Identification of Lutheran Blood Groups and Genetic Variants within *KLF1* among Thai Blood Donors

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Abstract

Background: Lu^a and Lu^b are inherited as codominant allelic characters resulting from a single nucleotide variant (SNV) of the basal cell adhesion molecule (*BCAM*) gene. Red cells of the dominantly inherited suppressor of the Lutheran antigens In(Lu) phenotypically appear as Lu(a−b−) by the haemagglutination test. In(Lu) resulted from heterozygosity for mutations within the erythroid-specific Krüppel-like factor 1 (*KLF1*) gene. This study aimed to determine the frequency of the Lu(a) and Lu(b) phenotypes and genotypes and genetic variants of the distinct In(Lu) among Thai blood donors. **Material and Methods:** Samples from 334 Thai donors were phenotyped with anti-Lu^a and anti-Lu^b. These DNA samples and an additional 1,370 donor DNA samples with unknown Lu(a)/Lu(b) phenotypes were genotyped using an in-house PCR-SSP. In the case of the three Lu(a−b−) donors, the *BCAM* and *KLF1* genes were analysed by PCR and sequencing. **Results:** A total of 331 of the 334 donors were Lu(a−b+), while the other observed phenotype, appearing as Lu(a−b−), was found among three donors. Of those three Lu(a−b−) donors with the *LU*02/02* genotype, we identified *KLF1* variant alleles, consisting of two variants: c.[304T>C, 1001C>G] and c.[304T>C, 519_525dupCGGCGCC], leading to the In(Lu) phenotype, and one homozygous variant (c.304T>C) mutation. Also, only one Thai donor was genotyped as *LU*01/02*, confirmed by serology test and DNA sequencing. **Conclusion:** In this study, we identified *KLF1* variants to be included in Lutheran typing analysis in Thai populations. Therefore, the application of genotyping and phenotyping methods has simultaneously been in use to screen and confirm the rare Lu(a+) and In(Lu) phenotypes.

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Introduction

The Lutheran (LU) blood group system (International Society of Blood Transfusion, ISBT No. 005) currently consists of 26 antigens [1], which are located on two isoforms of red cell membrane glycoproteins (basal cell adhesion molecule, B-CAM, CD239) belonging to the immunoglobulin superfamily (IgSF) of adhesion/receptor molecules [2, 3]. These antigens are numbered LU1 to LU29: four antithetical pairs – LU1(Lu^a)/LU2(Lu^b), LU6/LU9, LU8/LU14, and LU18(Au^a)/LU19(Au^b) – and three antigens (LU10, LU11, and LU15) are declared obsolete [1]. The Lu$_{null}$ or Lu(a−b−) phenotype is inherited as a recessive allelic character in which no Lutheran antigens can be detected in red cells. Thus, individuals possessing this phenotype may generate an antibody against Lu glycoproteins, anti-Lu3, which reacts equally strongly with all red cells except those of the Lu$_{null}$ phenotype [3]. Lutheran antibodies, anti-Lu^a and anti-Lu^b, are either naturally occurring or immune and are IgM, IgG, or IgA. Clinically significant findings for both antibodies have shown them to be involved in the formation of mild delayed hae-

molytic transfusion reactions (HTRs) [4–6]. There were some cases of haemolytic disease of the fetus and new-born (HDFN) recorded, and they were not severe enough to require any treatment beyond phototherapy, despite detecting either raised bilirubin or a positive direct anti-globulin test (DAT) [5].

The LU (now *BCAM*) gene is located on chromosome 19 at 19q13.32 and is organised into 15 exons distributed over approximately 12.5 kb. The Lu[a] and Lu[b] antigens have codominant allelic relationships and represent a single nucleotide variant (SNV), rs28399653, c.230A>G (p. His77Arg) in exon 3 of the *BCAM* gene [7, 8]. The true Lu(a−b−) phenotype is the recessive type and results from either homozygous or double heterozygous mutations responsible for inactivating the *BCAM* gene [9].

