Twin 1	D17S1852	299	309*	Twin 1	D17S1852	299	309*
Twin 2	D17S1852	299	303	Twin 2	D17S1852	299	309*
Twin 1	D2S125	90	96	Twin 1	D2S125	90	96
Twin 2	D2S125	90	96	Twin 2	D2S125	90	96
Twin 1	D2S2211	256*	256	Twin 1	D2S2211	256*	256
Twin 2	D2S2211	248	256	Twin 2	D2S2211	256*	256
Twin 1	D2S337	292*	304	Twin 1	D2S337	292*	304
Twin 2	D2S337	296	304	Twin 2	D2S337	292*	304
Twin 1	D3S1267	97	109	Twin 1	D3S1267	97	109
Twin 2	D3S1267	97	109	Twin 2	D3S1267	97	109
Twin 1	D6S257	175*	179	Twin 1	D6S257	175*	179
Twin 2	D6S257	179	179	Twin 2	D6S257	175*	179
Twin 1	D8S284	297*	299	Twin 1	D8S284	297*	299
Twin 2	D8S284	283	299	Twin 2	D8S284	297*	299

Allele size was determined across 12 polymorphic microsatellite markers and rounded to the nearest whole number. Shading indicates a match in size of both alleles between twin 1 and twin 2. In buccal cell DNA, allele size is the same at only 3 loci, clearly indicating dizygosity. In white blood cell DNA, 100% of all alleles appear to be shared. However, at 8 informative loci (asterisks), the allele in white blood cells of twin 2 must come from twin 1, suggesting a net flow of blood from the latter to the former.

previously published.<sup>4</sup> Zygosity was determined by a 12 marker microsatellite test (Table 1) at the Australian Genome Research Facility (AGRF, Melbourne). In buccal cell DNA, only three loci were shared by the twins. In leucocyte DNA, 12 of 12 loci were shared by the twins. This confirmed that the pair was dizygotic in a nonshared tissue and apparently monozygotic in blood. In addition, at eight of eight informative loci, the genotype in leucocytes of twin two belonged to twin one, indicating a net flow of blood from the latter to the former.

## Discussion

Chorionicity and amnionicity are ideally determined by ultrasound examination late in the first trimester. The timing of zygote splitting can then be estimated by determining the number of chorions, amnions and fetuses (Table 2). Traditionally, monochorionicity has guaranteed monozygosity, and opposite sex twins have guaranteed dizygosity. Same-sex dichorionic twins have remained of uncertain zygosity until postnatal evaluation can be performed.

Case reports over the last decade have made the precise establishment of zygosity more difficult. The obstetric

**Table 2** Chorionicity and amnionicity by time of zygote splitting

Zygosity	Twins	Time of split	Chorions	Amnions	Fetal mass
DZ	DC DA	No split	2	2	2
MZ	DC DA	Day 1–3	2	2	2
MZ	MC DA	Day 3–8	1	2	2
MZ	MC MA	Day 8–13	1	1	2
MZ	Conjoined	>Day 13	1	1	1

DZ, dizygotic; MZ, monozygotic; DC, dichorionic; MC, monochorionic; MA, monoamniotic.

mantra that ‘monochorionicity guarantees monozygosity’ has been disproven, albeit with exceptional rarity. Almost all cases of MCDZ twins have involved treatment with ART, often with microinjection techniques<sup>2,3</sup> and, in two cases, by spontaneous fertilisation following ovarian stimulation.<sup>5,6</sup> There are only three previous reports of spontaneous MCDZ twins<sup>1,7,8</sup> and in only one of those reports<sup>1</sup> is there clear histological and genetic evidence confirming the diagnosis.

Assisted reproductive technologies, particularly those involving microinjection techniques, may disrupt the zona pellucida and increase the risk of cell fusion at the

blastocyst stage. It is possible that trophoblasts from each embryo then fuse before implantation, resulting in a shared chorion/placenta surrounding two embryos derived from the nonfused inner cell masses.<sup>3</sup>

However, in spontaneous MCDZ twins, these events would occur more by a chance event of two blastocysts, in close proximity, fusing around the time of blastocyst hatching.

The term 'chimaera' refers to any single organism that is composed of two or more different populations of genetically distinct cells that originated from different zygotes. Despite having cells with potentially cross-reacting blood types, the two genetically different blood lines in DZ chimaeric twins do not seem to recognise each other as foreign, despite the demonstration that these twins can have normal titres of isohaemagglutinins.<sup>9</sup> Therefore, these twins are likely to be a good immunological match for each other, despite their dizygosity.

Although fusion of the placental vascular circulations of same-sexed DZ twins will result in leucocyte and erythrocyte chimaerism, it is possible that there could also be some germ cell chimaerism, so that each twin might subsequently produce some of his co-twin's gametes. The consequences for the female co-twin in a male-female MCDZ twin pair might be far more serious; she could develop as the equivalent of a bovine freemartin, virilised by the secretions of her male co-twin, and almost certainly sterile.<sup>10</sup> This has yet to be demonstrated in humans.

In summary, this is the second case report of histologically and genetically proven spontaneously conceived MCDZ twins. For these boys, the importance is their immunological tolerance for each other. For obstetricians, the importance is the recognition that, in exceptional circumstances, monochorionic twins can be

dizygotic, and this should be considered when twins are phenotypically distinct, including being of opposite sex.

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