Allelic Imbalance in a Neonate with Dispermic Chimerism

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Background:

- Chimerism is defined as the presence of two or more genetically distinct cell populations, acquired either artificially or naturally.
- Dispermic chimerism is a rare, natural form of chimerism described as the cellular fusion of two zygotes early in gestation.
- Investigation of a neonate with blood group AB born to a mother with blood group O and father blood group AB revealed the presence of dispermic chimerism demonstrating allelic imbalance.

Methods:

- ABO blood group typing was performed by:
  - solid phase automation
  - serologic tube testing
  - targeted next generation sequencing (NGS)
- HLA typing of 11 loci was performed by NGS.
- Short tandem repeat (STR) analysis was performed by PCR using peripheral blood and saliva-derived genomic DNA.
- Karyotyping was performed by culturing WBCs to metaphase and staining chromosomes

Results:

- The serologic ABO typing of the proband demonstrated group AB with atypical mixed field (MF) agglutination and disproportionately pellet sized anti-A versus anti-B reactions (Figure 1).

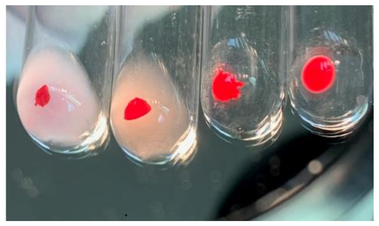


Figure 1. Proband tube ABORH hemagglutination reactions using (L to R) Anti-A, Anti-B, Anti-D, Anti-A,B antisera. Mixed-field agglutination pattern seen in Anti-A and Anti-B antisera. Stronger reactivity is observed in Anti-B (3+mf) than Anti-A (2+mf), indicative of a larger population of B cells than A cells.

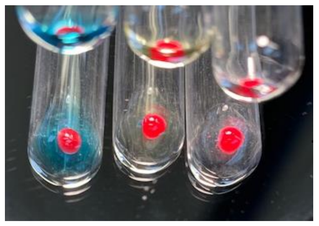


Figure 2. Paternal tube ABORH hemagglutination reactions using (L to R) Anti-A, Anti-B, Anti-D antisera. No observed mixed-field reactivity.

- The MF agglutination confirmed a dual population of cells. Specifically, the unexpected presence of separate populations of A and B red cells versus the typical A,B red cells observed in blood group AB individuals. The presence of group O red cells was ruled out by the complete hemagglutination of all cells in the Anti-A,B test tube (Figure 1).

- ABO NGS showed the inheritance of three alleles, *O101/B01/A102*, with unequal inheritance of the father's A (~25%) and B (~75%) alleles (Table 1).

Table 1. Results of serologic and molecular ABO typing

System	Proband	Mother	Father
ABO phenotype	AB*†	O	AB
ABO genotype	*O101/B01‡/A102*	*O175/O101*	*A102/B01*

* mixed field agglutination

† B antigen predominance over A antigen

‡ *B01* (~75%) predominance over *A102* (~25%)

Results:

- The disproportionate ABO inheritance is supported phenotypically by the hemagglutination reactions in the serologic ABO testing. The size of the pellet in the anti-B tube was discernably larger than that of the anti-A tube, indicating a larger population of B RBCs versus A RBCs (Figures 1 & 3).

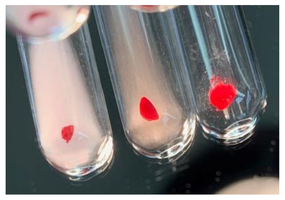


Figure 3. Proband tube ABORH hemagglutination reactions using (L to R) Anti-A, Anti-B, Anti-D antisera. Anti-B pellet (B RBCs) larger in size than Anti-A pellet (A RBCs). More "free cells" (B RBCs) in Anti-A tube.

- RBC phenotyping of the following blood group antigens was performed: C, c, E, e, K, Fy, Jk, M, N, S, and s. However, MF agglutination was not clearly observed. The phenotypic expression of chimerism appears to be limited to the ABO group of RBC antigens.

- Peripheral blood STR analysis demonstrated the presence of three alleles for 7 of the 21 loci examined (Table 2).