In addition to the recessive Lu$_{null}$ phenotype, the rare X-linked suppressor (XS2) and In(Lu) phenotypes usually manifest as the Lu(a−b−) phenotype detected by hae-magglutination tests due to the very low levels of Luther-an antigens presented in their red cells. Hence, individuals with the types of XS2 and In(Lu) do not produce anti-Lu3. The X-linked type found in a large Australian family was shown to be caused by a hemizygous mutation in the erythroid transcription factor *GATA1* [10, 11]. In another type governed by the erythroid transcription factor KLF1 (erythroid-specific Krüppel-like factor 1), the In(Lu) phenotype results from heterozygous inheritance of loss-of-function mutations in the dominant suppressor gene, *KLF1* [12], and In(Lu) is also characterized by the diminished expression of the high-prevalence antigen, AnWj, and the antigens for the Indian, P1PK, Land-steiner-Wiener, Knops, OK, RAPH, and I blood group systems [3, 12, 13]. The human *KLF1* gene is located on chromosome 19, encompassing three exons and two introns [14]. KLF1 contains two transactivation domains at the N-terminal end of the protein and three highly conserved zinc fingers at the C-terminal, which regulates the activity of several erythroid genes [14, 15].

Lu[b] is a high-prevalence antigen that is present in more than 99.9% of Southeast Asians. Its antithetical antigen, Lu[a], typically occurs very rarely or is absent from Asians [3, 16, 17], although it is widely distributed in certain populations (e.g., it is found in approximately 6% of Europeans (non-Finnish), 5% of Africans/African Americans, and 3% of Latino/admixed Americans) [3, 17]. The recessive Lu(a−b−) phenotype is extremely rare and is observed in all populations [3]. In three Thai reports, in which donor red cells were tested with anti-Lu[a] and anti-Lu[b], the following results were revealed: in 1967, 455 were tested, 452 were Lu(a−b+), one was Lu(a+b+), and two were Lu(a−b−) [18]; in 2001, 200 were tested and all were Lu(a−b+) [19]; in 2002, 500 were tested, 498 were Lu(a−b+) and two were Lu(a−b−) [20]. Two predominant Thai studies found two samples of blood that were apparently Lu(a−b−), but it is currently unproven whether these samples are either inherited Lu$_{null}$ or XS2/In(Lu) phenotypes, as identified by the suppressor gene mutations [18, 20]. In addition to considering the inheritance of these antigens, no study among Thai blood donors has ever been genetically investigated. This study aimed to determine the frequency of the Lu(a) and Lu(b) phenotypes and their genotypes and genetic variants of the In(Lu) phenotype among the Thai blood donors.

Materials and Methods

Sample Collection and Preparation
Ethylenediaminetetraacetic acid-anticoagulated samples were collected from the whole blood of 334 healthy Thai blood donors at the Blood Bank at Thammasat University Hospital (TUH) in Pathumtani, Central Thailand, between January and April 2022. Genomic DNA from these samples was extracted using the DNeasy Blood & Tissue Kit according to the manufacturer's instructions (QIAGEN, Valencia, CA, USA), then stored at −20°C until genotyping. An additional 1,370 DNA samples from unrelated healthy Thai blood donors obtained from our inventory of DNA banks (TUH) were used for *LU*01/LU*02* genotype screening by testing with in-house polymerase chain reaction sequence-specific primers (PCR-SSP).

Lu[a] and Lu[b] Phenotyping
A total of 334 blood samples were typed using the conventional tube technique (CTT). The reaction was tested by adding one drop of human anti-Lu[a] and anti-Lu[b] (CE-Immundiagnostika GmbH, Eschelbronn, Germany), one drop of 22% bovine albumin (National Blood Centre, Thai Red Cross Society, Bangkok, Thailand), and one drop of 2–3% red cell suspension in 0.9% normal saline to each tube before incubation at 37°C for 30 min. The mixtures were washed three times with normal saline, and two drops of the anti-human globulin (CE-Immundiagnostika GmbH) were added. After centrifugation, the reactions were read macroscopically, and the agglutination reactions were graded as 4+, 3+, 2+, 1+, and w+. After reading the negative reaction under a microscope, IgG-coated RBCs were added to check the validity of the antiglobulin test. Also, a DAT was performed for all samples that gave positive results to rule out false positive results, if any. In the case of apparent Lu(a−b−), column agglutination technology (CAT) – ID card antigen profile-I (P1, Le[a], Le[b], Lu[a], Lu[b]) – was additionally used for phenotyping as per the manufacturer's instructions (Bio-Rad, Hercules, CA, USA).

