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CASE REPORT

An unusual observation of tetragametic chimerism: forensic aspects

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Abstract A 41-year-old healthy Caucasian male showed an unidentifiable direct AB0 group and a B group by an indirect method revealing the presence of natural antibodies anti-A1 and anti-A2. Mixed fields with anti-B and anti-A+B antisera led to the conclusion that blood group B and 0 cell populations were present in a 1:1 ratio. A negative anamnesis for both transplantation and transfusion suggested a chimerism. DNA analysis of tissues revealed a tetragametic chimerism due to an apparent double parental contribution of nuclei in a phenotypically normal man.

Keywords Tetragametic chimerism · AB0 mixed agglutination · DNA investigation

Introduction

Chimeras are defined as individuals showing two or more genetically distinct cell populations. Two main different classes of chimerism are identifiable: (1) partial hematopoietic chimerism in dizygotic twins, due to twin–twin transfusion over vascular anastomoses between the two dichorionic placentas [1], (2) dispermic chimerism as a consequence of the fertilization of two maternal nuclei by two spermatozoa and the fusion of these products into one body, and (3) a third kind of chimerism which originates after allogenic bone marrow or stem cells transplantation [2, 3].

Here, a rare event of tetragametic chimerism is described which was generated by a double contribution of parental nuclei. This less common kind of chimerism is identified during routine AB0 blood grouping when mixed-field agglutination is observed by cell typing.

Case history

A 41-year-old healthy Caucasian male presented to hospital for the identification of serological blood group and Rh factor.

Results showed an unidentifiable AB0 blood group using a direct method (Fig. 1) and a B blood group by an indirect method that revealed the presence of the natural antibodies anti-A1 and anti-A2.

Rh phenotyping analysis was unremarkable because of the same expression in the propositus as in his parents: (CcDee, Kell neg.)

Mixed fields were observed with anti-B and anti-A+B sera as normally verified when two different B and 0 red cell populations are present at the same time.

This mixed-field pattern led to the conclusion that two distinct red blood cell (RBC) populations (B and 0) were simultaneously present in the proportion of 50% for each.

The principal hypothesis was that this was due to chimerism, but prior bone marrow transplantation [4] from an AB0 compatible donor or prior transfusion of 0 red cells in a propositus with blood group B were excluded.

The blood group of the propositus was identified as a B Rh positive with mixed field 0 Rh positive from paternal origin (paternal blood group was 0 Rh positive and maternal B Rh positive).

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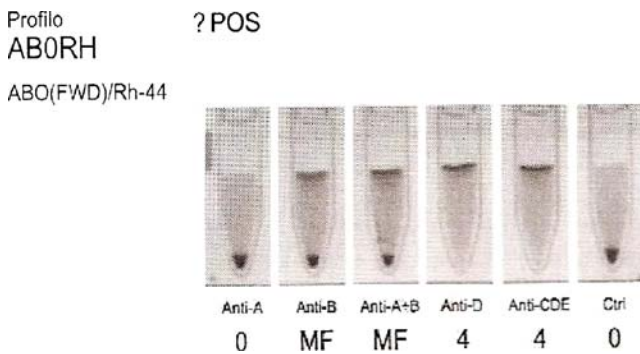

Fig. 1 Direct AB0 blood grouping. Mixed field reactions are evident

The man had no clinical history of therapeutic transplantation or a transfusion, and the absence of clinical symptoms suggested the validity of the principal hypothesis.

However, to explain the origin of the different RBC populations, two possibilities were to be considered, chimerism and mosaicism. Mosaicism is assumed to be far more common arising from single zygotes by embryonic mutation, while in a chimera, two or more different populations of genetically distinct cells originate from different zygotes.

To discriminate chimerism from mosaicism, DNA polymorphisms of tissues derived from different germ layers were investigated. DNA from buccal swabs of the propositus (ectodermally derived) and blood cells (mesodermally derived) were tested for autosomal and X sex chromosomes. The same analyses were carried out on his parents.

Y sex chromosome was tested to verify the paternity.

Materials and methods

Blood group typing

A 7-ml blood sample was included in a tube with EDTA and an automatic blood grouping was obtained using the Ortho Autovue Innova System (Johnson & Johnson Gateway® Hong Kong).

AB0 and Rh blood groups were tested by the direct method matching propositus red cells with anti-A, anti-B, anti-A+B, anti-D, and anti-CDE sera while the plasma was tested with standard A1, A2, B, and 0 red cells by an indirect method.

Dna isolation

Dna was extracted from peripheral blood and buccal swabs with a commercial DNA extraction kit (Gentra Puregene Blood Kit and Gentra Puregene Buccal Cell Kit—Qiagen Sciences, MD, USA) according to manufacturer's protocol.

