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ORIGINAL ARTICLE

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Identification of a novel high-prevalence red blood cell antigen on junctional adhesion molecule-A defines a new blood group system

Tosti J. Mankelow[1,2] | Louise A. Tilley[1] | Vanja Karamatic Crew[1] | Benjamin Jones[1] | Marina Izak[3] | Liora Muncher[3] | Vered Yahalom[4,5] | Nicole M. Thornton[1]

[1]International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, UK

[2]Component Development Laboratory, NHS Blood and Transplant, Bristol, UK

[3]Magen David Adom – National Blood Services Center, Ramla, Israel

[4]Blood Services and Apheresis Institute, Rabin Medical Center, Petach Tiqva, Israel

[5]Gray Faculty of Medical and Health Sciences, Tel Aviv University, Tel Aviv, Israel

Correspondence
Nicole M. Thornton, International Blood Group Reference Laboratory, NHS Blood and Transplant, Northway, Filton, Bristol BS34 7QH, UK.
Email: nicole.thornton@nhsbt.nhs.uk

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Abstract

Background and Objectives: Availability of new technologies has accelerated the pace of blood group discovery, with nine of 48 known systems being discovered in the past 5 years alone, and yet uncharacterized antigens remain. This study involved investigations of samples from a female patient presenting with a moderate strength pan-reactive antibody to a high-prevalence antigen present on all red blood cells (RBCs) tested other than her own and her brother's.

Materials and Methods: Samples underwent extensive serological testing; however, the antibody specificity could not be assigned to any known blood group antigens. Therefore, whole exome sequencing was performed alongside antigen expression studies to identify the gene variant responsible for this rare phenotype.

Results: Both individuals were found to carry a homozygous variant c.644G>A (rs755819660; gnomAD allele frequency 0.0000044) in exon 6 of the *F11R* gene, encoding junctional adhesion molecule-A (JAM-A). This variant encodes p.Arg215Gln located on the second extracellular immunoglobulin domain of JAM-A. Expression of JAM-A was demonstrated on all RBCs tested by flow cytometry, with cells of both the patient and her brother showing lower expression than control cells. Control (Arg215) and variant (Gln215) forms of JAM-A were expressed as both soluble Fc fusion proteins and on Chinese hamster ovary cells. In both cases, mouse monoclonal

Tosti J. Mankelow, Louise A. Tilley and Vanja Karamatic Crew shared first authorship.

anti-JAM-A recognized both forms, whilst the patient antibody only reacted with the Arg-215-JAM-A control.

Conclusion: These data demonstrate that JAM-A carries a new high-prevalence RBC antigen, lacking in the patient and her brother, thereby defining a novel blood group system encoded by *F11R*.

Keywords

blood group system, F11R, JAM-A, red blood cell antigen

Highlights

- We show that p.Arg215 in junctional adhesion molecule-A (JAM-A) defines a new high-prevalence red cell antigen, JIMI (JAMA1).
- Homozygosity for c.644G>A in *F11R*, encoding p.Arg215Gln, results in lack of this antigen and immunized individuals can produce an antibody that binds both red cells and platelets.
- Our findings establish a new blood group system, JAMA (International Society of Blood Transfusion system no. 49).

INTRODUCTION

Currently there are 398 recognized blood group antigens, of which 371 have known genetic backgrounds and are categorized into 48 blood group systems [1]. Historically, antigens could only be discovered using serology, but the development of modern molecular biological techniques, particularly next generation sequencing (NGS), has greatly increased the pace of novel blood group antigen and system discovery [2–6]. Despite this greater understanding of blood group antigens and their genetics, some patients still present with antibodies to unrecognized antigens outside of the known blood group systems, requiring extensive serological and molecular investigations for resolution. There are many more proteins known to be expressed on the red blood cell (RBC) plasma membrane than there are blood group systems, suggesting that there may still be much more to be discovered.

Junctional adhesion molecule-A (JAM-A), also known as JAM-1, is encoded by the *F11R* gene [7]. JAM-A is a member of the immunoglobulin (Ig) superfamily and is widely expressed on epithelial and endothelial cells, platelets, RBCs and other haematopoietic cells [8]. The protein consists of two extracellular Ig superfamily domains, an N-terminal V-set Ig domain (D1) and a C-terminal I-set Ig domain (D2), alongside a single transmembrane domain and a 39 amino-acid cytoplasmic domain [9]. JAM-A is a well-characterized adhesion molecule, whose extracellular domain can bind to itself in a *cis* (two JAM-A molecules on the same cell to form a dimer) or *trans* (two JAM-A molecules on different but adjacent cells) homophilic interaction, alongside a heterophilic interaction with the $\alpha_L\beta_2$ integrin [10–15]. The binding sites for both *cis* ($R_{59}V_{60}E_{61}$) and *trans* ($N_{43}N_{44}P_{45}$) homophilic interactions have been defined within JAM-A domain 1 [11, 12] whilst integrin $\alpha_L\beta_2$ is known to bind domain 2 [15, 16]. JAM-A is also known to interact laterally within the same membrane with various other integrin and tetraspanin proteins [17–19].

