This update of the Cromer (CROM) blood group system (Storry JR, Reid ME, Yazer MH. The Cromer blood group system: a review. *Immunohematology* 2010;26:109–17) includes additional variants to the Cromer system (ISBT021), both new antigens and new molecular bases underlying the null phenotype. The molecule on which the Cromer blood group antigens are carried, CD55 (DAF), is an important receptor for the malaria parasite, *Plasmodium falciparum*, and the role of CD55 in health and disease continues to expand. *Immunohematology* 2021;37:118–121. DOI: 10.21307/immunohematology-2021-017.

**Key Words:** Cromer, CROM, CD55

The Cromer blood group system antigens are carried on CD55 (also known as DAF), a molecule that continues to demonstrate increasing polymorphism. CD55 is an important regulatory protein within the complement cascade and has increasingly been shown to play a role in hemostasis. CD55 is one of a few blood group–carrying proteins that are attached to the red blood cell (RBC) membrane through a glycosylphosphatidylinositol (GPI) linkage, and thus the Cromer “null” (IFC−, Inab) phenotype arises not only from variation in the CD55 coding sequence but also as a consequence of disease, such as paroxysmal nocturnal hemoglobinuria (PNH).

One of the fascinating observations regarding the nucleotide variation giving rise to Cromer blood group system antigens is that many of them have been identified in a unique folk group. With the major advances in genome analysis worldwide, we can now see that our serologic discoveries have specifically captured this variation long before it was seen as a regional marker.

**New Cromer Blood Group Antigens**

Since the publication of the original review, the Cromer blood group system has continued to expand, and a further five high-prevalence antigens have been described (Table 1).

An antibody reactive with all RBCs except for the patient’s own and IFC− RBCs was identified in the plasma of an Australian woman. Molecular analysis of CD55 revealed homozygosity for c.389G>A, encoding a change p.Arg130His in the second complement control protein (CCP) domain. The antigen was named CROZ (CROM16) after the proband’s Australian heritage. The antigen CRUE (CROM17) was assigned after a classic serologic investigation of an antibody in the plasma of a Thai woman. The patient’s RBCs reacted somewhat more weakly with antisera to other Cromer antigens, and molecular investigation revealed that she was heterozygous for two novel CD55 alleles. On one allele, substitution of c.650T>G, encoding p.Leu217Trp, in CCP3 was identified. On the other allele, mutation of c.639G>A introduced a novel stop codon, p.Trp213Ter.

CRAG (CROM18) was described following the identification of a Cromer-related antibody in the plasma of an elderly Greek woman. DNA analysis revealed homozygosity for c.173A>G, which encodes an amino acid substitution of p.Asp58Gly carried by CCP1.

The CROK antigen (CROM19) is somewhat of a conundrum. The antibody that led to the discovery of this antigen was originally defined as anti-IFC, that is, it reacted with all RBCs except for those lacking CD55 (IFC− or Inab phenotype), but it reacted only weakly with WES(a+b−) RBCs. The patient’s RBCs were negative with all available antibodies to Cromer antigens. The weaker reactivity with WES(a+b−) RBCs could be explained in part by the molecular basis, which showed that the proband (of Druze origin) was homozygous for CD55 c.245T>C, encoding a change p.Leu82Pro. The WESb polymorphism is defined by c.245T (p.Leu82), and the substitution c.245T>G changes p.Leu82Arg and creates WESA (Table 2). Based on the serologic reactivity, which showed that substitution of p.Leu82 per se was insufficient for compatibility, it was concluded that p.Leu82 encoded both for WESb and for a novel high-prevalence antigen, which was named CROK. Although no expression of CD55 was observed by flow cytometry testing on the RBCs of the patient, using two different anti-CD55 clones (JS11KSC2.3, NaM16-4D3), adsorption and elution studies with anti-Drα and anti-IFC showed that the proband did express some Cromer antigens/DAF protein. The same mutation was identified in her sister, her daughter, and her son.

A long-standing serologic mystery was solved by whole exome sequencing on DNA samples from a 103-year-old...
Cromer system update

Corsican woman and her son. Analysis revealed homozygosity in the patient’s CD55 for c.713G>A that encoded p.Gly238Glu in CCP4. DNA from her son showed heterozygosity for the nucleotide variant. Her antibody, first investigated in 2002, was subsequently shown to be compatible with IFC− RBCs, and the antigen defined by her antibody was named CORS (CROM20).

The Cromer Null Phenotype

Three new silenced CD55 alleles have been described in patients who have made anti-IFC (Table 3).

Anti-IFC in a young Moroccan woman with a history of miscarriages led to the identification of homozygosity for a single nucleotide insertion c.366_367insA in CD55, resulting in a frameshift and premature transcription termination.

Another case of anti-IFC was identified in a young Kashmiri woman following the birth of her first child. She had a history of two spontaneous abortions, although the underlying
cause was unknown. Her serologic picture was complicated, however, because her RBCs not only lacked CD55 but were also Yt(a−) and MER2−. DNA analysis revealed homozygosity for two novel nucleotide changes, c.147G>A (silent), and c.148G>T, predicted to encode p.Glu50Stop.

The third Cromer null allele to have been described since the original review was identified in the CRUE− proposita described earlier, who was heterozygous for an allele carrying the mutation c.639C>A that introduced a novel stop codon: p.Trp213Ter.

