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CASE REPORT

CRIMINALISTICS

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Congenital Tetragametic Blood Chimerism Explains a Case of Questionable Paternity*

ABSTRACT: Human chimerism is the presence of ≥2 cell populations in one person that contain genetic material from more than one zygote. Chimerism may be either acquired by transfusion or transplantation of donor cells, or congenital arising from embryo fusion or dizygotic twin–twin transfusion. We encountered a 4-year-old boy with developmental hip dysplasia whose preoperative (serologic) blood group was AB, but whose red cell agglutination was atypical ("mixed field") and caused us to study the patient's parents' ABO blood groups. Parental blood groups (AB and O) suggested possible nonparentage. An alternative explanation of the findings was that the child was chimeric or mosaic. Molecular cloning and genotyping of his ABO locus in leukocytes revealed two heterozygous genotypes: A102/O01 and B101/O01. Other loci, each of which possessed three distinct alleles, unambiguously showed transmission of two alleles from either the child's mother (e.g., HLA-A) or two alleles from the child's father (e.g., D8S1179). Findings indicate that the child is a tetragametic chimera.

KEYWORDS: forensic science, chimerism, ABO, HLA, STR, paternity, DNA typing

A human chimera is a person whose cells have originated from two (or more) zygotes. A chimera differs from a mosaic, which is derived from one zygote (1). Chimerism may be acquired, following transfusion or transplantation of donor cells to a patient, or it may be congenital, arising from fusion of dizygotic twin embryos or by embryonic twin–twin transfusion. When only blood cells are evaluated, the term "blood chimerism" is used. It is usually detected by finding two red cell populations ("mixed-field" agglutination) during a patient's serologic blood grouping. Then, when family studies are carried out to clarify a nontransplanted patient's blood group, nonparentage may be suspected as a result of the congenital blood chimera's parental blood groups.

Congenital chimerism, also called tetragametic chimerism, can demonstrate evidence of four gametes (two zygotes) in a variety of body tissues. Most tetragametic chimeras, other than those found by blood serotyping, are discovered because they have evidence of male and female sex characteristics. Clinical studies may reveal true hermaphroditism: two cell populations are cytogenetically (X/X and X/Y) and four gametes can be observed at autosomal loci in some tissues.

Materials and Methods

A 4-year-old boy was to undergo surgery for congenital hip dysplasia, but his preoperative blood group produced atypical serologic results ("mixed-field agglutination": some red cells fail to agglutinate with anti-A and anti-B reagent antibodies). In order to ascertain his ABO type, the patient's parents were typed (AB and O), and these findings suggested nonparentage—an AB × O mating should produce only A or B children. Clinical history, however, indicated that the boy was the twin of a female child who had died of asphyxia at birth. Therefore, the possibility was suggested that the living child was chimeric and prompted further laboratory studies.

After obtaining informed consent, 5.0 mL blood samples were collected for serologic studies from the two adults and the child in vacuum tubes containing no preservative or anticoagulant. In addition, 5 mL blood samples were collected in EDTA for DNA studies.

Blood Grouping

Forward and reverse ABO blood typing were performed by standard hemagglutination tube tests at room temperature with two commercial antisera (monoclonal: Immucor/Gamma® [Dartmouth, Nova Scotia, Canada] and human sources: Brother® [Bodei Bio-Company, Changchun, China]). Standard gel centrifugation test cards were used as well (DiaMed®; DiaMed AG, Cressier, Switzerland).

ABO Genotyping

After genomic DNAs were extracted from blood by a modified salting out technique, a commercial genotyping reagent was used (BAGene; Amstgerichtsstraβe1-5, 35423 Lich, Germany), which consisted of a multiplex of eight polymerase chain reactions (PCRs) with sequence-specific primers (PCR-SSPs) designed to detect nucleotide sequence differences specific for O1, O2, A2, and B alleles of the ABO locus. Additionally, an ABO genotyping method was applied that was previously reported by our laboratory (2). This consisted of four PCR-SSPs that detect O1, A, B, and A201 alleles.