JAM-A has a well-established role as an adhesion molecule and is involved in signalling and scaffolding in tight junctions in epithelial and endothelial cells [20, 21]. In haematological cells, JAM-A is primarily involved with platelet aggregation and adhesion [22]. JAM-A negatively regulates $\alpha_{IIb}\beta_3$ integrin via its interactions with Csk kinase, keeping the integrin in the inactive form [23]. During activation, signalling events phosphorylate the cytoplasmic domain of JAM-A, disrupting its interaction with Csk kinase in turn allowing $\alpha_{IIb}\beta_3$ integrin activation [23]. JAM-A has also previously been shown to be present on the surface of RBCs [8] but, like other adhesion molecules; intercellular adhesion molecule 4 (ICAM-4), basal cell adhesion molecule (BCAM) and CD44, its function in erythrocytes is currently unknown.

The aim of this study was to characterize an antibody to an unidentified high-prevalence RBC antigen lacking in a patient and her brother. We present serological, genetic and protein expression as evidence to establish a new blood group system encoded by *F11R*, comprising a single high-prevalence antigen carried on JAM-A.

MATERIALS AND METHODS

Samples and ethics

Blood samples were procured, and the study conducted according to NHS Blood and Transplant (NHSBT) Research and Development governance requirements and ethical standards, in accordance with the Declaration of Helsinki. Blood samples from a pregnant patient of Arab ethnicity (with no known history of transfusion) and her brother were originally investigated in 2013 due to the presence of a suspected antibody to a high-prevalence antigen in her plasma. Further samples from the patient and her brother were investigated in 2020 during a subsequent pregnancy. Both the patient and her brother were reported to be healthy with normal RBC parameters (haemoglobin concentration, mean cell volume, mean cell haemoglobin and RBC distribution width) and platelet parameters (platelet count and mean platelet volume). Blood samples from voluntary NHSBT donors, who

consented to the use of their blood for research purposes, were used as controls.

Serology

Standard agglutination techniques were used for assessment of antibody reactivity and RBC phenotyping [24]. For the indirect antiglobulin test (IAT), a low-ionic strength saline (LISS) tube method was used, with polyspecific anti-human globulin (Millipore) as the secondary antibody for detecting human primary antibodies (in-house International Blood Group Reference Laboratory reference collection). Eluates were prepared using Gamma ELU-KIT™ II elution kit (Immucor). Agglutination was scored on a scale of 0 (negative) to 5+ (strongest positive). For individual enzymes or chemical treatments, one volume of washed packed RBCs was incubated with two volumes of papain (NHSBT Reagents) for 3 min, four volumes of trypsin (Sigma), pronase (Sigma) or 0.2 dithiothreitol (DTT; NHSBT Reagents) for 30 min, chymotrypsin (Sigma) for 60 min, according to manufacturer's instructions. Following all treatment incubations, RBCs were washed at least four times with phosphate-buffered saline (PBS) until the wash supernatant was clear.

Next generation sequencing

Genomic DNA was extracted using a commercial isolation kit (QIAamp DNA blood mini kit; Qiagen). Library preparation, indexing and target enrichment for whole-exome sequencing was carried out using Illumina DNA prep with exome enrichment. Paired-end (2 × 87 cycle) single-plex sequencing was carried out on a MiSeq (Illumina). Secondary data analysis, including alignment of reads against human reference genome hg19, was performed using MiSeq Reporter v2.5.1 and variants called and annotated using BaseSpace Variant Interpreter (Illumina). Selected alignments were further visualized using Integrative Genomics Viewer (IGV v2.17) [25].

