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Genetic variants within the erythroid transcription factor, KLF1, and reduction of the expression of Lutheran and other blood group antigens: review of the In(Lu) phenotype

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Key Words

Kruppel-like factor 1, KLF1

Inhibitor of Lutheran, In(Lu)

Serology

Blood group genomics

Abbreviations

BCAM: Basal Cell Adhesion Molecule (carries Lutheran blood group system antigens)

CDA: Congenital Dyserythropoietic Anaemia

CD44: CD44 antigen glycoprotein (carries Indian blood group system antigens)

GATA1: GATA Binding Protein 1

IN: Indian blood group system

In(Lu): Inhibitor of Lutheran (phenotype)

ISBT: International Society of Blood Transfusion

KLF1: Erythroid-specific Krüppel-like factor 1

KN: Knops blood group system

LU: Lutheran blood group system

LW: Landsteiner-Wiener blood group system

RBC: Red Blood Cell

Abstract

Erythroid-specific Krüppel-like factor 1, or KLF1, is an integral transcriptional activator for erythropoiesis. Genetic variants within *KLF1* can result in a range of erythropoietic clinical phenotypes from benign to significant. The In(Lu) phenotype refers to changes in the quantitative expression of blood group associated red cell surface molecules due to *KLF1* variants which are otherwise clinically benign.

These clinically benign *KLF1* variants are associated with a reduced expression of one or more red cell membrane proteins/carbohydrates that carry blood group antigens for the LU (Lutheran), IN (Indian), P1PK, LW (Landsteiner-Wiener), KN (Knops), OK, RAPH and I blood group systems. This is of significance during routine serologic blood typing when expression falls below the test sensitivity and therefore impacts on the ability to accurately detect the presence of affected blood group antigens. This is of clinical importance since the transfusion requirements for individuals with the In(Lu) phenotype differ from those of individuals that have a true Lu_{null} phenotype. With this review we summarise the current body of knowledge with regard to the In(Lu) phenotype and associated *KLF1* variants. Our review also highlights discordant reports and provides insights for future research and management strategies.

Serological heterogeneity in blood group expression of In(Lu) individuals has been shown, but studies are limited by the low prevalence of the phenotype, and therefore the small numbers of samples. They are further limited by availability and inconsistent application of serological reagents and varying tes algorithms. With the advent of genome sequence-based testing, an increasing list of In(Lu) associated *KLF1* variants are being revealed. The spectrum of effects on blood group expression due to these variants warrants further attention and a consistent methodological approach of studies in larger cohorts is required.

We propose a recently reported testing framework of standardised serological studies, flow cytometry and variant analysis be adopted; and that the international databases be curated to document *KLF1* variability and the resultant In(Lu) red cell blood group expression. This will provide better classification of *KLF1* variants affecting blood group expression and allow for phenotype prediction from genotype, accurate typing of In(Lu) individuals, and better transfusion management of related challenging transfusion scenarios.

Introducing erythroid-specific transcription factor, KLF1

Cellular differentiation is a genetically driven process involving activation of an array of genes and pathways within each distinct tissue type. An integral transcriptional activator in the pathway of progenitor cells committing to erythropoiesis is erythroid-specific Krüppel-like factor 1, or KLF1. Initially referred to as EKLF, KLF1 was discovered from murine-based studies in 1993 [1] and our understanding of the significance for KLF1 in human erythropoiesis has progressed over the last 10 years; elucidated from serologic, genomic and transcriptomic studies [2, 3].

In humans, the KLF1 protein is a product of the *KLF1* gene, it being located on chromosome 19 and composed of three exons and two introns [4]. The translated 362 amino acid protein contains two transactivation domains at the N-terminal of the protein and three zinc fingers at the C-terminal [3] . These highly conserved zinc fingers come into contact with DNA at 9bp motifs which conform to the degenerate motif, CCMCRMCCN; where M=C or A, R=A or G and N=any base [1]. KLF1 thereby regulates the activity of over 650 erythropoietic genes [5]. It is therefore unsurprising that genetic variants within *KLF1* can result in a range of erythropoietic phenotypes; from clinically insignificant morphological changes up to clinically significant and pathologic conditions.