DNA Analysis
The *KLF1* exons 1–3 and *BCAM* exons 3–4 were PCR-amplified using the oligonucleotide primers shown in Table 1. For each PCR reaction, 3 μL of genomic DNA (50 ng/μL) was amplified in a total volume of 40 μL using 0.75 μM forward primer and 0.75 μM reverse primer for each reaction. The PCR was performed with 20 μL of 2× PCR reaction mixture (Phusion High-Fidelity PCR Master Mix, New England BioLabs, MA, USA) and 11 μL of sterile distiled water in a T100 Thermal Cycler (Bio-Rad). PCR conditions were as follows: for *BCAM*, 98°C for 30 s, (98°C for 30 s, 63°C for 40 s, 72°C for 30 s) × 35 cycles, 72°C for 5 min, producing a 1,039-bp amplicon; for *KLF1*, 98°C for 30 s, (98°C for 10 s, 69°C for 60 s) × 10 cycles, (98°C for 10 s, 65°C for 50 s, 72°C for 90 s) × 25 cycles, 72°C for 5 min, producing a 2,692-bp amplicon. PCR products were separated on a 1.5% agarose gel containing SYBR

Table 1. Oligonucleotide primers used for PCR procedures and sequencing

Name of primer	Sequence of primer (5′ to 3′)	Gene target	PCR product size, bp
SEQ-BCAM-F	TCAAGAACTGATAGGGAATGGGG	Exons 3–4, *BCAM*	1,039
SEQ-BCAM-R	TCCTAGGACACAGGAGTCTTCCC		
SEQ-KLF1-F	CCTTCTTTGGAGACCCAATGTC	Exons 1–3, *KLF1*	2,692
SEQ-KLF1-R	AGAGGGTCCATTCGTGGGA		
LU-F	AACTGATAGGGAATGGGGGC	Exon 3, rs28399653, c.230A	214
LU-A-R	CTCAGCCGAGGCTAGGT		
LU-F	AACTGATAGGGAATGGGGGC	Exon 3, rs28399653, c.230 G	214
LU-B-R	CTCAGCCGAGGCTAGGC		
HGH-F	TGCCTTCCCAACCATTCCCTTA	*HGH* gene (internal control)	434
HGH-R	CCACTCACGGATTTCTGTTGTGTTTC		

Bp, base pair; HGH, human growth hormone.

Safe DNA Gel Stain (Invitrogen, Paisley, UK), electrophoresed in 1× TBE buffer at 100 V, and visualised under a blue light transilluminator. Thereafter, amplicons were purified using a gel extraction kit (GeneJET Gel Extraction Kit; Thermo Scientific, Waltham, MA, USA), and eluted fragments were then sequenced by next-generation sequencing-based technology on the Illumina Platform (BTSeq™ Services; Celemics Inc., Seoul, Korea). Regarding P^1/P^2 genotype determination, a noncoding region of *A4GALT* targeting SNPs rs5751348 was analysed according to the method described by Thinley and colleagues [21].