Site directed mutagenesis, Fc fusion expression and serological inhibition, and Chinese hamster ovary (CHO) cell expression

PCR derived site-directed mutagenesis of JAM-A complementary DNA (cDNA) was performed as previously described [26]. Either Gln215 or Arg215 JAM-A cDNA was inserted into both pTEV and pcDNA3.1 vectors. Human embryonic kidney (HEK) 293T cells were transfected with pTEV using Polyethylenimine Max (Polysciences) for transient expression of the two extracellular Ig domains of JAM-A as Fc fusion proteins. JAM-A Fc was purified 5 days post-transfection from culture supernatant on protein A-Sepharose and concentration was determined by enzyme-linked immunosorbent assay against a known standard curve of murine IgG (IgG2a). For serological inhibition assays, 10 µL of JAM-A Fc fusion protein (at 1 mg/mL) (or 10 µL PBS) was mixed with 90 µL plasma (0.1 mg/mL final concentration) and incubated for 30 min at room temperature (RT) before testing.

Stable transfections of Chinese hamster ovary (CHO) cells were made with pcDNA3.1 using polyethylenimine max and cellular clones with highest membrane expression of JAM-A were detected by flow cytometry.

Flow cytometry and JAM-A Fc inhibition

Flow cytometry tests were performed using 0.25 µL packed RBCs or 3×10^5 CHO cells per test. Incubations were for 1 h, with shaking at RT followed by three washes, all performed in PBS 0.1% bovine serum albumin (BSA). Then, 15 µL CSTEM27 mouse anti-JAM-A (Fisher) at 1 µg/mL, murine IgG2a isotype control at 1 µg/mL, 5 µL patient plasma, 5 µL AB plasma and 10 µL eluate were used per test as was either 15 µL 40 µg/mL goat anti-mouse IgG or goat anti-human IgG PE conjugate secondary (Stratech). After resuspension in PBS 0.1% BSA, flow cytometry was performed on a Navios (Beckman Coulter). Data analysis was performed using Kaluza software (Beckman Coulter). For blocking with soluble Fc proteins, 0.5 µL 15 µg/mL CSTEM27 was mixed with 0.5 µL 1 mg/mL Fc proteins (0.5 mg/mL final concentration) or 0.5 µL PBS and incubated for 30 min at RT before adding to cells suspended in 14 µL PBS 0.1% BSA as the primary antibody. Alternatively, 4.5 µL patient plasma was mixed with 0.5 µL 4 mg/mL Fc proteins (0.4 mg/mL final concentration) or 0.5 µL PBS and incubated for 30 min at RT before adding to cells as the primary antibody.

Platelet antibody adsorption and elution

Platelets were washed in PBS and 300 µL packed platelets were added to 600 µL plasma, mixed and incubated at 37°C for 1 h. Following centrifugation for 5 min at 2438*g*, the supernatant was removed and incubated with fresh washed platelets twice more. The antibody was eluted from platelets using Gamma ELU-KIT™ II (Immucor) in accordance with manufacturer's instructions (for RBC), except centrifugation steps were 3 min at 2790*g*.

RESULTS

Serology

On initial referral, the patient's plasma was found to contain antibodies to a high-prevalence antigen, reacting moderate strength by LISS IAT with all untreated and papain-treated cells, except her own and her brother's cells. Antibody reactivity was still observed when tested against cells treated with trypsin, pronase and DTT, but not with chymotrypsin-treated cells. An active eluate was made and found to be incompatible with a large selection of null phenotype cells. The patient's and her brother's cells were tested with an extensive selection of rare antisera to high-prevalence antigens, but all were reactive.

Despite in-depth serological investigations, the specificity of the antibody remained unknown. Further samples received in 2020, during a new pregnancy, presented with similar results and the antibody specificity was still serologically unresolved.

NGS and JAM-A amino-acid substitution modelling

To identify the gene responsible for the rare phenotype, whole exome sequencing of the patient's and her brother's DNA was performed. No variants in known blood group genes were found to explain the phenotype. Sequencing data were filtered to identify shared rare (<0.01) homozygous coding variants, predicted to encode surface-exposed changes in putative erythrocyte-expressed membrane proteins, which could explain the observed lack of a high-prevalence RBC antigen. Only one variant fit these stringent criteria, with both individuals found to carry the same homozygous variant c.644G>A (rs755819660; gnomAD global allele frequency 0.0000044) in exon 6 of the *F11R* gene, encoding JAM-A. This protein is known to be expressed on the erythrocyte plasma membrane, and the observed variant encodes a p.Arg215Gln substitution, predicted to be externally exposed, providing a highly plausible candidate for further investigation. In silico analysis of an X-ray crystal structure, Protein Data Bank reference 1NBQ [9] shows Arg215 to be situated in the second extracellular Ig domain of JAM-A, with the R chain pointing out into the aqueous environment with the substitution to Gln being conservative with little structural alteration (Figure 1). The apparent conservative nature of this change is supported by other prediction tools, including POLYPHEN-2 [27] (benign; 0.021), SIFT [28] (tolerated; 0.76) and AlphaMissense [29] (likely benign; 0.112).

