Letter to the Editor
Transfusion Medicine

Ann Lab Med 2014;34:259-262
http://dx.doi.org/10.3343/alm.2014.34.3.259
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

The First Known Case of Blood Group Chimerism in Monochorionic Dizygotic Twins in Korea

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Dear Editor,

Monochorionic (MC) dizygotic twins (DZT) are rarely conceived naturally [1]. However, the number of pregnancies conceived by assisted reproductive technology (ART) has increased from 9,864 in 1994 to 29,733 in 2006, thus multiple birth rate increased from 10/1,000 live births in 1980's to 27.5/1,000 live births in 2008 in Korea [2]. All MC DZT are theoretically chimeric for blood cells because blood stem cells might be exchanged through the common (MC) placenta [1, 3]. To date, there is no report on MC DZT exhibiting blood group chimerism in Korea. There has been only one reported instance of blood group chimerism in a dizygotic dichorionic pregnancy, even though IVF-ET is widely used in Korea [2, 4]. We report blood group chimerism in sex-discordant MC DZT conceived via IVF-ET, initially misdiagnosed at birth as having blood subgroup B3.

The sex-discordant MC DZT conceived via IVF-ET were born at a tertiary university hospital and assigned the blood subgroup B3. Twenty-three months later, the male twin (propositus) visited the same hospital for a preoperative workup prior to hydrocele surgery. Approximately a half of his red blood cells (RBCs) agglutinated strongly (4+) with anti-B reagents on forward typing, whereas the remaining RBCs did not (mixed field agglutination). His serum reacted with A_1 RBCs only. His father, mother, and older sister had typical B, O, and B phenotypes, respectively (Fig. 1). Retrospective medical record review revealed that the propositus did not have any history of transfusion and bone marrow transplantation and, interestingly, he and his twin sister were MC twins.

To confirm blood group chimerism, the following tests were performed on the twins and their father, mother, and sister, after obtaining their informed consent: 1) ABO serological testing using a micro-column agglutination technique (Bio-Rad, Cressier sur Morat, Switzerland); 2) ABO genotyping via direct sequencing of exons 6 and 7; 3) allelic separation of the ABO gene via cloning; and 4) short tandem repeat (STR) marker analysis. All tests were performed as described previously [5, 6]. The results are summarized in Fig. 1, 2, and 3. In addition, STR analysis of buccal swabs, hair follicle material, and peripheral blood leukocytes from the propositus and his twin sister, was performed to discriminate between whole-body and blood chimerisms. Six loci (D21S11, D13S317, D2S1338, TPOX, D18S51, and FGA) yielded three or more peaks in DNA obtained from peripheral blood leukocytes, but not in DNA obtained from buccal swabs or hair follicles (Table 1). This result confirmed that chimerism in these MC DZT was confined to blood cells.

A chimera is defined as an organism carrying cells from two

Received: August 23, 2013
Revision received: October 25, 2013
Accepted: January 25, 2014

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or more zygotes [7]. When chimerism is confined to the lymphohematopoietic system and occurs spontaneously in twins, the condition is termed "twin blood chimerism" [7]. The twins described in the present report displayed overt blood group chimerism because of their MC history. The blood cells were approxi-

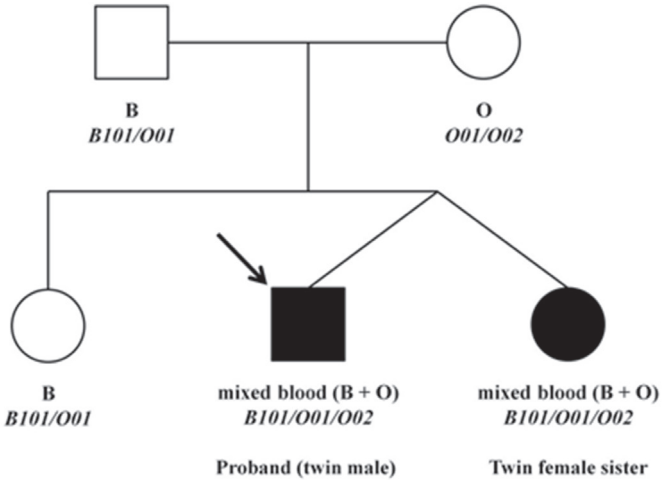

Fig. 1. Blood groups of the propositus and his family. The phenotypes and genotypes of the propositus' family members (except his twin sister) were normal. Mixed blood of types B and O, caused by the genotype *B101/O01/O02* (as revealed by cloning and direct sequencing of exons 6 and 7) was observed in the propositus (arrow) and his twin sister.