*LU*01 and LU*02 Genotyping*

Additional donor DNA samples were analysed using the in-house PCR-SSP method to screen the *LU*01/LU*02* genotypes. The primer pair sequences for each amplicon are shown in Table 1. Two tube reactions were performed, one for the *LU*01* allele (c.230A) and the other for the *LU*02* allele (c.230G). The constituents of the PCR mixtures (10 μL volume per reaction) were as follows: 1 μL (50 ng/μL) of genomic DNA, 1 μM common forward primer (LU-F), 1 μM reverse primer (LU-A-R or LU-B-R), 0.3 μM*HGH* primers (HGH-F and HGH-R), and 5 μL of the 2× PCR reaction mixture (GoTaq® Hot Start Colorless Master Mix; Promega, Madison, WI, USA). The genomic regions of interest were PCR-amplified from DNA template 95°C for 5 min, (95°C for 30 s, 69°C for 60 s) × 10 cycles, (95°C for 10 s, 62°C for 50 s, 72°C for 30 s) × 25 cycles, 72°C for 5 min, producing a 214-bp amplicon (*LU*01* or *LU*02* allele) and a 434-bp amplicon (HGH-internal control). The amplicon separation and visualisation were performed as described above. To validate our in-house PCR-SSP technique for *LU*01/LU*02* genotyping, random DNA samples from 170 genotyped blood donors were independently sequenced.

Statistical Analysis

The prevalence of the observed phenotypes was described using descriptive statistics and expressed in percentages and 95% confidence intervals (CI). The genotype and allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium (HWE) was calculated by comparing expected and observed genotype frequencies using the Chi-square (χ^2) statistic. All statistical analyses were conducted using SPSS, Version 16.0 (SPSS Inc., Chicago, IL, USA), and p values ≤0.05 were considered statistically significant.

Results

Red Cell Phenotyping by CTT and CAT

A total of 334 blood donor samples were typed for Luᵃ and Luᵇ antigens. The Lu(a−b+) phenotype was commonly observed in 99.10% of donors ($n = 331/334$, 95% CI, 0.9740–0.9981), while the other phenotype, Lu(a−b−), was found in 0.90% of donors ($n = 3/334$, 95% CI, 0.0019–0.0260). Among the three Lu(a−b−) donors, P1, Leᵃ, Leᵇ, Luᵃ, and Luᵇ were additionally tested by CAT, and the prevalence of those phenotypes detected is shown in Table 2. All three donors were Lu(a−b−) and Le(a−b+) phenotypes, whereas only one donor showed a weak reaction for P1 antigen.

Mutation Analysis by DNA Sequencing

A total of three Lu(a−b−) donor samples were selected by the partial *A4GALT* and *BCAM* and the whole *KLF1* gene analysis. Table 2 shows the genomic DNA sequencing results of these donors. The molecular basis for a serologically weakened P1(+) phenotype of BZL286 donor was determined to be the heterozygous genotype at rs5751348 (G/T), representing the P^1/P^2 genotype, while the others (BZL008 and BZL116) had the P^2/P^2 genotype. Moreover, all donors had the *LU*02/02* genotype (c.230G/G). DNA sequencing revealed that one donor (BZL286) was found to have a single mutation (homozygous) for the mutant *KLF1* allele, with a missense c.304T>C (p.Ser102Pro) mutation. The remaining two donors were found to carry additional mutations in *KLF1*. One donor (BZL008) had a homozygous missense c.304T>C (p.Ser102Pro) and a heterozygous missense c.1001C>G (p.Thr334Arg) mutation, while the other donor (BZL116) had a heterozygous missense c.304T>C (p.Ser102Pro) and a homozygous duplication of c.519_525dupCGGCGCC (p.Gly176ArgfsX179). The variant alleles defined by these polymorphisms are c.[304T>C, 1001C>G] and c.[304T>C, 519_525dupCG-

Table 2. Phenotypes, alleles, and genotypes for three In(Lu) donors

Donor	Phenotypes			Genotypes		KLF1 mutations			
	Lutheran	Lewis	P1PK	*A4GALT*	*BCAM*	Nt change	Aa change	exon	rs number
BZL008	Lu(a−b−)	Le(a−b+)	P1(−)	*P2/P2*	*LU*02/02*	c.304T>C	p.Ser102Pro	2	rs2072597
						c.1001C>G	p.Thr334Arg	3	rs483352841
BZL116	Lu(a−b−)	Le(a−b+)	P1(−)	*P2/P2*	*LU*02/02*	c.304T>C	p.Ser102Pro	2	rs2072597
						c.519_525dupCGGCGCC	p.Gly176ArgfsX179	2	rs483352838
BZL286	Lu(a−b−)	Le(a−b+)	P1(+)	*P1/P2*	*LU*02/02*	c.304T>C	p.Ser102Pro	2	rs2072597

Aa, amino acid; Nt, nuclotide.