JAM-A expression on RBC

Flow cytometry with mouse monoclonal anti-JAM-A was used to investigate JAM-A expression on control RBCs ($n = 8$) and those of the patient and her brother (Figure 2). All RBCs tested were positive (Figure 2a), with consistent expression levels across controls (six fresh and two frozen samples). However, JAM-A expression was lower in both the patient and her brother, with the patient having the lowest expression of all samples tested (Figure 2b). Reactivity of control RBCs with patient plasma was comparable to that seen with mouse anti-JAM-A, but patient plasma did not react with patient's own RBCs (Figure 2c), or her brother's (Figure 2d).

Soluble JAM-A expression and inhibition assays

As the two extracellular domains of JAM-A are Ig domains, it was possible to express both Arg215 and Gln215 forms as soluble Fc fusion proteins for inhibition assays with the patient plasma [30]. When tested serologically, Arg215-JAM-A Fc blocked agglutination of control RBCs by the patient plasma, but Gln215-JAM-A Fc did not (Figure 3a), indicating that the patient antibody selectively binds JAM-A Arg215. No serologically active commercial anti-JAM-A antibodies were available; therefore, to show that both soluble versions of JAM-A were biologically active, they were tested in a flow cytometry inhibition assay against monoclonal mouse anti-JAM-A. Mouse anti-JAM-A or patient plasma was incubated individually with either Arg215 or Gln215-JAM-A Fc prior to flow cytometric analysis. Again, Arg215-JAM-A Fc inhibited binding of the antibody within the patient plasma (Figure 3b-iii) although it required a higher concentration of the protein than when tested serologically. No inhibition of patient

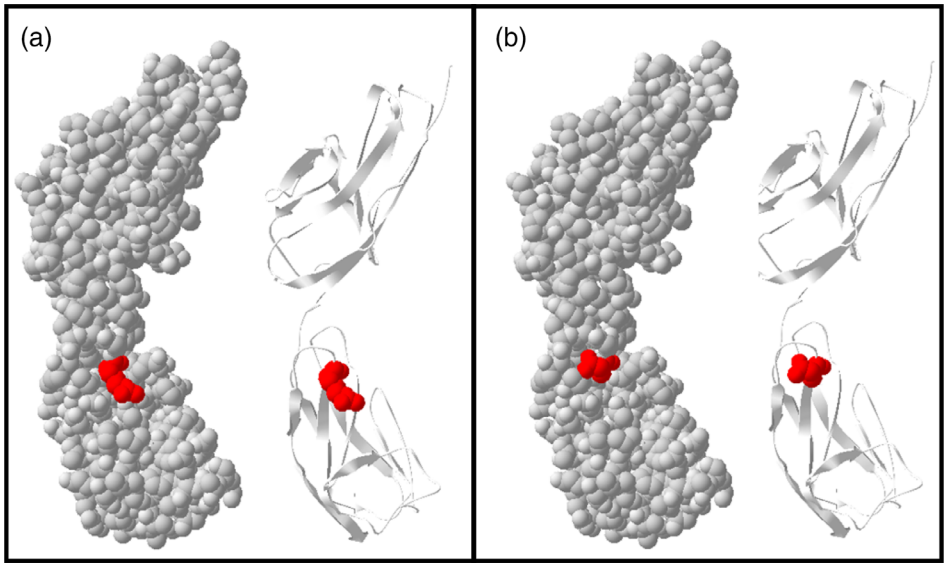

FIGURE 1 p.Arg215Gln substitution visualized on the crystal structure of junctional adhesion molecule-A (JAM-A). Protein Data Bank reference 1NBQ [9] was used to visualize the JAM-A p.Arg215Gln substitution. Arg215 is surface exposed (a); the substitution to Gln215 (b) is conservative, with the R group in both cases extending into the aqueous environment.

