HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Case Report

Rh E^{w} antigen in a multi-transfused patient with sickle cell disease

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Introduction

The Rh blood system is the second in clinical importance in transfusions and gestations[1]; however, it is the most complex with more than 50 antigens already having been described.[2] Of the antigens, D, C, c, E and e, whose alloantibodies react at 37 °C and cause hemolysis, are responsible by the vast majority of intercurrences in transfusions and gestations.[3] The E antigen is expressed by the *RHCE* gene and differs from the e antigen by a single nucleotide polymorphism (SNP) (676C>G; Pro226Ala).[4]

The E^{w} antigen, a rare variant of the E antigen, was described for the first time in 1955.[5] According to some reports in the literature, it is only found in Caucasians with a frequency of less than 0.1%.[5–9] A hemolytic disease of the newborn (HDN) caused by anti-E^{w} maternal antibodies was reported in two of these reports[5,7]; in another, an anti-E alloantibody was found in an E^{w} positive patient, after receiving E positive red blood cells (RBCs).[6] Although the antigen has been described only in Caucasians (and at a very low frequency), in this study, we found the phenotype with a molecular basis in a patient of mixed ethnicity (neither White nor Black) and sickle cell disease (SCD), highlighting the high level of miscegenation in Brazil.

Case report

We report on the case of a 30-year-old male patient with A RhD+ blood type and SCD and a history of more than 100 packed RBC transfusions at the Hemocentro Regional de Uberaba/Fundação Hemominas. He was of mixed ethnicity (White and Black) as were his ancestors (parents and grandparents). In 2009, his results of RBC alloantibody screening were positive; however, the antibody (present as a low titer) was not identified. In the same year, his erythrocyte phenotyping was performed by the Immunohematology Center of the Fundação Hemominas (Belo Horizonte, Brazil) and defined as Rh [C-c+ (Cw−); E+ (weak positive) e+]; K−; Fy (a+b+); Jk (a+b+); S-s+.

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Table 1 – Primer sequences used in polymerase chain reaction-restriction fragment length polymorphism for *RHCE*E/e* genotyping.

Primer	Primer sequence (5′→3′)
CEI4 (sense)	GGCAACAGAGCAAGAGTCCA
CEX5 (antisense)	CTGATCTTCCTTTGGGGGTG

In 2014, after he gave his consent, a new blood sample was collected, with the RBCs submitted to a repeat phenotyping test using the gel centrifugation technique and the ID-DiaClon Rh-Subgroups+K card (Diamed-BioRad Latin America, Lagoa Santa, Minas Gerais, Brazil), with monoclonal antibodies,[8] while the leucocytes had the DNA extracted using the Flexigene kit (Qiagen, Hilden, Germany). In both tests, the manufacture's recommendations were followed. The DNA was submitted to RBC genotyping by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[10] and BeadChip *wRHCE2.1* (Bioarray Solutions, Immucor, Warren, NJ, USA), also following the manufacture's recommendations.

PCR was performed with 50–100 ng of DNA, 0.25 μM of each primer, 0.25 mM of each dNTP, 2.5 mM of $MgCl_2$, 1.0 U Taq DNA polymerase and buffer 1×. PCR amplification was performed in a Veriti™ 96-well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The following conditions were used: denaturation at 95 °C for 15 min, followed by 35 cycles at 95 °C for 30 s, 62 °C for 30 s and 72 °C for 40 s, and a final extension step of 72 °C for 10 min. Amplified products were analyzed by electrophoresis in 1.5% agarose gel in Tris-Borate EDTA buffer (TBE). For enzyme digestion, the PCR product was incubated with *Mnl* I restriction enzyme overnight at 37 °C according to the manufacture's recommendation. All reagents used in the PCR reactions were obtained from Applied Biosystems, Foster City, CA, USA. The RFLP analysis was performed after electrophoresis in 3.5% agarose gel in TBE. Primer sequences used in the PCR are described in Table 1.

As one result of phenotyping (Rh(E-e+)) and genotyping (*RHCE*E/RHCE*e*) was discrepant, and he had not received transfusions of red blood cells in the 12 months preceding the last blood collection, his DNA sample was submitted to a sequencing of the *RHCE* gene, in order to identify the cause of the discrepancy.