Clinical Significance of Antibodies

In all of the cases described herein, there had been an immunizing event—pregnancy, transfusion, or both—that was the likely cause of antibody production. However, the clinical significance of the antibodies, with regard to transfusion where it had occurred, was unremarkable enough not to be commented on in the reports. It has been well documented that antibodies to Cromer blood group system antigens decrease in titer over the duration of a pregnancy, and this finding has been attributed to adsorption by the placenta. Complement regulators including CD55 are expressed early on the syncytiotrophoblast and are thought to be important in protecting the developing fetus from complement-mediated attack. Furthermore, it has been shown that spontaneous abortion or early termination occurs in almost half of women with PNH, who lack the GPI-linked complement regulators, CD55 and CD59 (reviewed in Regal et al.17).

The malarial parasite Plasmodium falciparum, an enemy of the RBC, has been shown to use a number of different RBC membrane components to attach to and invade the RBC. Egan et al.18 have shown recently that CD55 is also an important ligand for P. falciparum invasion both in laboratory strains of the parasite and in different clinical isolates. In experiments using RBCs from two genetically characterized, IFC− (Inab phenotype) individuals, no invasion could be observed with the RBCs of one person, and invasion was considerably inhibited with the RBCs of the other, suggesting that CD55 was critical.

CD55 and Disease

Of interest, while reviewing the literature, was the fact that the two young women in whom new null alleles were identified had both suffered from two or more miscarriages.9,13 Although a comprehensive review of the role of CD55 in pregnancy is beyond the scope of this blood group system update, significantly low expression levels of CD55 mRNA were identified in women suffering from spontaneous abortion in one study.16 Complement regulators including CD55 are expressed early on the syncytiotrophoblast and are thought to be important in protecting the developing fetus from complement-mediated attack. Furthermore, it has been shown that spontaneous abortion or early termination occurs in almost half of women with PNH, who lack the GPI-linked complement regulators, CD55 and CD59 (reviewed in Regal et al.17).

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In the 2010 Immunohematology review, the authors listed 11 individuals confirmed or suspected to lack CD55, either genetically or transiently.1 Of these, four individuals were reported to have gastrointestinal disorders, including protein-losing enteropathy, and in a fifth individual, capillary angioma was reported. A recent study by Ozen et al.19 looked at 11 patients with an early-onset protein-losing enteropathy that was apparently autosomal-recessively inherited. Whole exome sequencing identified homozygosity for five different CD55 nonsense mutations in these families (Table 3) and conclusively showed that CD55 deficiency was the underlying cause of a

### Table 3. Molecular bases for the new CD55<sup>nu</sup> (IFC−, Inab phenotype) alleles

<table>
<thead>
<tr>
<th>ISBT allele name</th>
<th>Nucleotide change</th>
<th>Exon</th>
<th>Amino acid change</th>
<th>Reference</th>
<th>rs number&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Null phenotype identified in</th>
<th>Occurrence in gnomAD&lt;sup‡&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>CROM*01N.04</td>
<td>c.366_367insA</td>
<td>3</td>
<td>p.Thr123Asnfs*6</td>
<td>8</td>
<td>No rs</td>
<td>Moroccans</td>
<td></td>
</tr>
<tr>
<td>CROM*01N.05</td>
<td>c.148G&gt;T</td>
<td>2</td>
<td>p.Glu50Ter</td>
<td>13</td>
<td>rs773074921</td>
<td>Pakistanis</td>
<td>3/30,616 alleles in South Asian population only</td>
</tr>
<tr>
<td>CROM*01N.06</td>
<td>c.639G&gt;A</td>
<td>5</td>
<td>p.Trp213Ter</td>
<td>9</td>
<td>rs1391706310</td>
<td>Thais</td>
<td>3/18,392 alleles in East Asian population only</td>
</tr>
<tr>
<td>Not assigned</td>
<td>c.149_150delIAInsCCTT</td>
<td>2</td>
<td>p.Glu50Alafs*12</td>
<td>19</td>
<td>rs1135402916</td>
<td>Turks</td>
<td>No data</td>
</tr>
<tr>
<td></td>
<td>c.109delG</td>
<td>2</td>
<td>p.Gly37Alafs*24</td>
<td></td>
<td>rs1135402915</td>
<td>Turks, Syrians</td>
<td>No data</td>
</tr>
<tr>
<td></td>
<td>c.800G&gt;C</td>
<td>6</td>
<td>p.Cys267Ser</td>
<td></td>
<td>rs1135402917</td>
<td>Turks</td>
<td>No data</td>
</tr>
<tr>
<td></td>
<td>c.287-1G&gt;A</td>
<td>3</td>
<td>Exon skipping</td>
<td></td>
<td>rs1135402918</td>
<td>Turks</td>
<td>No data</td>
</tr>
</tbody>
</table>

ISBT = International Society of Blood Transfusion.

†Reference single nucleotide polymorphism cluster identification number.

‡Genome Aggregation Database.
severe clinical syndrome they called the CHAPLE syndrome (CD55 deficiency with Hyperactivation of complement, Angiopathic thrombosis, and Protein-Losing Enteropathy). The syndrome is much more complex than the originally reported protein-losing enteropathy, and thrombosis both in capillaries and major vessels is not uncommon. In response to the report of Ozen et al., another group commented that some patients with CHAPLE syndrome also suffered from predisposition to glomerular injury, all of which points to the important role of CD55 in the maintenance of complement homeostasis.

References