Table 1 DNA profiles in blood and buccal swabs from the propositus and his parents

STR locus	Father	PPropositus blood	Propositus buccal swabs	Mother
Amel	XY	XY	X (Y)	XX
D8S1179	10/15	10/15	10/15	13/15
D21S11	29/29	29/34.2	29/34.2	30.2/34.2
D7S820	11/11	11/12	11/12	10/12
CSF1PO	11/12	11/(12)	11/12	11/11
D3S1358	14/15	15/17	15/(17)	15/17
THOI	6/9.3	(6)/7/9.3	6/(7)/9.3	6/7
D13S317	8/12	(8)/9/12	8/(9)/12	9/12
D16S539	12/12	12/13	12/13	13/13
D2S1338	17/19	(17)/19/20	17/(19)/20	17/20
D19S433	15/15.2	13/(14)/15(15.2)	13/(14)/(15)/15.2	13/14
vWA	18/18	17/18/(19)	(17)/18/19	17/19
TPOX	8/11	8/11	8/(11)	8/8
D18S51	12/13	(12)/13	(12)/13	12/13
D5S818	11/13	(11)/13	11/13	11/13
FGA	22/22	22/25	22/(25)	22/25
FES	10/11	10/(11)/12	10/(11)/(12)	10/12
F13A1	3.2/14	3.2/6	3.2/6	4/6
D19S253	10/11	6/10/(11)	N.D.	6/10
LPL	9/11	9/11	9/11	9/11
F13B	6/8	6/7/(8)/(9)	(6)/(7)/8/9	7/9
HPRTB	13	(10)/11/(13)	10/(11)/13	10/11
DXS8377	46	(41)/45/(46)	41/(45)/46	41/45
DXS8378	10	(10)/11/(11.2)	10/(11)/11.2	11/11.2