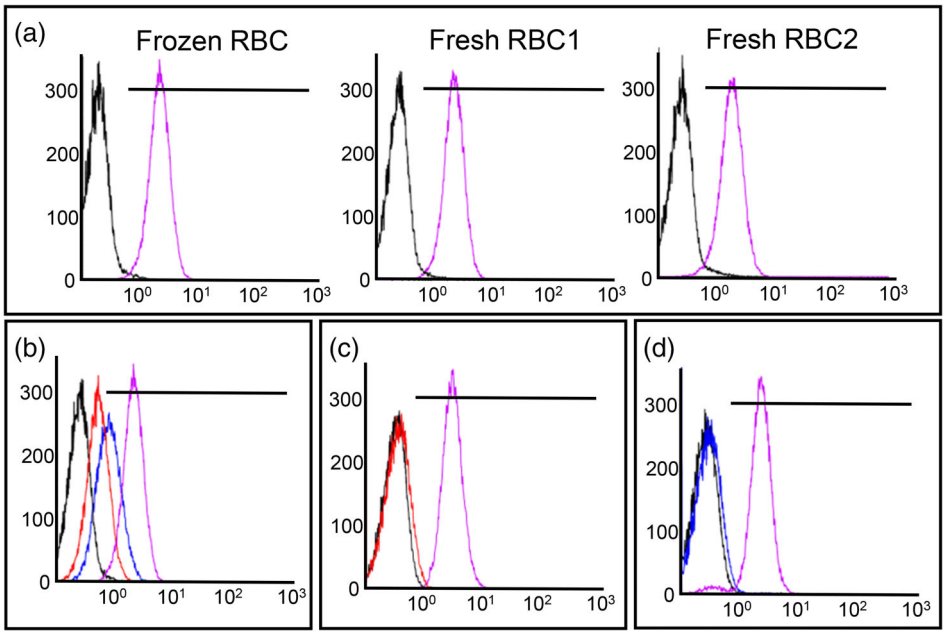

FIGURE 2 Junctional adhesion molecule-A (JAM-A) expression measured by flow cytometry on the patient, her brother and control red blood cells (RBC) with mouse anti-JAM-A and patient plasma. (a) When incubated with mouse anti-JAM-A, all control RBCs tested (purple; $n = 8$) were positive with similar expression levels observed. Three representative examples shown (one frozen, two fresh RBC). (b) Mouse anti-JAM-A also positively labelled patient RBCs (red) and those of the patient's brother (blue), although lower expression was observed in both patient and her brother as compared to control RBCs (purple). (c and d) When incubated with patient plasma, control RBCs (purple) were positive, as compared to negative reactions with (c) patient RBCs (red) or (d) the patient's brother (blue). In all cases negative control: isotype mouse immunoglobulin G (IgG2a) control (a and b) or AB plasma (c and d) shown in black. Positive gate indicated by horizontal black line.

plasma binding was seen with Gln215-JAM-A Fc (Figure 3b-iv); however, both JAM-A Fc proteins inhibited binding of mouse anti-JAM-A (Figure 3c-iii,iv). These results show that both JAM-A Fc proteins are biologically active, and that the patient's antibody specifically binds Arg215-JAM-A but not Gln215-JAM-A.

JAM-A expression in CHO cells

JAM-A is widely expressed in epithelial, endothelial and haematopoietic cells, and on a wide range of human cell-lines [8]. Historically, most JAM-A expression studies have been performed using the JAM-A negative CHO cell-line [15, 16, 31, 32], so CHO cells were stably transfected with full-length JAM-A constructs encoding either Arg215 or Gln215. CHO cells transfected with Arg215-JAM-A or Gln215-JAM-A were both positive when tested by flow cytometry with mouse anti-JAM-A, but native CHO cells were negative, showing that both clonal cell-lines express JAM-A (Figure 4a). Testing with patient eluate demonstrated antibody binding only to Arg215-JAM-A CHO cells, but not to Gln215-JAM-A or native CHO cells (Figure 4b). This corroborates the findings of the antibody inhibition studies with JAM-A Fc fusion proteins, providing clear evidence that the patient's antibody binds to Arg215-JAM-A, characterizing a new high-prevalence blood group antigen.

Adsorption of patient antibody with platelets

As JAM-A is expressed on platelets, the antibody in the patient's plasma could potentially bind platelets as well as RBC. When incubated with control platelets, the antibody was successfully adsorbed from patient plasma (Figure 5a-i,ii). When eluted from platelets, the antibody was able to agglutinate control RBC but not those of the patient (Figure 5a-iii,iv). In contrast, when tested in an identical manner, no adsorption of anti-D from plasma was observed, as RhD protein is not expressed on platelets (Figure 5b). These data clearly demonstrate that the anti-JAM-A in the patient's plasma specifically binds JAM-A on both RBC and platelets.

DISCUSSION

Here we report characterization of a new high-prevalence antigen defined by c.644G in *F11R* encoding p.Arg215 in the JAM-A protein. We demonstrate the absence of this antigen in a patient and her brother, caused by homozygosity for *F11R* c.644G>A (p.Arg215Gln). The patient's antibody, formed during pregnancy, is directed to Arg215-JAM-A. Our evidence indicates JAM-A represents a new blood group system, currently comprising a single antigen of high prevalence.