All 10 exons of *RHCE* were sequenced. Amplification was performed with *RHCE*-specific primers designed to target flanking intronic regions (7) and sequencing analysis was performed in a 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Primer sequences and annealing temperature used are described in Table 2.

Three polymorphisms were identified by sequencing (361A>T, 380C>T and 383G>A) compatible with the *RHCE*cE.15.02/RHCE*ce* genotype (Figure 1). The *RHCE*cE.15.02* allele is a rare variant, leading to a weakened expression of the E antigen.

Table 2 – Sequences and annealing temperature of the primers used in the sequencing of the *RHCE* gene.

Exon	Primer sequence (5′→3′)	Annealing temperature
1 (S)	catagacaggccagcacag	55 °C
1 (AS)	cctgctatctgctcctgtga	
2 (S)	ctcgtccttctcgccatct	55 °C
2 (AS)	ggattccttgtgatacacggagta	
3 (S)	atcctggctctccttctca	55 °C
3 (AS)	caagtgatcttccctcctcaa	
4 (S)	tgaactttctccaaggaccat	55 °C
4 (AS)	aatttagcaaacactactcaaagaag	
5 (S)	tggagcaggagtgtgattct	55 °C
5 (AS)	gtgaccacccagcattctt	
6 (S)	agaggtggtttcaggatcag	55 °C
6 (AS)	agccaaagcagagagcatta	
7 (S)	ccattgatgtgagtacacatt	50 °C
7 (AS)	gtaggggctggacataatt	
8 (S)	agccagggagaggacccttg	60 °C
8 (AS)	gggaaggagatggggcaaatag	
9 (S)	aaggatttctgttgagacact	50 °C
9 (AS)	agcaagtcaacatatataccca	
10 (S)	cccagggaggtgcagtataa	56 °C
10 (AS)	gcgtttctcacgtacaaatgc	

(S): sense; (AS): antisense.

Discussion

In the present case, although the patient was genotyped as *RHCE*E/RHCE*e*, his respective phenotype was defined as E+ (weak positive) and E negative in the first and the second phenotyping tests, respectively. This discrepancy was due to the presence of three polymorphisms (361A>T, 380C>T and 383G>A) identified in the *RHCE* gene sequencing, responsible for the E^w antigen. A different molecular basis had already been identified: 500T>A; Met167Lys.[6,9] As already cited, there is a report of anti-E alloimmunization in a E^w positive patient after receiving E positive erythrocytes.[6] It is thus advisable to transfuse only E negative RBCs in these individuals.

Our results clearly reinforce the importance of erythrocyte phenotyping and genotyping as complementary tests in the transfusion routine of multi-transfused patients or candidates for chronic transfusions of RBCs, as in some cases, the detection of a certain allele by one molecular technique does not necessarily mean its complete or partial expression. In these situations, DNA sequencing is paramount, including in other cases where RBC phenotype and genotype discrepancies cannot be solved by molecular methodologies currently used in the routine and the patient has no history of recent transfusions.

Lastly, it is important to highlight the singularity of this case, considering that the phenotype has been detected in about 1:1000 Caucasians, with no reports in other ethnicities; however, here it was identified in a patient with sickle cell disease, which originates from the Black population. As the patient was mixed and reported that his parents and grandparents had the same ethnicity, this shows the high level of miscegenation of the Brazilian population.

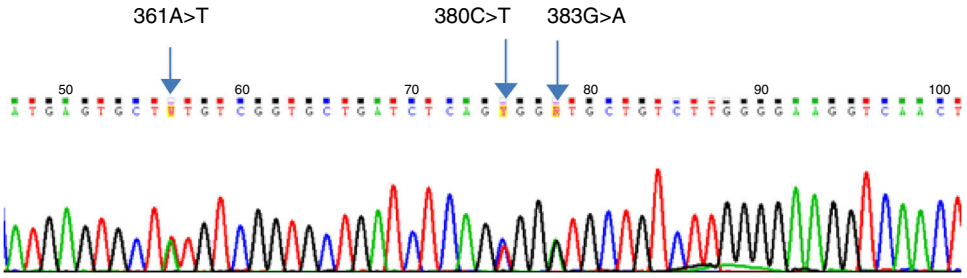

Figure 1 – Result of the *RHCE* gene sequencing, with three polymorphisms compatible with the *RHCE*cE.15.02/RHCE*ce* genotype.

Conflicts of interest

The authors declare no conflicts of interest.

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