The frequency of *KLF1* variants can range from 0.0004% to 37%, varying in different global geographical locations [6-8]. This includes common and rare variants, with or without a clinically significant consequence. *KLF1* genetic variants have been stratified from class 1 to 4 according to the clinical phenotype which is related to the variant type, its location within the gene and the consequence on the transcribed protein [3]. Class 1 variants are mostly neutral polymorphisms. An example being the p.Ser102Pro variant which occur at a frequency of 37% in the Genome Aggregation Database (gnomAD) and is known to not impact on blood group antigen expression [8, 9]. Class 2 variants are the most interesting in terms of unpredictable phenotypic impact. They are missense variants in the DNA-binding domain and result in protein variants with reduced or sometimes altered DNA-binding affinities. However, these tend to be less phenotypically severe than loss of function variants, i.e. class 3 where stop codon or frameshift variants result in truncated proteins that lack the DNA-binding domain. Class 4 is reserved exclusively for an alteration of a highly conserved residue (p.Glu325Lys) in the DNA-binding domain which causes dominant and pathogenically severe Congenital dyserythropoietic anaemia (CDA) type IV [3]. *KLF1* variants in classes 2 to 4 are not compatible with life at a homozygous level and are therefore detected at a heterozygous level in individuals, as reflected in gnomAD [8]. We note one interesting reported case of severe neonatal anemia with kernicterus due to compound heterozygosity for class 3 variants [10].

The primary focus of this review will be the *KLF1* class 2 and 3 variants which are not known to be associated with pathogenic clinical manifestations at a heterozygous level. Although otherwise considered clinically benign, these variants do still cause reduced expression of one or more red cell membrane proteins that carry blood group antigens to give what is now called the In(Lu) phenotype (see next section). This impacts on the ability to accurately detect the presence of affected blood group antigens by routine serologic blood typing, as expression falls below the test sensitivity. This review focuses primarily on those variants that have been described in blood group screening studies, performed on the blood of healthy blood donors. We summarise historical findings attributed to *KLF1* variants affecting blood group antigens, which reveal a possible spectrum of phenotypes even within the class 2 and 3 *KLF1* variants.

The In(Lu) phenotype and clinical significance

The classic example for the impact of the class 2 and 3 *KLF1* variants is for the Lutheran system (LU), designated ISBT 005 [9]. The Lutheran blood group system is known to comprise at least 25 blood group antigens which are carried on two isoforms of the red cell membrane glycoprotein which is termed Basal cell adhesion molecule (BCAM). Lutheran antigens show variable antigenic strength, even within families and even between red cells of an individual [11, 12].

Polymorphisms in the *BCAM* gene define the LU blood group antigens, e.g. the primary antithetical antigens Lu^a and Lu^b. Homozygous inactivating variants result in the recessive Lu_{null} phenotype, Lu(a–b–) [11, 13]. In contrast, class 2 and 3 *KLF1* variants cause an apparent dominantly inherited Lu(a–b–) phenotype where BCAM expression is reduced to such an extent that LU antigens are difficult to detect by agglutination methods. Other than KLF1, GATA Binding Protein 1 (GATA1) is another transcription factor that has been implicated in an apparent Lu(a–b–) phenotype. In this case, a hemizygous variant in the X-linked *GATA1* gene leads to serological characteristics of both the recessive Lu(a–b–) and In(Lu) phenotypes [14, 15]. Determining the cause of apparent loss of LU antigens is beneficial for transfusion management.

For accurate blood group typing, it is important to determine whether red cells that are presenting with an apparent phenotype of Lu(a–b–) are a true Lu_{null} phenotype. Although very rare, Lu_{null} individuals have been detected by their development of anti-Lu3, thereby requiring transfusion of antigen-negative red cells [13]. In contrast, it is not necessary to transfuse patients with the In(Lu) phenotype with Lu_{null} red cells, as such patients are not at risk of being alloimmunised if transfused with matching Lutheran typed red cells. Given that Lu_{null} red cells are rare, this ensures that such

donations can be reserved for patients with true Lu_{null} phenotypes. Paradoxically, there is evidence to suggest that red cells with the In(Lu) phenotype can be provided to patients with the Lu_{null} phenotype as the weakened antigen expression reduces the risk of alloimmunisation in the non-matched recipient [16].

Red cells with the In(Lu) phenotype are also of particular importance during transfusion management of individuals that have developed anti-AnWj antibodies (AnWj antigen discussed in subsequent section on phenotypic effect of In(Lu)). For these individuals, providing AnWj-negative red cells for transfusion is challenging and the physiological responses in such patients are variable: some individuals suffer no adverse effects when transfused incompatible units [17, 18], while others have been affected by severe red cell haemolysis [19]. As AnWj-negative red cells are extremely rare, In(Lu) blood has been safely substituted to meet transfusion requirements [19].