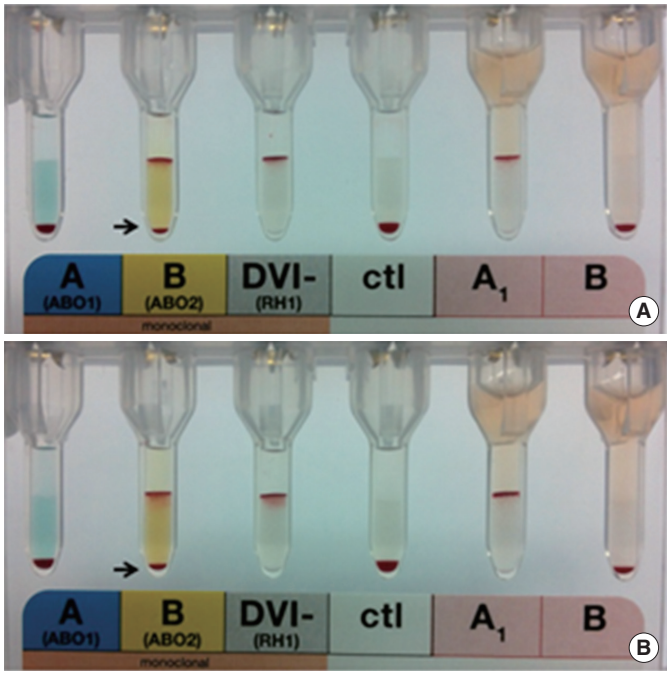

Fig. 2. Results of the monoclonal gel test showing two populations of blood cells on typing of the propositus. A mixed-field agglutination pattern was observed by using the anti-B reagent (arrows), indicating the simultaneous presence of two red blood cell populations (Twin, male, A). The same pattern was observed in the twin sister of the propositus (Twin, Female, B).
Abbreviations: DVI, RhD^{VI} variants; ctl, control.

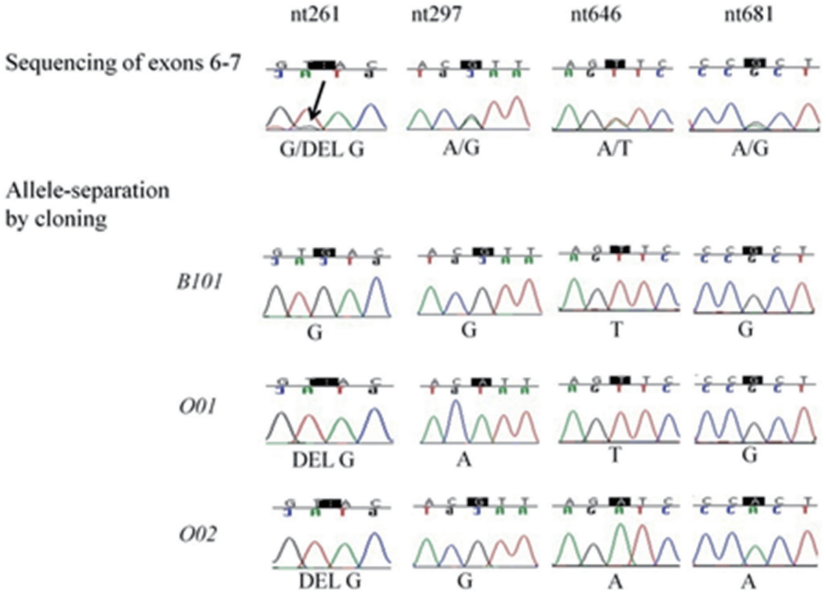

Fig. 3. Nucleotide sequences in the region of nucleotides 261 (arrow), 297, 646, and 681 in ABO exons 6 and 7. The top chromatogram was produced by routine sequencing of exons 6 and 7. The presence of both *O01*- and *O02*-allele-specific nucleotides and B-allele-specific nucleotides (arrow) is evident. The bottom three chromatograms were obtained via sequencing conducted after cloning, and conclusively show *B101*-, *O01*-, and *O02*-allele-specific nucleotides.
Abbreviation: nt, nucleotide.

