Case Report: Monochorinic Dizygotic Twins With Chi 47,XY,+21 /46,XX In Peripheral Blood Cells

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Case report

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Abstract

Background: A 38-year-old woman conceived twins by in-vitro fertilization (IVF). Ultrasound examinations showed signs of monochorionic twins.

Case presentation: Standard karyotyping of peripheral blood cells from the twins showed chimerism 47,XY,+21/46,XX, mainly 46,XX line. Single nucleotide polymorphism microarray (SNP-array) analysis of buccal membrane cells from the twins showed a single cell line 46,XX in the twin girl and a single cell line 47,XY,+21 in the twin boy. Three-year follow-up after the birth showed that the twin girl had normal development, while the twin boy had Down Syndrome.

Conclusion: This is the first report on monochorionic dizygotic (MCDZ) twins by IVF with normal development/Down Syndrome respectively. To evaluate prenatal diagnosis of twins, early determination of chorionic status is important, and the amniotic fluid karyotyping is preferred rather than chorion villus sampling (CVS) or cord blood. It is important to perform genetic analysis of different tissues of MCDZ twins. Future research is needed to identify the causes and long-term consequences of chimerism in twins.

Introduction

After the first report of monochorionic dizygotic (MCDZ) twins by Souter et al.[1], more and more MCDZ twins have been reported worldwide, especially for pregnancies with assisted reproductive technology (ART). Here we report a case of MCDZ twins with chimerism of normal and abnormal karyotypes of peripheral blood cells but independent genotype of buccal membrane cells. This is the first report on MCDZ twins with normal development/Down Syndrome respectively.

Case Report

A 38-year-old woman conceived twins by in-vitro fertilization (IVF). Ultrasound examinations performed at weeks 6, 7, and 8 of gestation showed signs of monochorionic twins. Figure 1 show an ultrasound scan at 7 weeks' gestation.

At week 32⁺² of gestation, the twins were delivered, showing normal external genitalia. One of the twin babaies was a phenotypically normal girl with a birth weight of 1950 g. The other baby was a boy with a birth weight of 1450 g, who had typical Down Syndrome facial features.

Methods

Cytogenetic studies

Peripheral blood samples were collected from both parents and twins when they were 2-day-old and 3-year-old respectively. Standard karyotyping was performed.

SNP-array analysis

When the twins were 3-year-old, buccal membrane cells samples were collected. CytoCMA-12 and ISCAN (Illumina, America) were used for the single nucleotide polymorphism microarray (SNP-array) analysis to determine genotypes.

Results

Cytogenetic studies

The peripheral blood karyotypes of the father and mother were 46,XY,9qh+ and 46,XX, respectively. The twins were cohort chimerism karyotype, showing fewer cells of male Down Syndrome and more cells of normal female karyotype, eg.chi 47,XY,+21/46,XX. Table 1 summarized the results of peripheral blood karyotypes of the parents and the twins at 2-day and 3-year old. Figure 2 showed the chimerism karyotypes of the twins at 2-day and 3-year old respectively.

Table 1 Result of karyotyping for peripheral blood samples

	Father	Mother	Twin boy	Twin girl
	46,XY,9qh+	46,XX		
2-day-old			47,XY,+21[25]/46,XX[75]	47,XY,+21[14]/46,XX[86]
3-year-old			47,XY,+21[11]/46,XX[89]	47,XY,+21[13]/46,XX[87]

SNP-array analysis

The results from buccal membrane cells evidently revealed a single cell line 46,XX in the twin girl and a single cell line 47,XY,+21 in the twin boy. Figure 3 showed the results from buccal membrane cells of the twin boy at 3-year-old.

Discussion

The term chimerism in an individual refers to the coexistence of cells derived from more than one genetically distinct zygote. It can be caused by iatrogenic procedures (organ transplantation or blood transfusion), feto-fetal or feto-maternal transfusion, or the fusion of two separately fertilized oocytes [2]. The present report showed the chimerism karyotype: 47,XY,+21/46,XX of peripheral blood cells from the twins. SNP-array analysis of buccal membrane cells revealed a single cell line 46,XX in the twin girl and a single cell line 47,XY,+21 in the twin boy. According to the previous reports [1–3], an early fusion of two separately fertilized embryos occurred and the outer masses subsequently formed the placenta. The fusion before implantation lead to a single placenta with vascular anastomosis allowing an intrauterine exchange of cord blood stem cells. Hence, a blood chimerism exists in both twins with two different types of blood cells. The embryonic lineage remained spatially distinct. This phenomenon exists because of the