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TABLE 1—*The paternity test for the identification of the relationship between the proposita with A3B3 blood type and the parents with AB and O blood types as discussed in this study.*

Loci	Child	Mother	Father	Two-parent Paternity Index
ABO phenotype	A3B3	O	AB	
ABO genotype (SSPs)	B1O1 or A1/B1/O1*	O1O1	A1B	
ABO genotype (sequence-based typing [SBT])	B101/O01 (Direct SBT) A102, B101, O01 (Clonal SBT)	O01/O01	A102/B101	
HLA-A	2, 31, 33	2, 31	24, 33	/
HLA-B	7, 58	7, 62	48, 58	/
HLA-DRB1	13, 14, 15	15	13, 14	/
D8S1179	10/12 [14]†	10/14	12/14	3.9936
D21S11	30.2/31	30.2/31	30.2/31	4.5495
D7S820	7/11	7/11	11	2.7203
CSF1PO	12	11/12	10/12	1.3390
D3S1358	15	15/16	15	3.0525
TH01	7/9	8/9	7/9	1.7531
D13S317	8/10[9]	10	8/9	1.6072
D16S539	12	12	8, 12	2.3288
D2S1338	24/25	19/24	23/25	7.9365
D19S433	13/15.2	13/15.2	13/16.2	1.1471
vWA	16/18 [15]	16/18	15/16	2.8818
TPOX	7/10	7	10/11	19.0114
D18S51	12.2/22	13.2/22	12.2	21.9780
D5S818	10/11	10	11/12	4.9019
FGA	20.2/24	21.2/24	20.2/21.2	714.2857
Amelogenin	X, Y	X	X, Y	
Cumulative parentage index				50877164482
Plausibility percentage				99.9999

*Two PCRs with SSPs genotyping results from different reagents.
†Genotypes in brackets refer to minor-intensity haplotypes. They were disregarded in calculating parentage indices.

Direct Genomic Sequencing of Encoding Region and the 5′ Regulatory Region Containing the CBF/NF-Y Enhancer at ABO Gene Locus

Five separate PCRs were carried out to amplify the promotor, all seven exons, introns 2, 5, 6, and part of introns 1, 3, 4 of the ABO gene (3). Genomic DNAs of the trio were sequenced in the 5′ regulatory region containing the CBF/NF-Y enhancer with a pair of PCR primers (Pro-F:5′ggaaacaaatcctacccctac-3′ located at −nt3806 to −nt3826; Pro-R:5′gtgctgcctgtgcctgttac-3′ located at −nt3612 to −nt3631 in 5′UTR of the ABO gene). Reaction volume for PCR was 50 μL containing 1× buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$), 10 nmol dNTP, 5 pmol each primer, 300–500 ng genomic DNA, and 0.5 U AmpliTaq Gold™ DNA polymerase (Applied Biosystems, Branchburg, NJ). Different sizes of enhancer PCR produce fragments obtained by gel purification, and PCR products of the ABO gene were directly sequenced by kit (BigDye® Terminator, V3.1; Applied Biosystems, Foster City, CA) on an ABI PRISM 377 genetic analyzer (all ABI from Applied Biosystems, Foster City, CA).

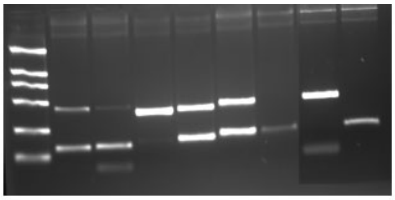
Cloning and Sequencing of PCR Products of the ABO Genes of the Trio

We amplified a 2.1-kb fragment spanning a region from intron 5 to exon 7 of the ABO gene from the trio's genomic DNAs. The PCR forward primer was 5′ctggaagggtggtcagagga-3′, and the reverse primer was 5′gttactcacaacaacaggacggac-3′. After purification using extraction (Montage™; Millipore, Bedford, MA), three separate PCR products were pooled together and ligated into the pGEM-T Easy Vector (Promega, Madison, WI). Ligation products were transformed into JM109 competent cells, and the cells were cultivated in 2YT with ampicillin for 24 h. More than 40 positive clones were obtained randomly from the trio. The recombination plasmids were isolated with the Plasmid Miniprep kit (Axygen, Union City, CA). These alleles were sequenced from both directions by two forward primers: cgccagggttttcccagtcacgac, ctgccagcgcttgtaggcgcgca and a reverse primer 5′-gagcggataacaatttcacacagg-3′; on an ABI 3730 sequencer.

