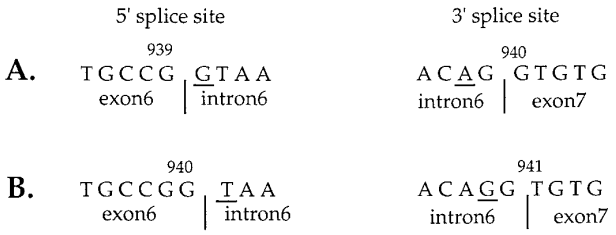

Fig 1. Exon6-intron6 and intron6-exon7 junctions in the *RH* gene. (A) Exon6/exon7 boundary was found at positions 939/940, as we had previously reported.[2] (B) Setting exon6/exon7 boundary at positions 940/941, as suggested by Huang,[1] results in abnormal 5′ (GT → TA) and 3′ (AG → GG) splice sites. Invariant +1 and −2 residues at the 5′ donor and 3′ acceptor splice sites, respectively, are underlined.

gested[2] that this boundary was located between nucleotides 939/940. We have recently analyzed the genomic fragment encompassing exon6 to exon7 from *RHD*, *RHCE*, and *RH* variant genes.[3] Sequencing of both exon6-intron6 and intron6-exon7 junctions indeed confirmed our previous result identifying exon6/exon7 boundary at positions 939/940 (Fig 1A). Moreover, setting the exon6/exon7 boundary at positions 940/941, as suggested by Huang (Fig 1B), would disrupt the splice consensus sequence at the invariant +1 and −2 residues at the 5′ donor and 3′ acceptor splice sites, respectively, thus preventing correct intron6 splicing.

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Use of the Polymerase Chain Reaction–Sequence Specific Oligonucleotide Technique for the Detection of the K1/K2 Polymorphism of the Kell Blood Group System

To the Editor:

The molecular cloning and sequencing of the Kell blood group protein led to the subsequent elucidation of the KEL gene into 19 discrete exons.[1] Further characterization of this gene showed that the polymorphism of K2 (k) and K1 (K) was due to a single base nucleotide substitution C701T on exon 6. This substitution disrupts the N-glycosylation motif for the K1 allele.[2] Anti-K has been implicated as a major cause of hemolytic disease of the newborn (HDN). Recently, molecular methods have been described for determining KEL genotypes using polymerase chain reaction (PCR)-based techniques, including allele-specific primers[3,4] and restriction fragment length polymorphism.[5] In this letter, we describe a PCR–sequence specific oligonucleotide (PCR-SSO) procedure for KEL genotyping based on a previously described protocol[6] and present frequency data for the K1 and K2 alleles in a white population.

A panel of serologically typed individuals, consisting of 10 K−k+ (K2K2), 8 K+k+ (K1K2), and 7 K+k− (K1K1) individuals, were initially used to establish the parameters of the K1/K2 PCR-SSO technique. Subsequently, a panel of 261 random white individuals was tested to determine the KEL genotype frequencies. Genomic DNA was extracted from peripheral blood leukocytes using a standard salting out and ethanol precipitation procedure. All primers and probes were designed in-house (Table 1) and synthesized on an Oligo 1000 (Beckman Instruments, Fullerton, CA). PCR consisted of 100 ng of genomic DNA, 25 pmol of primer KEL-6 S and KEL-6 AS, 10 mmol/L Tris-HCl pH 8.8, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.1% Triton X-100, 200 μmol/L of each dNTP, and 1 U of Dynazyme DNA Polymerase (Finnzymes, Espoo, Finland) in a final volume of 25 μL. PCR conditions were 94°C for 5 minutes and then 35 cycles of 30 seconds at 94°C, 60 seconds at 58°C, and 30 seconds at 72°C, with a final cycle for 5 minutes at 72°C. This yielded a PCR-amplified product of 145 bp. Genotype identification was determined by immobilizing the PCR product on a nylon membrane (Genescreen Plus Hybridisation Transfer Membrane; DuPont, NEN Research products, Boston, MA) and hybridizing with the specific probes KEL-K1 and KEL-K2 at 42°C for at least 2 hours. An internal control probe, KEL-IC750, was used to verify that the PCR amplification and SSO procedure had been successful. The TMAC wash temperatures for each probe were 63°C, 60°C, and 57°C, respectively, followed by detection using the DIG-detection system (Boehringer Mannheim, Mannheim, Germany). Each PCR included DNA from individuals typed as K1K1, K1K2, and K2K2 to validate the specificity of the probes.