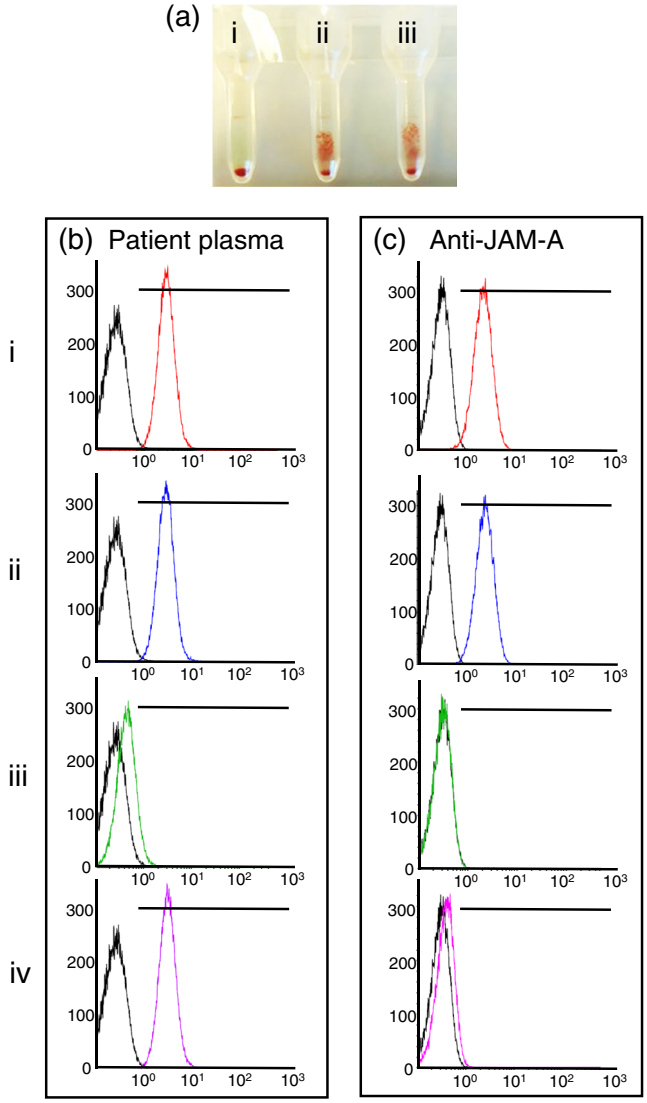

FIGURE 3 Arg215 and Gln215-junctional adhesion molecule-A (JAM-A) soluble Fc fusion proteins in serological and flow cytometry inhibition assays. (a-i) Arg215-JAM-A Fc inhibited the patient antibody, preventing agglutination of control red blood cells (RBCs), but (a-ii) Gln215-JAM-A Fc or (a-iii) Lutheran Fc (control) did not inhibit. Patient's plasma (b) or mouse anti-JAM-A (c) was incubated with PBS (i, red), Lutheran control (ii, blue), Arg215 (iii, green) or Gln215 (iv, purple) Fc fusion protein before being used to test RBCs in a flow cytometry assay. Negative control (secondary antibody only) shown in black in all cases. Mouse anti-JAM-A binding was inhibited with both Arg215 and Gln215 Fc fusion proteins (c-iii and c-iv), but not with PBS or Lutheran Fc controls (c-i and c-ii). Reactivity of RBCs with patient plasma was inhibited by Arg215 Fc (b-iii) but not with Gln215 (b-iv) or PBS or Lutheran Fc controls (b-i and b-ii). Positive gate indicated by horizontal black line in all plots.