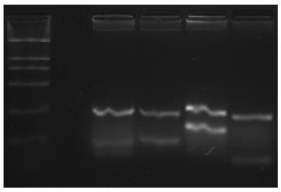
HLA Typing by SSP-PCR

Human leukocyte antigen (HLA) Class I (A, B) and II (DRB1) alleles were amplified with commercially available kits (UniTray®-

FIG. 1—*Monoclonal gel test card, presenting two populations of ABO red blood cells, the smaller of which is of blood group A (arrows).*



Marker	O 1	Non O1	O 2	Non O2	B	Non B	A 2	Non A2	
DL2000	+	+	-	+	+	+	-	+	BO 1



Marker	A	B	O	A201	
DL2000	+	+	+	-	A, B, O

FIG. 2—*Agarose gel electrophoretic fractionation of the proposita's ABO characteristics determined by two reagents. Left testing result is based on eight independent PCR procedures, of which four are allele-specific (O1, O2, B, A2) and the remaining four are non-allele-specific (non-O1, non-O2, non-B, non-A2). A, B, and O alleles were simultaneously determined by four allele-specific including A-specificity shown in the right figure. In the bottom line, the names are listed for each column, determining positive reactions.*

96; Dynal Biotech A.S.A., Oslo, Norway) by SSP. The PCR products were identified using agarose gel electrophoresis and detected through ethidium bromide intercalation under UV light. If a band is present in the gel, the pattern of bands identifies the HLA. UNIMATCH PLUS 3.2 Analysis software (Invitrogen, Carlsbad, CA) was used for HLA typing.

Short Tandem Repeat PCR Multiplexes

DNA samples from the trio were amplified using an ABI AmpF/short tandem repeat (STR) Identifiler PCR kit according to the manufacturer's instructions. The loci and results are listed in Table 1. The PCR products were analyzed with an ABI PRISM 3100 DNA sequencer, and STR allele calls were made using ABI GENOTYPER 3.7 software. Paternity indices were calculated using the parent's Chinese Han population allele frequencies of STR locus.

Results

Except for the child's hip dysplasia, the trio was healthy and had no history of transfusion or transplantation. Serologically, by two different tests, the child's red cells phenotyped A3B3 in mixed-field reactions, using either anti-A or anti-B reagents. Two distinct cell populations appeared to be present (Fig. 1). Neither anti-A nor anti-B were detected on reverse (serum) typing. The child's mother typed as O+ and her husband typed AB+.

Genomic typing results are shown in Table 1. The two adults showed a maximum of two alleles at the ABO locus, but cloning and genotyping the child demonstrated three alleles at the ABO locus in two separate heterozygous types: A102/O01 and B101/O01 (Fig. 2). The child, however, possessed three alleles at HLA-A, DRB1*, D8S1179, D13S317, and vWA loci. Clearly, at the HLA-A and vWA loci, the child inherited two alleles from his mother, whereas at the HLA-DRB1* and D13S317 loci, he inherited two alleles from his father. Neither adult could be excluded as a parent of the child, and the two-parent paternity index was $>5 \times 10^{10}$.

Discussion

The child presented here is similar to two other chimeras that presented with weak ABO types (4,5). The reason for weak expression may be inheritance of a hybrid A01-O02 allele that encodes for a low-activity A transferase or a minor population of B101/L02 cells causing a Bel phenotype in an AelBel individual.

Mosaics may demonstrate three alleles in a cell population, but not four. The third allele may be the result of a cytogenetic anomaly arising in one zygote such as trisomy. When trisomy occurs, the extra chromosome arises from one parent. Unlike mosaics, chimeric people show either four gametes (four different alleles/locus) or other evidence that two zygotes contributed alleles.

Two popular hypotheses explain chimerism. First, dizygotic twin–twin transfusion may give rise to blood chimerism. However, most blood chimeras have not had their other tissues examined sufficiently to rule out the possibility that they are actually "whole-body" chimeras. Second, hermaphroditic chimeras are thought to arise by fusion of male and female (dizygotic) twin embryos. Neither these two hypotheses nor others are well supported at this time, and some embryologists believe that chimerism may be more common than the number of reported cases suggest.

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