immaturity of the immunological fetal system [3]. The proportion of chimerism highly depends on the directivity and quantity of vascular anastomosis. In fact, ART increases the risk of preimplantation fusion, especially when several embryos are placed with a close physical proximity. Zona pellucida (ZP) disruption and various cell culture procedures can lead to changes on the cell surface, or induced ovulation and extended blastocyst culture are promoting multiple birth and embryo fusion [3–5]. This hypothesis that under certain conditions, fusion of preimplantation mammalian embryos can be induced *in vitro* was supported by studies of Tarkowski and Wojewodzka [6].

Seiko Fumoto et al.[7] reported a case of chimerism of peripheral blood and buccal membrane cells, and presumed that any organ could become chimeric with various degrees of admixture of two cell types in MCDZ twins, depending on the timing and the sites of the contact of the two fetuses. Bogdanova et al. [2] suggested that early fusion of embryos occurred because of a successful 'bone marrow transplantation' during pregnancy. It demonstrated that bone marrow stem cells can differentiate into mature cells of various organs. Therefore, more research should be focused on the chimerism of other cells and tissues, not just blood cells.

It is now recognized that monochorionic pregnancies increase the risk of maternal, fetal, and neonatal complication, which including congenital anomalies, fetal growth restriction, twin-twin transfusion syndrome (TTTS) and other perinatal and maternal complication [3, 8]. Chimerism of MCDZ twins has brought more complicate implications: prenatal misdiagnosis because of the chimerism in blood and false interpretation of diverse genetic analyses of twins. To evaluate prenatal diagnosis of twins, early determination of chorionic status is important, and the amniotic fluid karyotyping is preferred rather than chorion villus sampling (CVS) or cord blood which reflects only the cell line in hematopoietic tissue.

Chimerism is also an important subject in immunology. It's believed that microchimerism may play a role in the etiopathogenesis of some autoimmune diseases [5, 9–11]. The repeated karyotyping showed approximately the same distribution in the twins: ~20% XY,+21 cell line and ~ 80% XX cell line. This could point towards a permanent state. In the peripheral blood cells, twin girl had only a small proportion of male 'donor' cells, while the female 'donor' cell line predominated in the twin boy. Follow-up until the twins were 3-year-old, the twin boy showed Down Syndrome typical phenotype and normal external genitalia, while the twin girl had normal intelligence, growth, and genitalia development. These findings confirmed earlier reports that major proportion of blood cells in the mixture does not necessarily indicate the true genetic cell type [4, 12]. However, the same comparison can be carried out between the freemartin phenomenon in cattle [13, 14] and in human [2, 15]. The prevalence of genital anomalies in sex discordant twins suggests an association with intrauterine cell trafficking [5]. This means that close and long-term follow-up of genital anomalies is recommended in chimeric twins.

In summary, we report a rare case of MCDZ twins after IVF. The twins, a healthy girl and a Down Syndrome boy, are alive. For the peripheral blood cells, the contribution of predominated 'XX' cell line in the boy and the extra "+21" in the girl should be closely monitored in the future follow-up. We cannot exclude the existence of a low level of chimerism in other tissues. Such cryptic chimerism-generating

genetic in tissues should be investigated, especially in genital system, in addition to the effect of possible hormone transfer through the vascular anastomoses. It is important to perform genetic analysis of different tissues of MCDZ twins. Intrauterine cell trafficking between twins and the long-term effects of chimerism are subjects that need further exploration. A challenging task for further research is to identify the causes and long-term consequences of chimerism in twins.

Declarations

Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Chongqing Health Center for Women and Children. Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

We declared that there was no conflict interest for this research.

Funding

Not applicable

Authors' contributions

Hongrui Chen and Yingyign Wang analyzed and interpreted the patient data regarding cytogenetic studies. Mengjie Shen performed the SNP-array analysis and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Fetal ultrasound obtained at 7 weeks' gestation. The absence of a white chorionic membrane between the fetuses indicates monozygotic twins.

A

B

C

D

Figure 2

Chimerism karyotypes of the twins. G-banded karyotype of the twins' peripheral blood showing an admixture of 46,XX (A) and 47,XY,+21 (B) (chi 47,XY,+21/46,XX) at 2-day old. C and D showing a chimerism karyotype of the twins at 3-year old.