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Table 1. Short tandem repeat analysis of DNA isolated from the blood, buccal swab, and hair root cells of the propositus and his twin sister

DNA polymorphism	Propositus			Twin sister		
	Blood	Buccal swab	Hair root cells	Blood	Buccal swab	Hair root cells
D8S1179	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12
D21S11	29, 30, 32.2	30, 32.2	30, 32.2	29, 30, 32.2	29, 30	29, 30
D7S820	11	11	11	11	11	11
CSF1PO	7, 11	11	11	7, 11	7, 11	7, 11
D3S1358	16, 18	16, 18	16, 18	16, 18	16, 18	16, 18
TH01	9, 10	9, 10	9, 10	9, 10	9, 10	9, 10
D13S317	10, 11, 12	11, 12	11, 12	10, 11, 12	10, 12	10, 12
D16S539	12	12	12	12	12	12
D2S1338	20, 23, 24, 25	20, 24	20, 24	20, 23, 24, 25	23, 25	23, 25
D19S433	14	14	14	14	14	14
vWA	17, 19	17, 19	17, 19	17, 19	17, 19	17, 19
TPOX	8, 9, 11	8, 11	8, 11	8, 9, 11	9, 11	9, 11
D18S51	12, 15, 17	15, 17	15, 17	12, 15, 17	12, 17	12, 17
D5S818	12, 13	12, 13	12, 13	12, 13	12	12
FGA	22, 23.2, 24	22, 23.2	22, 23.2	22, 23.2, 24	23.2, 24	23.2, 24
X, Y	X, Y	X, Y	X, Y	X, Y	X	X

Alleles shown in bold indicate a double maternal and/or double paternal contribution, confirming that propositus and his twin sister exhibit twin blood group chimerism.

mately 50% O and 50% B (Fig. 2). However, the twins were initially misdiagnosed as having blood subgroup B3 at birth, and the propositus was only later shown to exhibit blood group chimerism.

Clinical, serological, and molecular studies of patients and their families may be useful in distinguishing blood group chimerism from ABO subgroups such as B3. The present case was characterized by using the following criteria: an MC DZT, no evidence of subgroup B3 in his parents, distinct double cell populations in micro-column, more than three ABO alleles, and three or more STR peaks in six loci. Thus, the propositus was suspected to exhibit blood group chimerism rather than to be of subgroup B3. The flow cytometry for the detection of A/B antigens of red blood cells might also be useful in discriminating between chimerism and ABO subgroups [8].

In terms of clinical implications, blood chimerism in MC DZT may be a risk factor for the development of particular complications such as twin-to-twin transfusion syndrome. Blood group chimerism may also cause confusion, if a blood transfusion is required or an ABO discrepancy occurs [1, 5, 6]. Thus, accurate diagnosis of twin blood chimerism is essential.

ART such as IVF-ET is widely used in Korea, and the frequency of MC DZT may be increasing [2]. The use of ART, including IVF-ET, may affect cell fusion, adhesion, and embryo proximity [9], and is likely to increase the probability of monochorionicity in DZT. Thus, many Korean MC DZT are likely to have blood group chimerism. Unexpectedly, this is the first reported case of blood group chimerism in MC DZT conceived via IVF-ET in Korea, suggesting that blood group chimerism in MC DZT may be overlooked in Korean hospitals and blood banks.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

We would like to thank Hye Ryoen Park for her technical assistance. This work was supported by a research grant from the Research Institute of Medical Sciences, Chonnam National University (2012-CURIMS-DR001).

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