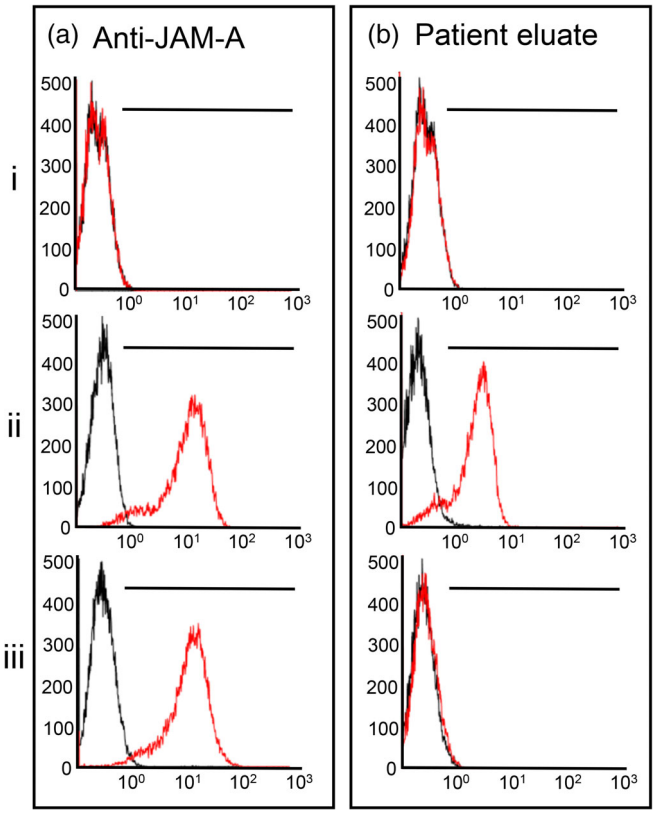

FIGURE 4 Flow cytometry on Chinese hamster ovary (CHO) cell junctional adhesion molecule-A (JAM-A) transfectants. Flow cytometry was performed on native CHO cells alongside CHO cells transfected with either Arg215-JAM-A or Gln215-JAM-A. (a) Mouse anti-JAM-A (red) does not react with (i) untransfected CHO cells but reacts with both CHO cells transfected with Arg215-JAM-A (ii) or Gln215-JAM-A (iii). (b) An eluate made from patient's plasma (red) reacts only with CHO cells transfected with Arg215-JAM-A (ii) but not with native CHO cells (i) or those transfected with Gln215-JAM-A (iii). Negative control (secondary antibody only) shown in black in all panels. Positive gate indicated by horizontal black line.

Although JAM-A is known to be expressed on RBC membranes, its function on RBCs or erythroid precursor cells is unknown. JAM-A may play a similar role as hypothesized for other adhesion molecules on RBCs, such as BCAM and ICAM-4 [33, 34], in stabilizing erythroblastic islands during erythropoiesis [35]. JAM-A is known to regulate the function of the ICAM-4 ligand and integrin $\alpha_{IIb}\beta_3$ [23, 36], and also directly binds integrin $\alpha_L\beta_2$ [15, 16]. Both these integrins are expressed on erythroid precursors [37], suggesting a possible adhesive and regulatory role for JAM-A within the erythroblastic island. The p.Arg215Gln substitution reported here is within domain 2 of JAM-A and will have no effect on its *cis* and *trans* homophilic interactions, which are mediated by domain 1 [11, 12]. Although the $\alpha_L\beta_2$ binding site on domain 2 of JAM-A has yet to be identified [15, 16], adhesion is known to be affected by glycosylation at Asn185 [38], located at the opposite end of domain 2 to Arg215 [9]. In contrast to JAM-A knockout mice, which exhibit thrombotic and other dysfunctions [39, 40], no associated pathology is reported in either the patient or her brother. Both exhibited routine RBC and platelet haematological parameters falling within normal range, suggesting no significant impairment of JAM-A function. Therefore, we hypothesize that any function for JAM-A, particularly within the erythroid lineage, is unaffected by the p.Arg215Gln substitution. Nevertheless, although predicted to be a conservative substitution, p.Arg215Gln does appear to