Table 3. The genotype, allele and MAF, and the HWE for the *BCAM* SNP rs28399653

Population (N)	Allele (N)	Frequency	Genotype (N)	Frequency	MAF	HWE	
						χ^2	*p* value
Total (1,704)	*LU*01* (1)	0.0003	*LU*01/01* (0)	0.0000	0.0003	0.0006	0.9997
	*LU*02* (3,407)	0.9997	*LU*01/02* (1)	0.0006			
			*LU*02/02* (1,703)	0.9994			
Known Lu(a) and Lu(b) (334)	*LU*01* (0)	0.0000	*LU*01/01* (0)	0.0000	0.0000	0.0000	1.0000
	*LU*02* (668)	1.0000	*LU*01/02* (0)	0.0000			
			*LU*02/02* (334)	1.0000			
Unknown Lu(a) and Lu(b) (1,370)	*LU*01* (1)	0.0004	*LU*01/01* (0)	0.0000	0.0004	0.0086	0.9957
	*LU*02* (2,739)	0.9996	*LU*01/02* (1)	0.0007			
			*LU*02/02* (1.369)	0.9993			

HWE, Hardy-Weinberg equilibrium; *N*, number; MAF, minor allele frequency.

GCGCC], respectively. The occurrence of electropherogram peaks and the positions of these mutations are shown in Figure 1.

*LU*01 and LU*02 Genotyping by PCR-SSP*

An in-house PCR-SSP method was designed, and the genotype *LU*01/LU*02* alleles displayed by agarose gel electrophoresis are shown in Figure 2. A total of 1,704 DNA samples, containing 334 known and 1,370 unknown Lu(a) and Lu(b) phenotypes, were genotyped for the *BCAM* c.230A>G. The allele and genotype frequencies of these samples are shown in Table 3. The observed genotype frequencies in the overall population studied were found to be conformed to the HWE test ($p > 0.05$) and have a very low minor allele frequency (MAF), *LU*01* as < 1%. Overall, there were only two genotypes, with frequencies of 0.0006 and 0.9994 for *LU*01/02* and *LU*02/02*, respectively. The count of the *LU*01* allele was 1 and the *LU*02* allele was 1,703. A rare genotype of *LU*01/01* was not observed in this study. In the 334 known Lu(a) and Lu(b) population, all DNA samples were *LU*02/02* genotype. One hundred and seventy such random samples were subjected to and confirmed by independent DNA

sequencing. These verifications of the PCR-SSP results remained constant across the genotyping results obtained by DNA sequencing. In addition to one donor possessing the *LU*01/02* genotype, the donor's red cells were selected for Lu(a)/Lu(b) phenotyping, and the results were confirmed as Lu(a+b+), corresponding with the genotype.

Discussion

Blood group phenotype distribution varies globally across different populations and ethnic groups, but limited data exist for ethnic distributions of Lu(a) and Lu(b) phenotype, allele, and genotype frequencies in Thailand. Here, we investigated the frequency of Lu(a) and Lu(b) phenotypes among blood donors in the Central region of Thailand using CTT. The study demonstrated that the frequencies of these phenotypes were related to their published frequencies in Thai cohorts [18–20], supporting a very rare or absent Lu(a) antigen frequency in Thais. In accordance with Asian ancestry, this frequency is similar to those in Saudi Arabian, Indian, and Chinese populations [22–24]. The In(Lu) phenotype was found in three of 334