History of In(Lu) discovery

It was the apparent loss of expression of both the Lu^a and Lu^b in the Lutheran blood group system that resulted in the first study of the In(Lu) phenotype. This occurred in 1961, when immunohematologist Dr Mary N. Crawford serendipitously discovered that her own red cells appeared to phenotype as Lu(a–b–). This apparent phenotype was also detected across three generations of her family and Dr Crawford gave the first description of a dominant inheritance for this apparent Lu(a–b–) phenotype [20] . Further testing of these serologically Lu(a–b–) red cells by laborious adsorption and elution techniques demonstrated that these cells actually do express the Lu^a and/or Lu^b antigens, albeit very weakly, therefore raising the suggestion of an inhibition to their full expression. Thereby, this characteristic marked reduction, but not loss, in Lutheran antigens, was termed the In(Lu) phenotype, an abbreviation of Inhibitor of Lutheran [21]. While investigating the original Lu(a–b–) phenotype, Dr Crawford also identified suppression of the P1 (P1PK blood group system) antigen [22]. Over the next 11 years, similar observations were identified in other families displaying comparable Lu(a–b–) inheritance and serological patterns [23, 24].

Other phenotypic effects of In(Lu)

From further findings in the early 1980s, it was accepted that corresponding reduction of another red cell antigen occurred alongside the In(Lu) phenotype. This antigen was studied under various names including p80 (based on its size), A3D8 antigen (based on monoclonal antibody reactivity) and Pgp-1 (phagocytic glycoprotein-1) [14]. Convergence of knowledge now has this glycoprotein structure known as CD44, which carries the Indian (IN) blood group system [14, 15]. Historical

serological evidence indicates that CD44 expression is similar between the In(Lu) members of the same family but dissimilar compared to other families [25]. The combination of serologic Lu(a–b–) and reduced CD44 is a hallmark of the In(Lu) phenotype and is consistent for KLF1 class 2 and 3 variants [2, 26-28]. This insight has allowed for the use of anti-CD44 antibodies in mass-screening exercises to identify potential In(Lu) individuals [26, 29-33].

Historic serology studies also established that the In(Lu) phenotype is further characterized by reduced expression of the high frequency antigen called AnWj, thought to be located on the CD44 glycoprotein. AnWj is also decreased on In(Lu) cells [18] and it has been known since 1991 that the genetic locus for this antigen is not linked to the LU blood group system [34]. The AnWj-negative phenotype has recently been linked to a missense mutation in the *SMYD1* gene based on genetic and statistical evidence, however, functional proof is still required. The SMYD1 protein is proposed to act as a transcription factor during early erythropoiesis but the underlying mechanism leading to the loss of AnWj remains unresolved [35].

Other than the characteristic blood group profiles described above, historic serology studies provide evidence that red cells with the In(Lu) phenotype may also have reduced or altered expression of other blood group antigens in the P1PK, LW (Landsteiner-Wiener), KN (Knops), OK, RAPH and I systems (Table 1) . For the P1PK system, the A4GALT transferase produces both P1 and Pk antigens [36], but only P1 is impacted to variable degrees [22, 27, 32, 37]. This suggests that KLF1 may not directly target *A4GALT* but may act on a transferase that produces Lactotriaosylceramide or Paragloboside which are precursors of the P1 antigen in the paragloboside series [22, 38, 39].

For the LW system, the impact of *KLF1* variants on LW antigen expression has not only been demonstrated in In(Lu) RBCs [28, 40], but also appears to be reflected in CDA where an associated LW(a–b–) phenotype is caused by the single class 4 KLF1 variant, p.Glu325Lys [41].

The findings reported for the KN Blood Group system are ambiguous and may not only be caused by the specific underlying *KLF1* variants, but also genetic differences in *CR1* such as the H/L haplotypes associated with high and low CR1 copy numbers [12, 28, 42]. For the OK system, our recent study of In(Lu) in Australian blood donors suggests that the *BSG* transcription rate changes observed in cultured In(Lu) erythroblasts in the ground-breaking 2008 Singleton study (see next section) may also be observable on mature erythrocytes [2, 28]. For the RAPH system, findings from a family study in the 1980's suggested that the In(Lu) phenotype slightly decrease the expression of the MER2 antigen [43]. Finally for the I system, serological investigations in the 1970's and 80's reported reduced levels of the i antigen on In(Lu) RBCs compared to normal adult cells [10, 31]; the latter

study also reported a slight associated increase in I antigen. The mechanism behind these observations is unclear, however the reduction of neighbouring structures associated with In(Lu) may allow the antibody better access to the antigen, thus producing an increased level of binding.

Despite these reports, studies have been thwarted by the low prevalence of In(Lu), small numbers of samples and variable application of serological reagents and/or testing algorithms, admittedly also due to the availability of reagents at that point in history.