Table 2. Results of peripheral blood STR analysis

STR loci	Proband	Mother	Father	Chromosome location
D12S391	**17, 19, 21**	19, 21	17, 21	12p13.2
D1S1656	**15, 16, 17.3***	16, 16	15, 17.3*	11q42.2
D7S820	**9, 10, 12***	8, 10	9, 12*	7q11.21-22
D13S317	9, 10	9, 12	8, 10	
D5S818	11, 11	11, 11	11, 11	
FGA	23.2, 24	23.2, 25	24, 24	
TH01	6, 7	6, 7	6, 9	
D19S433	**13, 14, 15.2**	13, 15.2	13, 14	19q12
D18S51	12, 15	12, 14	15, 16	
D21S11	28, 28	28, 31	28, 29	
D8S1179	11, 11	11, 12	11, 11	
AMEL	X, X	X, X	X, Y	
TPOX	8, 9	8, 11	8, 9	
CSF1PO	**9, 10, 11***	10, 12	9, 11*	5q33.3-34
D16S539	10, 13	13, 13	10, 11	
vWA	**16, 17, 18**	16, 18	17, 18	12p13.31
D3S1358	15, 16	15, 16	15, 16	
D2S1338	18, 23	17, 23	18, 22	
D10S1248	14, 15	14, 15	15, 15	
SE33	**27.2, 30.2*, 31.2**	14, 31.2	27.2, 30.2*	6q14
D22S1045	15, 17	15, 15	11, 17	
D2S441	10, 14	10, 14	10, 10	

* Extra parental allele contribution

Bold and Highlight indicates marker with third allele present

Results:

- Saliva STR analysis did not detect additional STR alleles in any tested loci markers, ruling out chimerism in the saliva derived cells.

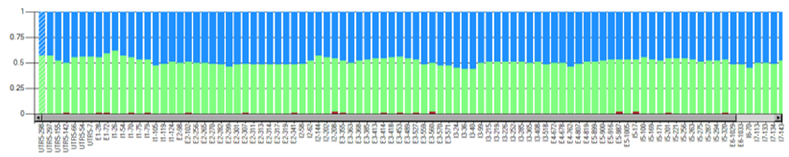
- Furthermore, the HLA typing of the proband indicated presence of more than two alleles for HLA-A, HLA-DPA1, and HLA-DPB1 loci. The additional alleles were from the non-inherited paternal HLA haplotype (Table 4).

Table 4. Results of peripheral blood HLA typing

	Proband		Mother		Father	
Haplotype			c	d	a	b
HLA Loci						
A	*	*	02:01	24:02	11:01	24:02
B	46:01	35:21	35:21	44:02	46:01	48:01
C	01:02	15:02	15:02	05:01	01:02	08:01
DRB1	09:CWA	14:06	14:06	04:02	09:CWA	09:01
DRB3		01:01	01:01			
DRB4	01:03			01:03	01:03	
DQA1	03:02	05:03	05:03	03:01	03:02	03:02
DQB1	03:03	03:BGCWT	03:BGCWT	03:ADAJH	03:03	03:03
DPA1	*	*	01:CDTE	01:CDTE	02:02	02:01
DPB1	*	*	04:HJMR	04:BCREN	05:RGPW	13:AMJWN

*Alleles not reported due to high background and allelic imbalance.

Paternal HLA allele balance:



Proband HLA allele imbalance:

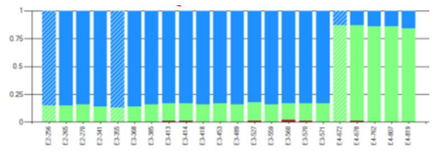


Figure 4. HLA allele balance seen in the paternal HLA-A locus (top) versus unusual alleleic imbalance seen in the proband HLA-A locus (bottom).

- Cytogenetics revealed an ordinary 46,XX karyotype with normal G-banding patterns.

Conclusion:

Cumulatively, the serologic and molecular analyses support the findings of dispermic chimerism with an imbalance of allele inheritance. The proband inherited one maternal and two paternal haplotypes or alleles. The proband is an otherwise normal and healthy female neonate.