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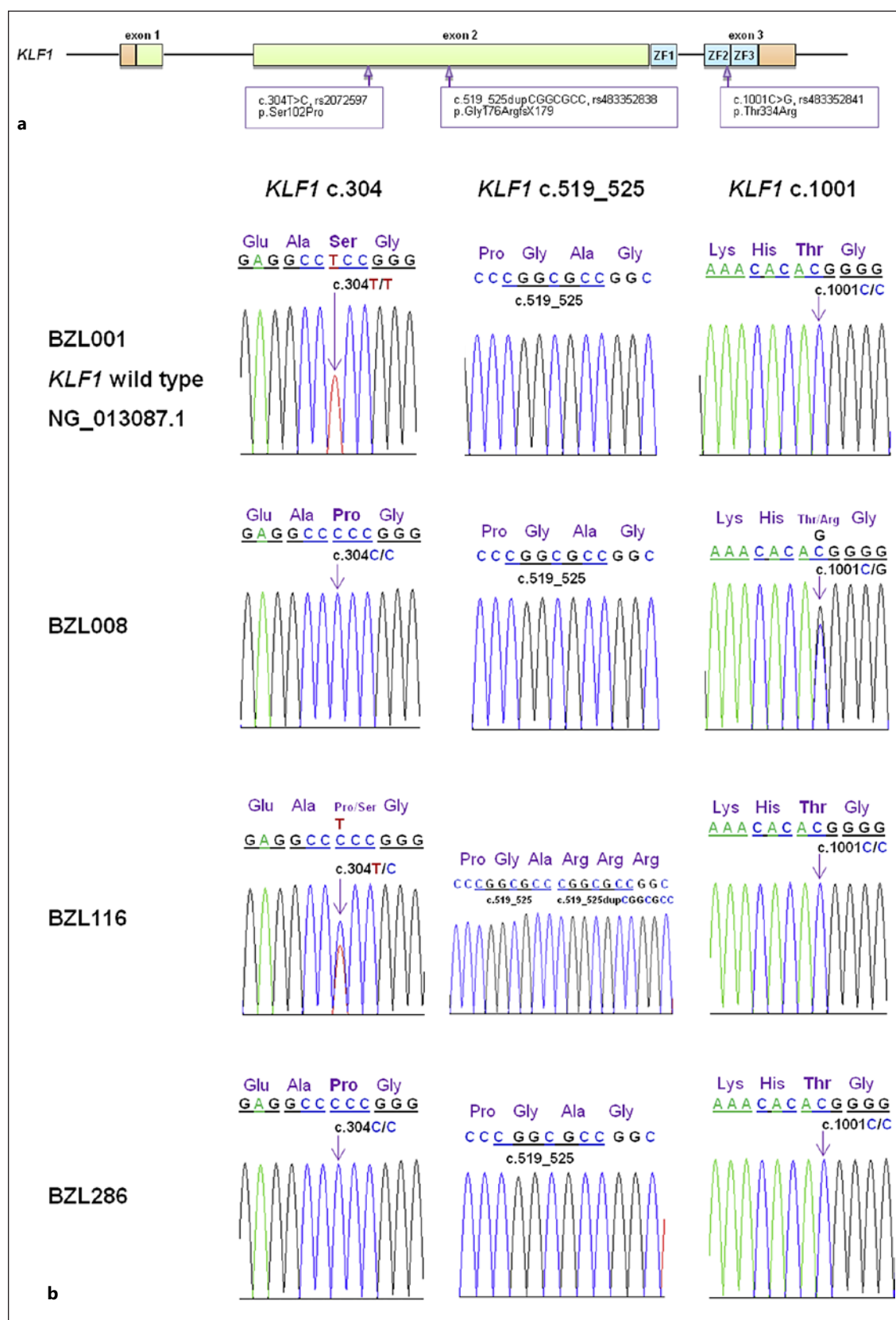
Fig. 1. The *KLF1* gene analysis. **a** Schematic representation of the *KLF1* gene with the location of the mutation variants indicated with an arrow. Exons: orange = non-coding regions; green = coding regions; blue = zinc fingers (ZF1, ZF2, ZF3). **b** Comparison of the partial *KLF1* sequence between the reference (NG_013087.1) and the three apparent Lu(a−b−) phenotypes.