The In(Lu) phenotype is linked to variants in *KLF1*

From Dr Crawford's first description of the In(Lu) phenotype, it was another 47 years until the causative gene locus was identified to be *KLF1* [2, 20]. In 2008, Singleton and colleagues achieved this by observing the transcriptome of cultured erythroblasts from four In(Lu) phenotype blood donors, comparing these to control erythroblasts. At incremental stages of maturation, differences in transcription rates were observed between the two groups, leading the team to hypothesize that a mutated erythroid transcription factor was the cause of this pattern of results. Sequencing performed on 24 archived In(Lu) phenotype samples revealed 21 of the 24 samples to have heterozygous variants in the *KLF1* gene. Nine different loss-of-function variants in *KLF1,* that results in reduced or abolished protein function, were described from these samples [2]. This confirmed that the serological phenotype is caused by erythropoietic suppression of antigen expression during red cell development, rather than by the action of a dominantly inherited inhibitor gene as previously hypothesized [2]. The study also identified that there is more than one *KLF1* variant that can result in an In(Lu) phenotype; but does not address any variability within the phenotype caused by each variant.

Population studies on the In(Lu) phenotype and *KLF1* variants

With the discovery that the transcription factor KLF1 impacts on blood group phenotypes, genotyping for *KLF1* variants became an important part of investigations into the In(Lu) phenotype. Population studies in this field have however been few in number.

The first retrospective study in 2012 was conducted on historic samples in the rare blood collection at the *French National Reference Centre for Blood Groups* and pioneered the use of flow cytometry analysis to confirm In(Lu) [26]. The group identified 10 causative *KLF1* variants, (seven novel) in 10 propositi samples. A more recent study in 2017 investigated the In(Lu) phenotype in American

patients and blood donors. Interestingly, this study included a stored sample from the Crawford index case and showed that this first In(Lu) phenotype arose from heterozygosity for the *KLF1*BGM06* allele where a nucleotide change NM_006563.3:c.954dupG predicts the p.Arg319Glufs*34 [27]. This study did not, however, follow suit in using flow cytometry to confirm In(Lu) but relied on serological testing by standard tube methods.

We recently conducted the first population screening within Australia and identified the In(Lu) phenotype in four out of 8036 Queensland blood donors (frequency = 1 in 2,009) [28]. These four samples, and two additional historical In(Lu) samples, were analysed further. Our approach included serological screening to identify negative or very weak Lu serology, sequencing to identify *KLF1* variants and comprehensive flow cytometric analysis of RBC proteins (n=14) and surface carbohydrates (n=6). Through this approach we identified five different *KLF1* variants (two novel) in the six samples.

As expected from such a rare phenotype, all of the above mentioned studies were limited by the number of In(Lu) samples included. Although each study identified several *KLF1* variants, analysis of the phenotypic impact of these variants were mostly limited to the LU, IN and P1PK systems in the retrospective studies. Even with the small number of In(Lu) samples analysed, Keller *et al* did note the variable impact of *KLF1* variants, consistent with the *KLF1* variant classes as defined by Perkins *et al* in 2016 [3, 27]. In our study of an Australian blood donor population, our testing algorithm allowed us to further identify variability even within the In(Lu) phenotype [28].

KLF1 variants listed with curated Blood Group Allele Tables

All reported *KLF1* variants known to impact on blood group antigen expression are registered on the allele nomenclature tables, curated and maintained by the International Society of Blood Transfusion (ISBT) working party for Red Cell Immunogenetics and Blood Group Terminology [9]. The current ISBT listing shows at least 65 *KLF1* variants that have been associated with altered blood antigen expression and these, as well as others which are under review, are summarised here in Table 2. Because KLF1 variants are so rare, only a few are represented in international databases such as gnomAD. The frequency and coding region location of these variants are presented in Figure 1.

The original study by Singleton *et al.* from the Bristol Institute for Transfusion Sciences defined the first nine allelic variants in the Table. These are designated *KLF1*BGM01 to 09*. The next variant listed on the Table was detected in individuals with the severe CDA type IV [25]. This is the NM_006563.3:c.973G>A change which defines the *KLF1*BGM10* allele and predicts a p.Glu325Lys substitution. Interestingly the individuals with this *KLF1*BGM10* allele may not have an altered Lutheran phenotype but do have reduced expression of CD44, AQP1 (Colton system) and ICAM4 [40, 41]. With the exception of *KLF1*BFM10*, all other listed variants are associated with the In(Lu) phenotype, having reduced expression of Lutheran blood group antigens.