Values in parentheses refer to minor intensity alleles

N.D. not determined

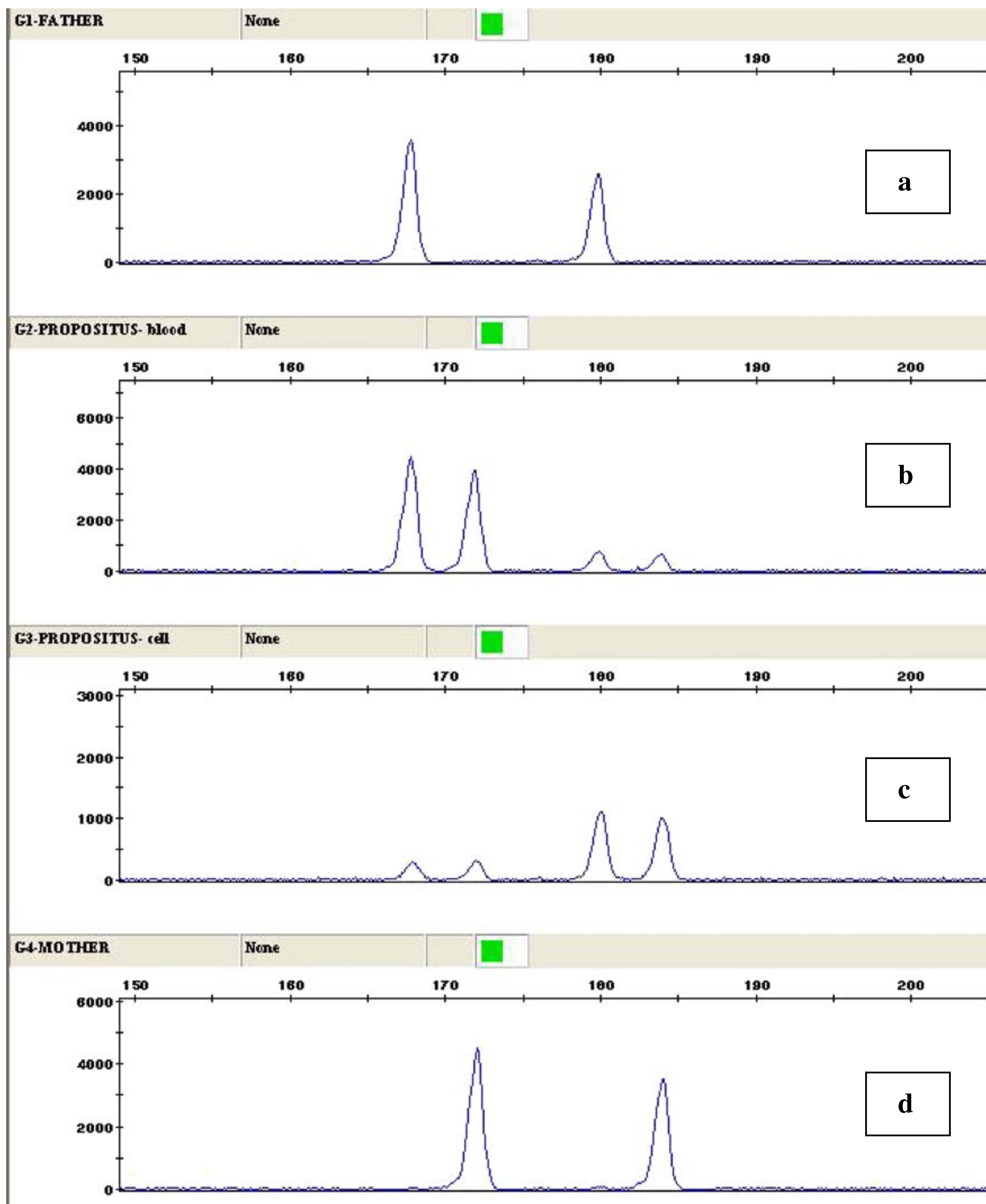

Fig. 2 Electropherogram of the HumF13B genetic marker showing both parental contribution. **a** Father; **b** Propositus blood sample; **c** Propositus buccal swabs; **d** Mother

Cytogenetic analysis from the blood sample revealed a normal karyotype of 46, XY [5].

Short-tandem-repeat microsatellite markers

Short-tandem-repeat (STR) typing was performed by employing commercial kits AmpFlSTR Identifiler and AmpFlSTR Y-filer [6] (Applied Biosystems USA), five more autosomal markers, and three X-chromosome microsatellites as shown in Table 1. Amplifications were performed according to the instructions provided in the kit users' manual with the recommended DNA amount (1.0–2.5 ng) and a final polymerase chain reaction (PCR) volume of 25 µl.

Polymerase chain reaction products were mixed with 24.5 µl of Hi-Di formamide and 0.5 µl ROX 500/LIZ 500 size standards and denatured at 95 for 3 min. The

samples were subjected to denaturing capillary gel electrophoresis using ABI PRISM 310 Genetic Analyzer (Applied Biosystems USA), and data were analyzed using the GeneMapperID v3.2 software.

Singleplex reactions for the HumF13B, HumFES, HumF13A1, and HumLPL loci were performed as previously described [7], while HPRTB, DXS8377, DXS8378 loci were studied as described by Fracasso et al. [8].

Results

The results are shown in Table 1. In 13 out of 20 examined autosomal loci, chimerism was detected.

Among the autosomal markers studied, D19S433 and HumF13B (Fig. 2) revealed a pattern consistent with a double paternal and maternal DNA contribution.

The same pattern appeared on the X chromosome where HPRTB, DXS8377, and DXS8378 loci showed a chimeric pattern (Table 1).

Analysis of peak size showed that the mixed allelic patterns were one type in blood, while the opposite arrangement was present in DNA derived from the buccal swabs (Fig. 2) indicating that the propositus was a tetragametic chimera as a result of a biparental contribution of genetic markers.

The Y-chromosome polymorphisms revealed identical haplotypes for the propositus and his father confirming the paternity (data not shown).

Cytogenetic analysis of a blood sample from the propositus demonstrated a normal karyotype of 46, XY (data not shown).

Discussion

This case represents a relatively uncommon example of tetragametic chimerism in a phenotypically normal and asymptomatic male.

Because of the lack of clinical symptoms or other phenotypic attributes, such as patchy skin coloration or differently colored eyes, this kind of chimerism often remains undetected.

A dual-cell population in blood and buccal swabs with cells deriving from two different germ-cell layers (ectoderm and mesoderm) should indicate that chimerism concerns the propositus whole body.

The different allelic pattern between the two cell lines suggests a clonal origin beginning early in embryonic development.

Such cases of tetragametic chimerism are discovered in the population with low frequency and high technical difficulty, while natural chimerism may concern up to 10% of the population as referred in literature [9].

Usually, some difficultly in blood grouping leads to additional investigations, and as in this case, further studies have to be performed to confirm evidence of chimerism [10]. Due to the apparent rarity of tetragametic chimerism and the importance of the use of molecular techniques to demonstrate its presence, this condition may be underdiagnosed.

Human chimeras are impossible to differentiate from single-genotype individuals by routine observation and very difficult to identify even when applying the best biomedical technologies,

Most chimeras are discovered in some genotyping situation to carry three or four, instead of one or two, alleles at multiple loci, but if a single cell line predominates in the blood, the chimeric state may not be detected unless family studies are undertaken.

Even then, the findings may be misinterpreted as ruling out maternity [11] or paternity and lack of identity in criminal investigations.

Generally, it is not possible to find a mixed allelic pattern due to the proportionally low copy number in the peripheral blood and to the relative insensitivity of PCR-based techniques for detecting small quantities of DNA in whole blood.

These results suggest that in forensic casework, the scientist could misunderstand the interpretation of a mixed profile resulting from the testing of chimeric biological stains. For instance, three or four alleles at a locus could be indicative of two or more contributors. Consequently, without knowing the circumstances surrounding the case, the subsequent interpretation will be conditioned only on the STR analysis assumptions and could lead to false conclusions [12]. Molecular studies of chimerism on different tissues should be considered in such cases because many mosaic cell lines could be found to be chimeric if adequately tested. Autopsy specimens could be useful to check for chimerism in tissues other than blood.

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