Fig. 2. Representative gel electrophoresis of the PCR-SSP products of the samples 1, 2, and 3 known with the *LU*01/LU*02* genotyping. A 434-bp fragment of *HGH* was present in all lanes. *LU*01* and *LU*02* were deduced from the presence or absence of 214-bp amplicons. From left to right: lane M: 100 bp DNA molecular weight marker. Lanes 1A and 1B correspond to *LU*01/02* genotype, 2A and 2B and 3A and 3B correspond to *LU*02/02* genotype, and lanes 4A and 4B are non-template controls.
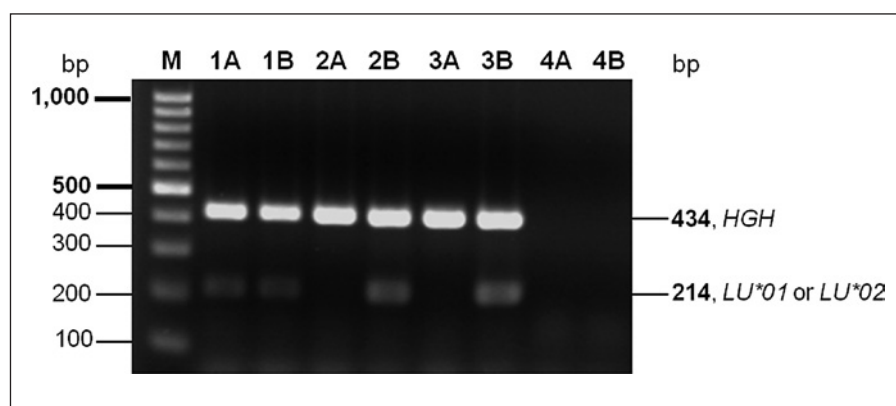
donors, or about 0.9%, similar to two published Thai studies [18, 20]. However, additional genetic relationships have not yet been directly elucidated in Thai donors. The absence of genotype information is probably one of the reasons In(Lu) individuals present phenotype-genotype discrepancy, so the application of molecular methods in blood typing can identify *KLF1* alleles through discrepancy resolution [25].

In the present study, we determined the genotype frequencies for the *LU*01* and *LU*02* alleles. Three apparent Lu(a−b−) donors detected by two serological techniques – CTT and CAT – were *LU*02/02* genotype; thus, full-gene *KLF1* sequencing has been required for those donors. Subsequently, we identified three known variants [12] – c.304C>T (*KLF1*BGM12*), c.1001C>G (*KLF1*BGM67*) and c.519_525dupCGGCGCC (*KLF1*BGM34*) – which were responsible for an amino acid substitution, p.Ser102Pro (rs2072597), p.Thr334Arg (rs483352841), and a reading frameshift creating a premature stop codon, p.Gly176ArgfsX179 (rs483352838), respectively. The *KLF1*BGM34* allele has been described by three separate groups as associated with In(Lu) phenotype [26–28]. Vrignaud et al. [29] described the *KLF1*BGM67* allele, and it is noted that another allele associated with the In(Lu) phenotype, *KLF1*BGM48*, involves the same triallelic SNV (rs483352841), but involves c.1001C>T (p.Thr334Met) [30]. Interestingly, the *KLF1* variant alleles found in two donors are characterised by combinations of the above mentioned polymorphisms in *cis*, as c.[304T>C, 1001C>G] and c.[304T>C, 519_525dupCGGCGCC]. Although the effects of missense variant c.304T>C have been reported to cause the In(Lu) phenotype in the early publication, this variant has been shown by three groups to have no impact on Lu^a and Lu^b expression [26, 31, 32]. Helias et al. [26] showed c.304T>C is relatively frequent in general population and thus could not be responsible for the rare In(Lu) phenotype. The potential bias was tested, and no association was found between the mutation and In(Lu) phenotype. Moreover, the study by Keller et al. [31] has reported that this c.304T>C