Summary and conclusion

In summary, the historical serology evidence indicates that *KLF1* variants impact on the expression of molecules carrying red cell antigens other than just for Lutheran and Indian systems. Serological heterogeneity observed for these blood group systems lead us to hypothesise that the impact on red cell antigen expression may be dependent on the exact *KLF1* variant. More specifically that some blood group molecules (such as BCAM and CD44) will be impacted by most *KLF1* variants while other blood group molecules (such as CR1) may only be impacted by a particular *KLF1* variant.

The spectrum of effects on blood group expression due to these variants warrants further attention. With the advent of genomic sequence-based testing, studies into *KLF1* variants have already revealed an array of alleles and this list is expected to increase [9]. A consistent methodological approach of studies in larger cohorts is required to correlate these *KLF1* variants with particular phenotypical changes.

We propose the testing framework of standardised serological studies, flow cytometry and variant analysis adopted in Fraser *et al* [28], and that an international database be established/curated to document variability to enable accurate prediction of the resultant In(Lu) red cell blood group expression from genotype. This will provide better classification of *KLF1* variants affecting blood group expression, accurate typing of In(Lu) individuals, and better transfusion management of related challenging transfusion scenarios.

Figures

Figure 1. Comparison of the gnomAD allele frequencies for 18 In(Lu)-associated *KLF1* alleles.

Only 18 of the 65 In(Lu)-associated *KLF1* alleles currently listed by the ISBT have allele frequency data available on gnomAD. The allele frequencies for these 18 alleles are presented in the upper lollipop plot section in order of CDS position (LRG sequence: NG_013087.1). Class 2 and 3 variants (as defined by Perkins *et al*. 2016) are violet and turquoise lollipops, respectively. Allele names associated with each lollipop are shown as grey italic text. The lower horizontal barplot shows CDS regions that are encode KLF1 functional domains. TAD = transactivation domain; ZF = zinc finger.

Tables

Table 1: Summary of blood group systems, other than Lutheran (LU) and Indian (IN), reported to have altered antigen expression associated with the In(Lu) phenotype

ISBT number	Blood Group System	Gene Name	Gene Product	Gene Product Role in Antigen Presentation	Reported Impact of In(Lu)	Research Study(ies)
3	P1PK	*A4GALT*	Lactosylceramide 4-alpha-galactosyltransferase (A4GALT)	Enzyme that synthesizes P_1 and Pk antigens on paragloboside & lactosylceramide respectively.	↓ P_1 antigen	**Crawford *et al*., 1974 [22]** Serological investigation identified lack (suppression) of P_1 antigen in 43 samples with dominant Lu(a–b–) type; 52 non Lu(a–b–) samples had P_1/P_2 distribution expected in general population.
						Shaw *et al*., 1984 [37] Family studies where serological investigation found the P_1/P_2 distribution in 45 dominant Lu(a–b–) samples to be different from that of 34 non Lu(a–b–) samples. Family tree analysis shows suppression of P_1 in one family.
						Kawai *et al*., 2017 [32] Serological investigation and genotyping identified 29 In(Lu) samples with significantly weakened P_1 expression.
						Keller *et al*., 2018 [27] Serological investigation and genotyping for seven In(Lu) case studies identified weakened P_1 expression in one historical sample from 1961.
16	LW	*ICAM4*	Intracellular adhesion molecule 4 (ICAM4)	Single-pass RBC membrane glycoprotein carrying the LW antigens	↓ ICAM4 protein	**Singleton *et al*., 2011 [40]** Promotor binding experiments using recombinant forms of wild type and KLF1 mutants identified weakened expression of ICAM4.
						Fraser *et al*., 2018 [28] Flow cytometric analysis and genotyping identified reduced ICAM4 levels on the surface of In(Lu) RBCs

						from Australian blood donors.
22	KN	*CR1*	Complement receptor type 1 (CR1)	Single-pass RBC membrane glycoprotein carrying the KN antigens	↓ Kn^a, Kn^a-like, Sl^a, McC^a and Yk^a antigens	**Daniels *et al*., 1986 [42]** Antibody titration analysis for dominant Lu(a–b–) and non Lu(a–b–) samples in 12 families. Reduced expression of Kn^a, Kn^a-like, Sl^a and McC^a observed (to varying degrees) in all In(Lu) sample; reduced expression of Yk^a observed in most cases.
					No impact	**Moulds *et al*., 1999 [12]** Serological investigation, genotyping and chemiluminescence immunoblotting of nine In(Lu) samples showed normal expression of CR1. Results for two In(Lu) siblings suggests possible variation within the In(Lu) phenotype. Moulds speculates that observations made by Daniels study (1986) are due to genetic differences in *CR1* (possibly the H/L haplotypes) and prolonged storage of the RBCs. Moulds data however shows poor correlation between CR1 copy numbers and H/L haplotyping.
					↓ CR1 protein	**Fraser *et al.,* 2018 [28]** Flow cytometric analysis and genotyping identified variability in the level of CR1 on In(Lu) RBCs from different Australian blood donors. Those with decreased levels of CR1 harboured L-haplotype associated variants at a heterozygous level; however haplotype phasing was not possible.
24	OK	*BSG*	Basigin	Single-pass type I RBC membrane protein carrying the OK antigens	↓ expression of *BSG*	**Singleton *et al*., 2008 [2]** Transcriptional analysis of cultured normal and In(Lu) erythroblasts identified a -1.7 and -1.3 fold change at days six (pronormoblasts) and 11 (normoblasts) respectively.
					↓ Basigin protein	**Fraser *et al.,* 2018 [28]** Flow cytometric analysis and genotyping identified

