The Cromer blood group system: an update

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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,[1] 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.[8]

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.[9] 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.[10]

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 WES^b polymorphism is defined by c.245T (p.Leu82), and the substitution c.245T>G changes p.Leu82Arg and creates WES^a (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 WES^b and for a novel high-prevalence antigen, which was named CROK.[11] 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-Dr^a 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

Table 1. High-prevalence antigens of the Cromer blood group system: molecular basis and distribution of the antigen-negative phenotype

Number	Name	Nucleotide change	Exon	Amino acid change	Reference	rs number*	Negative phenotype identified in	gnomAD† frequency of the variant allele (≥0.01%)
CROM1	Cr[a]	c.679G>C	6	p.Ala227Pro	2	rs60822373	African Americans	African 2%
CROM5	Dr[a]	c.596C>T	5	p.Ser199Leu	3	rs1135402914	Uzbekistani Jewish, Japanese	None
CROM6	Es[a]	c.239T>A	2	p.Ile80Asn	4	rs776347919	Mexicans, African Americans	None
CROM10	UMC	c.749C>T	6	p.Thr250Met	5	rs566298946	Japanese	East Asian 0.1%
CROM11	GUTI	c.719G>A	6	p.Arg240His	6	rs199705465	Native Chileans (5.3%)	Finn 0.02%
CROM12	SERF	c.647C>T	5	p.Pro216Leu	7	rs144692928	Thais	South Asian 0.1% East Asian 0.02% African 0.018%
CROM13	ZENA	c.726T>G	6	p.His242Gln	7	rs769586650	Turkish Syrians	None
CROM14	CROV	c.466G>A	3	p.Glu156Lys	7	No rs	Croatians	Data not available
CROM15	CRAM	c.740A>G	6	p.Gln247Arg	7	No rs	Somalians	Data not available
CROM16	CROZ	c.389G>A	3	p.Arg130His	8	rs756646491	Australians	None
CROM17	CRUE	c.650T>G	5	p.Leu217Trp	9	rs567156112	Thais	East Asian 0.1% (Vietnamese 1%)
CROM18	CRAG	c.173A>G	2	p.Asp58Gly	10	No rs	Greeks	Data not available
CROM19	CROK	c. 245T>C	2	p.Leu82Pro	11	No rs	Israeli Druze	Data not available
CROM20	CORS	c.713G>A	6	p.Gly238Glu	12	No rs	French Corsicans	Data not available

*Reference single nucleotide polymorphism cluster identification number.
†Genome Aggregation Database.

Table 2. Antithetical antigens in the Cromer blood group system: molecular basis and distribution of the low-prevalence antigen

High-prevalence antigen number	Name	Low-prevalence antigen number	Name	Nucleotide change	Exon	Amino acid change	Reference	rs number*	Low-prevalence antigen identified in	gnomAD† frequency of the low-prevalence allele (≥0.01%)
CROM2	Tc[a]	CROM3	Tc[b]	c.155G>T	2	p.Arg52Leu	2	rs28371588	African Americans	African 2.6% Latin American 0.1%
		CROM4	Tc[c]	c.155G>C		p.Arg52Pro	4		Europeans	European 0.03%
CROM9	WES[b]	CROM8	WES[a]	c.245T>G	2	p.Leu82Arg	4	rs147474393	African Americans, Finns	African 0.3% Finn 0.4%

*Reference single nucleotide polymorphism cluster identification number.
†Genome Aggregation Database.

Corsican woman and her son.[12] 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.[8]

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

Table 3. Molecular bases for the new $CD55_{null}$ (IFC–, Inab phenotype) alleles

ISBT allele name	Nucleotide change	Exon	Amino acid change	Reference	rs number†	Null phenotype identified in	Occurrence in gnomAD‡
*CROM*01N.04*	c.366_367insA	3	p.Thr123Asnfs*6	8 19	No rs	Moroccans	
*CROM*01N.05*	c.148G>T	2	p.Glu50Ter	13	rs773074921	Pakistanis	3/30,616 alleles in South Asian population only
*CROM*01N.06*	c.639G>A	5	p.Trp213Ter	9	rs1391706310	Thais	3/18,392 alleles in East Asian population only
Not assigned	c.149_150delAAinsCCTT	2	p.Glu50Alafs*12	19	rs1135402916	Turks	No data
	c.109delG	2	p.Gly37Alafs*24		rs1135402915	Turks, Syrians	No data
	c.800G>C	6	p.Cys267Ser		rs1135402917	Turks	No data
	c.287-1G>A	3	Exon skipping		rs1135402918	Turks	No data

ISBT = International Society of Blood Transfusion.
†Reference single nucleotide polymorphism cluster identification number.
‡Genome Aggregation Database.

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.[13]

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.[9]

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.[14,15]

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]).

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.

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

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.,[19] another group commented that some patients with CHAPLE syndrome also suffered from predisposition to glomerular injury,[20] all of which points to the important role of CD55 in the maintenance of complement homeostasis.

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