is not, by itself, associated with reduced expression of Lutheran antigens or the In(Lu) phenotype. Eernstman et al. [32] recorded that the c.304T>C distribution was in concordance with the frequencies observed in European populations, showing no correlation with the expression of CD44, band 3, Lu^a, Lu^b, HbF, and HbA2. In the current finding, we identified the c.304T>C variant with missense and frameshift variants, as mentioned above, which is in consonance with a previously published study of one Thai donor, c.[304T>C, 484insC] [25]. In contrast, homozygosity of the c.304T>C variant identified in the remaining donor did not seem to be a significant cause for the In(Lu) phenotype in this cohort. The haemagglutination test for this donor showed weakened expression (w+) of the P1 antigen by sensitive CAT, despite being the P^1/P^2 zygosity. Even though *KLF1* variants affect the expression of the P1 antigen [30], the strength of P1 reactivity could vary individually [3]. Currently, the variant c.304T>C is marked as obsolete in the table for Transcription Factor *KLF1* Alleles that caused the In(Lu) phenotype by the ISBT working party [12]. However, this study has not ruled out the XS2 phenotype in the donor that could be caused by mutations in the *GATA-1* gene [11]. The additional testing for the *GATA*BGM01* allele resulted in suggesting a further study.

The distribution of *LU*01/LU*02* genotype frequencies between known and unknown Lu(a) and Lu(b) phenotypes' populations in this cohort was like those expected in Asians [3] and those found in other Thai populations [18–20]. Both populations were in HWE and had a low MAF of the observed *LU*01* allele (<1%). Based on their MAF in this collection, the *LU*01* allele was confirmed as a rare variant among the Thai population. Even though the research on human genetic variation typically recommends a common MAF threshold at 5% to detect small effects [33], which would require substantially greater sample size of more than 1.1 billion Thai donors [1/(0.0003 × 0.0003)]. Only one donor was screened with the heterozygous *LU*01/02* genotype using the in-house PCR-SSP, which was confirmed by additional serology

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test and DNA sequencing. Moreover, robustness showed that the PCR-SSP and DNA sequencing displayed 100% concordance for *LU*01/LU*02* genotyping with rounded 10% samples of all donors. Therefore, this in-house assay was successfully validated based on its robustness, good concordance, and genotyping performance compared to the gold-standard DNA sequencing method. Concerning the unknown Lu(a) and Lu(b) population, it would be possible to identify In(Lu) individuals possessing the *LU*02/02* genotype, Lu(a−b+), predicted using PCR-SSP. Therefore, this study recommended that Lu^a/Lu^b antigen typing and In(Lu) status of Thai donors should be determined using combined serological and molecular techniques.

In Thailand, the Lu(a)/Lu(b) phenotyping is not routinely performed, nor have those antigens been included in the screening panels. An identification of Lu^a/Lu^b antibodies has not been reported in transfusion-dependent Thai patients [34], which seems to be in concordance with the rarity of the Lu^a antigen in the Thai population but also because of the use of unspecified Lu^a/Lu^b antigens in panel cells. Less frequently, mild and delayed HTRs can be caused by Lu^a/Lu^b antibodies [4–6]. Nevertheless, the risk of Lu^a and Lu^b alloimmunization in Thais could be low if it almost never takes place. Severe HTRs caused by anti-AnWj, if it occurs, could be transfused with In(Lu) red cell units without any clinical signs or symptoms of haemolysis [35, 36]. In this case, donors exist in whom the In(Lu) is greater in estimated detection probability than the AnWj-negative phenotype in Thai populations. Hence, our In(Lu) detection approach will possess several advantages in terms of reducing the risk of complications and avoiding transfusion risks to effectively transfuse the most appropriate blood components.

Conclusions

This is the first report of Lu^a and Lu^b phenotype, allele, and genotype frequencies in Thai populations and confirms that a high frequency of the *LU*02* allele (Lu^b). We found two distinct characteristics of *KLF1* variant alleles, consisting of two alleles (c.[304T>C, 1001C>G] and c.[304T>C, 519_525dupCGGCGCC]) in Thai donors with serologically apparent Lu(a−b−) that lead to the In(Lu) phenotype, which is important in blood group profiling of donors. However, the limitations of this study are a lack of *GATA-1* investigation and AnWj antigen typing that might have been helpful in defining the phenotype of the apparent Lu(a−b−) samples studied. With well-established blood group genotyping, the application of genotyping and phenotyping methods has simultaneously been in use, at least to screen and confirm the rare Lu(a+) and In(Lu) phenotypes.

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