						decreased levels of Basigin on In(Lu) RBCs from Australian blood donors; suggests that changes observed in erythroblast by Singleton study (2008) may also be observed on mature erythrocytes.
25	RAPH	*CD151*	CD151 antigen	Multi-pass RBC membrane glycoprotein carrying a single RAPH antigen	↓ MER2 antigen	**Garratty *et al.,* 1986 [43]** Serological investigation using two murine monoclonal anti-MER2 antibodies showed positive reactions for seven out of nine dominant Lu(a–b–) samples, i.e. strong expression of MER2 on RBCs for majority . Additional titration analysis for a large family in which In(Lu) was segregating found slightly reduced expression of MER2 for In(Lu) samples.
27	I	*GCNT2*	N-acetyllactosaminide beta-1,6-N-acetylglucosaminyl-transferase	Converts i antigen (dominant in cord blood/fetus) to I antigen (dominant in adults). These antigens are carbohydrate structures situated on the interior carbohydrate chains that present antigens for the ABO, H and LE systems.	↓ i antigen	**Crawford *et al*., 1974 [22]** Serological investigation using several anti-i sera found very strong reaction with cord cells, much weaker reaction with normal adult cells and Lu(a–b–) cells of the recessive type and negative/weak reaction with In(Lu) cells.
						Shaw *et al.,* 1984 [37] Study of 12 families where serological investigation using two selected anti-i sera found dramatic suppression of i antigen in all In(Lu) individuals whereas the I antigen was unaffected.

RBC: red blood cell

Table 2: *KLF1* variants that are presently known to cause the In(Lu) phenotype

Nucleotide Change†	Predicted Amino Acid Change	Allele Name¶	Reference	Variant Accession Number (dbSNP or ClinVar)
c.-124T>C	p.0	*KLF1*BGM01*	[2]	No submission
c.86A>G	p.Lys29Arg	*KLF1*BGM50*	[32]	No submission
c.109C>T	p.Gln37X	*KLF1*BGM39*	[32]	rs755193431
c.114delC	p.Asp38Glufs*53	*KLF1*BGM13*	[44]	No submission
c.151delC	p.Leu51SerfsX3	*KLF1*BGM44*	[32]	No submission
c.196G>T	p.Glu66X	*KLF1*BGM40*	[32]	No submission
c.199delA	p.Gly68AlafsTer236	Not on ISBT yet	[45]	No submission
c.204delC	p.Gly68GlyfsX169	*KLF1*BGM41*	[32]	No submission
c.262_284dup	p.Ala95AlafsX150	*KLF1*BGM51*	[32]	No submission
c.298G>T	p.Glu100Ter	*KLF1*BGM14*	[44]	No submission
c.304T>C, c.484insC	p.Ser102Pro, p.Lys162Glnfs*352	*KLF1*BGM15*	[44]	rs2072597 rs116107190
c.304T>C, c.1002del2	p.Ser102Pro, p.Thr334Glyfs*351	*KLF1*BGM16*	[44]	rs2072597 rs764608850
c.310_311insG	p.Ala104GlyfsX249	*KLF1*BGM32*	[26]	No submission
c.318T>G	p.Tyr106X	Not on ISBT yet	[27]	rs956697155
c.380T>A	p.Leu127Ter	*KLF1*BGM02*	[2]	No submission
c.421C>T	p.Arg141X	Not on ISBT yet	[28]	rs1426116895
c.472delG	p.Ala158ProfsX79	*KLF1*BGM52*	[32]	No submission
c.517_519delC	p.Pro173ProfsX	*KLF1*BGM23*	[32]	rs566095433
c.519_520insC	p.Gly174ArgfsX179	*KLF1*BGM33*	[26]	No submission
c.519_525dupCGGCGCC	p.Gly176ArgfsX179	*KLF1*BGM34*	[26, 46, 47]	rs483352838
c.533C>A	p.Ser178X	*KLF1*BGM53*	[32]	No submission
c.551_556GGA CCG>A	p.Gly184GlufsX167	*KLF1*BGM24*	[32]	No submission
c.569delC	p.Pro190Leufs*47	*KLF1*BGM03*	[2]	No submission
c.591C>G	p.Tyr197X	*KLF1*BGM35*	[26]	No submission
c.604G>A	p.Gly202Arg	Not on ISBT yet	[45]	rs756658473
c.621C>G	p.Tyr207Ter	*KLF1*BGM17*	[44]	No submission
c.637C>T	p.Glu213X	*KLF1*BGM25*	[32]	No submission
c.663delG	p.Leu222SerfsX15	*KLF1*BGM36*	[26]	No submission
c.796C>T	p.Arg266X	*KLF1*BGM42*	[32]	rs756046932
c.802C>T	p.Arg268X	*KLF1*BGM26*	[32]	No submission
c.809C>A	p.Ser270X	*KLF1*BGM20*	[48, 49]	rs558942739
c.826C>T	p.Gln276X	*KLF1*BGM45*	[32]	No submission
c.862A>G	p.Lys288Glu	*KLF1*BGM37*	[26]	No submission
c.868T>C	p.Tyr290His	*KLF1*BGM46*	[32]	No submission
c.874A>T	p.Lys292Ter	*KLF1*BGM04*	[2]	rs137852687
c.887T>C	p.Leu296Pro	*KLF1*BGM54*	[32]	rs754996390
c.892G>C	p.Ala298Pro	Not on ISBT yet	[49]	rs387907598

