Variant E Alleles (EIV) as a Cause of E Typing Discrepancies

Helene DePalma, Sunitha Vege, Christine Lomas-Francis, Randall W. Velliquette, Stella Chou, Connie M. Westhoff

Laboratory of Immunohematology and Genomics, New York Blood Center, New York

Division of Hematology, Children’s Hospital of Philadelphia, Philadelphia

INTRODUCTION

• Two homologous genes, RHD and RHCE, encode, respectively, the RhD and RhCE proteins.1
• Numerous variants of RH have been described, however RhE variants are uncommon.
• These variants are often discovered only after a serologic discordance between a historical and current type or when an E+ person makes alloanti-E.
• Four categories of E variants (Ei to EIV) have been described.2,3

CASE STUDY

We investigated eight samples:
• four donors whose RBCs historically tested E+ but typed E– on the current donation when tested on an automated instrument.
• one trial study sample reported to have variable E typing.
• three samples from patients with sickle cell disease (SCD) referred for routine RH genotyping.
• Ethnicity was available for seven of the eight samples: two were Caucasians (Cauc) and five were African American (AA).

MATERIALS AND METHODS

Serology testing
• RBC testing was performed by standard tube agglutination with commercial reagents according to manufacturer’s instructions.
• The clones used for the anti-E reagent formulations are shown in Table 1.

DNA testing
• Genomic DNA was isolated from WBCs from peripheral blood and used for genomic testing (manual PCR and automated assay).
• RHCE BeadChip prototype assay (BioArray/Immucor) was performed according to manufacturer’s instructions.

RESULTS: Serology

• RBCs from the four donors (samples 1-4) were non-reactive with anti-E formulated from clone 906 used on an automated instrument in routine use in blood centers.
• In tube testing, the donor RBCs demonstrated variable reactivity with four commercial anti-E reagents (Table 1).

RESULTS: Genomics

• DNA testing identified an exon 4 c.602G>C change, reported as RHCE*EIV, encoding amino acid change Arg201Thr.
• The RHCE*EIV allele is associated with weakened E antigen expression.

TABLE 1. REACTIVITY OF DONOR RBCS WITH COMMERCIAL ANTI-E REAGENTS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clone 906</th>
<th>Immucor Gammaclone (GAMA400)</th>
<th>Ortho BioClone (C2)</th>
<th>Bio-Rad Seraclone (MS260/MS12)</th>
<th>Quotient ALBAclone (DEM1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Donor</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td>2 Donor</td>
<td>0</td>
<td>W+</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td>3 Donor</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td>4 Donor</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

RESULTS: Genomics

• We report eight samples with the EIV variant antigen.
• RHCE*EIV was originally reported in Caucasians; here, we found the allele in five individuals of African descent.
• This study highlights:
  • Anti-E reagents formulated from different clones show variable reactivity with EIV RBCs.
  • E expression is not detected with the anti-E (formulated from clone 906) on the automated platform commonly used to type donors.
  • This poses a potential risk to E+ patients who may inadvertently receive such units.

REFERENCES