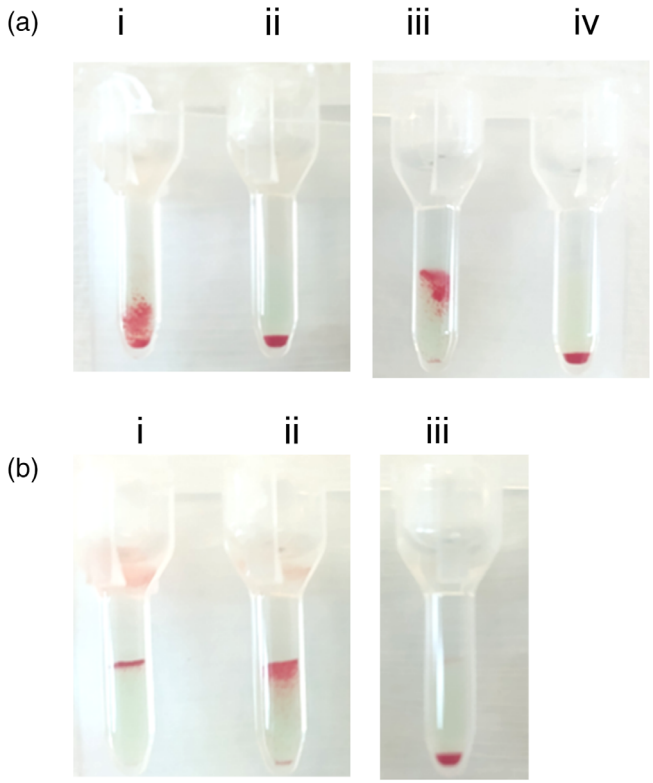

FIGURE 5 Platelet adsorption (and elution) of patient antibody. (a) Native patient plasma agglutinated control red blood cells (RBCs) (i), however, no agglutination was observed with plasma following incubation with platelets (ii) showing the antibody had been successfully adsorbed. The antibody subsequently eluted from platelets agglutinated control RBCs (iii) but not the patient's own cells (iv). (b) Plasma containing anti-D agglutinated D+ RBCs (i) but anti-D was not adsorbed by incubation with platelets (agglutination still observed) (ii). No anti-D was present in the eluate from the platelets, which did not agglutinate D+ RBCs (iii).

influence JAM-A expression level on mature RBCs, as indicated by the lower expression observed in the patient and her brother by flow cytometry (Figure 2b). Interestingly, Arg215 and Gln215-JAM-A expressed equally in both CHO and HEK cells (data not shown), suggesting the observed differences in JAM-A RBC expression is most likely to occur through membrane remodelling during erythroid terminal differentiation rather than during protein synthesis.

Although widely expressed on haematopoietic cells, JAM-A is primarily described in platelets [22, 29]. The identification of an RBC antigen on its surface therefore follows other recent discoveries of new blood group active proteins predominantly known for expression in other haematological cells: CD36 on platelets [41], MAL on leucocytes [3] and choline transporter-like protein 2 (CTL2) on granulocytes [2, 5]. This is unsurprising, as all haematological cells have a common precursor and thus express common proteins, albeit often at different expression levels. With advancing blood group identification techniques, it is likely that more overlap between antigens expressed on RBCs and other haematological cells will be found. However, it is important to note that antibody–antigen binding can differ depending on the cell type. For example, although Cs^a antigen on RBCs and human neutrophil antigen 3a (HNA3a) on neutrophils are defined by the same amino acid in CTL2, anti-HNA3a does not bind RBCs. However, anti-Cs^a binds RBCs and neutrophils, and both antibodies bind CTL2 on T-lymphocytes [2]. CTL2 is a multiple transmembrane spanning protein that has a relatively small extracellular domain when compared to the two extracellular Ig-domains of the single-pass membrane protein JAM-A. Therefore, it is likely that cellular size and shape, alongside interference from other membrane proteins, would affect JAM-A antigen presentation less than CTL2. This is supported by the observation that the anti-Arg215 JAM-A detected in this study clearly binds to JAM-A on both RBC and platelets (Figure 5). Any clinical significance of the anti-JAM-A antibody remains unclear; there was no evidence of haemolytic disease of the fetus and newborn (four live births from six pregnancies) and neither the patient nor her brother has any known history of transfusion. However, this, and any future anti-JAM-A antibodies, will have to be considered for their effects on both RBC and platelets. In general, when blood group systems are expressed on two or more haemopoietic cell types, clinical transfusion practice will need to consider the wider implications of any antibody present. Although the antibody may be elucidated from RBC investigations, it may also have clinical implications in fetal and neonatal alloimmune thrombocytopenia or in platelet or granulocyte transfusion.

In summary, this study provided the evidence to establish the 49th blood group system recognised by the International Society of Blood Transfusion, JAMA (ISBT049), currently comprising a single high-prevalence antigen, JIMI (JAMA1). This discovery, together with the 12 other new blood group systems ratified over the past decade, highlights the power of NGS and other new technologies in blood group exploration, and leaves us with the question of how much more there may still be to discover.

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T.J.M., V.K.C., L.A.T. and N.M.T. conceived, designed, coordinated the study and wrote the manuscript. B.J. performed serology experiments and platelet adsorption. L.A.T. and V.K.C. performed whole-exome sequencing, Sanger sequencing and data analysis. T.J.M. performed expression and flow cytometry experiments. M.I., L.M. and V.Y. performed initial serological study on the patient. All authors reviewed the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Genbank at https://www.ncbi.nlm.nih.gov/genbank/, reference number PZ337576.

ORCID

Tosti J. Mankelow https://orcid.org/0000-0003-3434-1973
Louise A. Tilley https://orcid.org/0000-0001-8244-4331
Vanja Karamatic Crew https://orcid.org/0000-0003-1412-9107
Nicole M. Thornton https://orcid.org/0000-0002-8635-0997

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