c.895C>T	p.His299Tyr	*KLF1*BGM05*	[2]	rs137852688
c.899T>C	p.Leu300Pro	*KLF1*BGM27*	[32]	rs763096758
c.902insT	p.Arg301LeufsX52	*KLF1*BGM28*	[32]	No submission
c.90G>A	p.Trp30Ter	*KLF1*BGM11*	[42]	rs1427628301
c.911C>A	p.Thr304Lys	Not on ISBT yet	[27]	No submission
c.914-1g>c	Splicing failure	*KLF1*BGM47*	[32]	No submission
c.939G>A	p.Trp313X	*KLF1*BGM55*	[32]	rs1364414353
c.947G>A	p.Cys316Tyr	*KLF1*BGM29*	[32]	No submission
c.948delC	p.Cys316Trpfs*326	*KLF1*BGM18*	[44]	No submission
c.954G>C	p.Trp318Cys	Not on ISBT yet	[28]	rs769526751
c.954dupG	p.Arg319Glufs*34	*KLF1*BGM06*	[2]	rs397514445
c.964C>A	p.Arg322Ser	*KLF1*BGM56*	[32]	rs376711350
c.968C>G	p.Ser323Trp	*KLF1*BGM30*	[26]	No submission
c.973G>A	p.Glu325Lys	*KLF1*BGM10*	[39]	rs267607201
c.977T>G	p.Leu326Arg	*KLF1*BGM21*	[26, 49]	rs397514634
c.983G>A	p.Arg328His	*KLF1*BGM08*	[2]	rs140252918
c.983G>T	p.Arg328Leu	*KLF1*BGM07*	[2]	No submission
c.991C>G	p.Arg331Gly	*KLF1*BGM09*	[2]	No submission
c.991C>T	p.Arg331Trp	*KLF1*BGM31*	[32, 50]	No submission
c.994A>G	p.Lys332Glu	*KLF1*BGM22*	[45]	No submission
c.1001C>T	p.Thr334Met	*KLF1*BGM48*	[32]	rs483352841
c.1004G>C	p.Gly335Ala	*KLF1*BGM57*	[32]	No submission
c.1012C>T	p.Pro338Ser	Not on ISBT yet	[51]	VCV000056892.1
c.1022G>A	p.Cys341Tyr	*KLF1*BGM43*	[32]	rs483352839
c.1040C>A, c.1045delT	p.Ala347Asp, p.Ser349Argfs*358	*KLF1*BGM19*	[44]	No submission
c.1048C>T	p.Arg350Cys	*KLF1*BGM49*	[32]	rs1321730417
c.1071C>A	p.His357Gln	*KLF1*BGM38*	[26]	VCV000040066.1

†The table is ordered by Nucleotide Change position (by first change position for multi-variant alleles). Nucleotide positions are relative to the LRG transcript sequence, NM_006563.3. ¶The ISBT allele tables are ordered by Allele Name, i.e. in order of discovery and reporting [9]. The *KLF1*BGM12* allele is also currently listed in the ISBT table but have been found not to be associated with the In(Lu) phenotype and this allele is now obsolete.

Declarations of interest

None.

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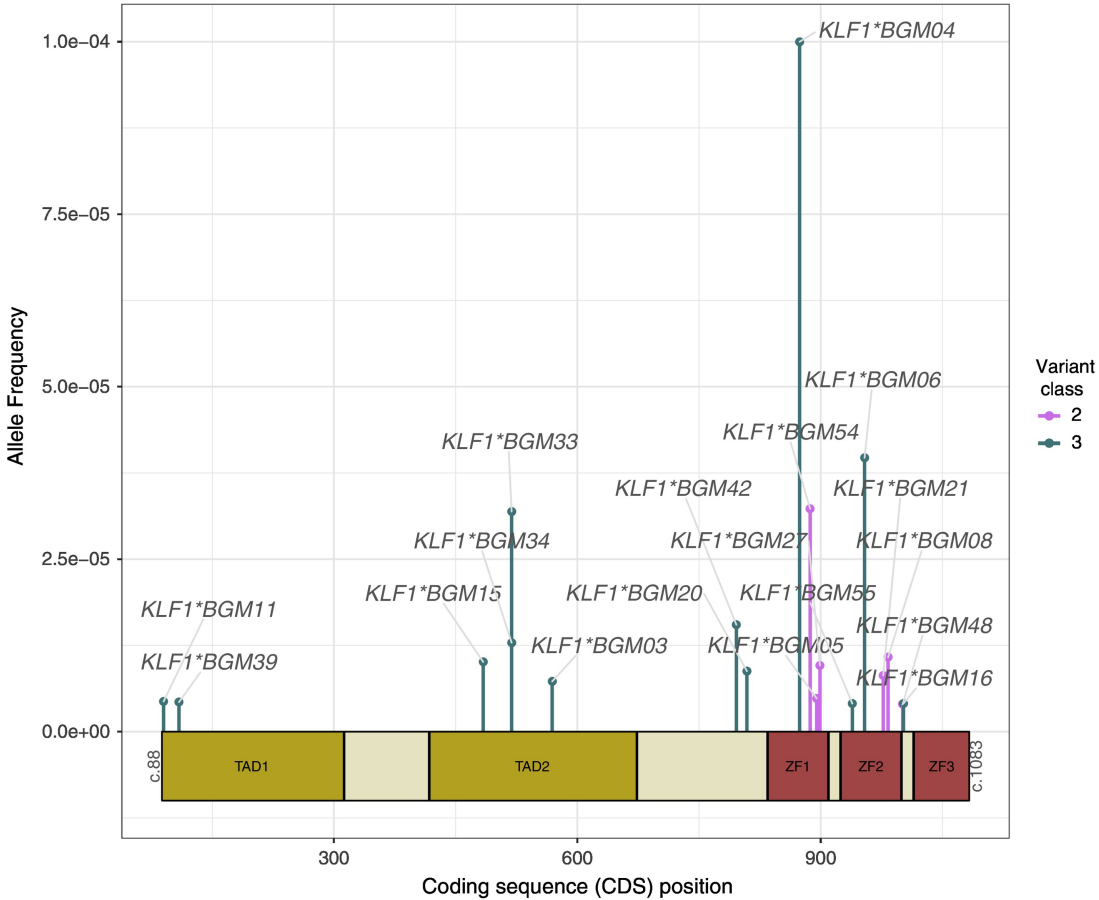
Highlights

- The variable impact of *KLF1* variants on blood group expression is ill-defined.
- Larger studies are required to enable intra-variant and inter-variant comparisons.
- A standardised testing framework is proposed to study In(Lu) and *KLF1* variants.
- Current databases need to log variability observed in In(Lu) due to *KLF1* variants.


1.0e−04
7.5e−05
5.0e−05
2.5e−05
0.0e+00
Allele Frequency
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KLF1*BGM06
KLF1*BGM33
KLF1*BGM54
KLF1*BGM42
KLF1*BGM21
KLF1*BGM34
KLF1*BGM27
KLF1*BGM08
KLF1*BGM15
KLF1*BGM20
KLF1*BGM55
KLF1*BGM11
KLF1*BGM48
KLF1*BGM03
KLF1*BGM05
KLF1*BGM39
KLF1*BGM16
c.88
TAD1
TAD2
ZF1
ZF2
ZF3
c.1083
300
600
900
Coding sequence (CDS) position
Variant class
2
3

Figure 1