Comparison of the KEL genotypes obtained by serology and the PCR-SSO procedure described here showed complete correlation. The genotype frequencies of the random population of 261 individuals showed 88% K2K2, 12% K1K2, and 0% K1K1. This correlates with previously reported KEL genotype frequencies of a Melbourne population, taking into consideration that the sample number is approximately four times larger.[7]

The PCR-SSO procedure described here enables accurate genotyping of the KEL genes, being both sensitive and reproducible. This technique is particularly useful for screening large numbers of samples and the possible application of KEL genotyping of fetal tissue for the management of HDN.

Table 1. Nucleotide Sequences of Kell Primers and Probes

Primer/Probe	Sequence (5′ → 3′)	Nucleotide Site
KEL-6 S	TTGGAGGCTGGCGCATCTC	650-668
KEL-6 AS	GTGTGTGTGGTCAGTAGGTC	775-795
KEL-K1	TTAACCGAATGCTGAGACT	692-710
KEL-K2	TAACCGAACGCTGAGACT	693-710
KEL-IC750	CTACCTAGGACCTCATCC	750-767

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Thrombopoietin in Upshaw-Schulman Syndrome

To the Editor:

In 1960, Schulman et al[1] reported on a patient with congenital thrombocytopenia and microangiopathic hemolytic anemia whose thrombocytopenia was transiently corrected by transfusion of normal plasma. These investigators speculated that the patient had a deficiency of thrombopoietic-stimulating factor, perhaps thrombopoietin (TPO). In 1978, a similar case was reported by Upshaw,[2] who suggested that thrombocytopenia and microangiopathic hemolytic anemia were due to increased consumption of platelets, probably in the same manner as in chronic thrombocytopenic purpura. Later, we observed another patient with this disease, and found that, in addition to plasma, another plasma component also produced a transient correction of platelet count.[3,4]

However, the question of the role TPO may play in this disease has never been addressed.

Recently, TPO, a ligand for the receptor encoded by the c-mpl proto-oncogene, has been purified, and it has been shown that TPO is involved in the regulation of megakaryocyte development and platelet production. It has become possible to accurately quantify the TPO level using the method of enzyme-linked immunosorbent assay.[5]

We measured the TPO level of nine serum samples obtained from five patients from various parts of Japan who were suffering from this disease. Table 1 shows the TPO levels of all the samples we measured. Before plasma infusion, when the patients' platelet count was low ($3.28 \pm 1.93 \times 10^4$/mm; mean ± SD) before plasma infusion (n = 9), the TPO level was as high (1.43 ± 0.89 fmol/mL) as that of normal adult serum (0.79 ± 0.35 fmol/mL).

We also had a chance to quantify TPO levels of four serum samples obtained when the patients' platelet counts increased after plasma infusion. Several days after plasma infusion, when the platelet count increased, the TPO level decreased to less than that of normal adult level (statistically not analyzed). The TPO levels of both the patients with idiopathic thrombocytopenic purpura (ITP; n = 12) and aplastic anemia (n = 7) are also included in the Table 1 as controls.

Table 1. TPO Levels of Upshaw-Schulman Syndrome

Patient No.	Sample	Date Obtained	Platelet Count ($\times 10^4/\mu$L)	TPO Level (fmol/mL)
1	Serum (before infusion)	September 30, 1995	6.5*	0.84†
	Serum (before infusion)	October 9, 1995	2.6*	1.13†
	Serum (before infusion)	October 20, 1995	5.5*	0.87†
2	Serum (before infusion)	February 8, 1996	1.0*	1.77†
3	Serum (before infusion)	April 10, 1996	3.1*	2.94†
	Serum (before infusion)	April 12, 1996	2.2*	2.81†
	Serum (after infusion)	April 17, 1996	32.1	0.42
	Plasma (used for infusion)			0.95
4	Serum (before infusion)	April 15, 1996	5.0*	1.19†
	Serum (after infusion)	April 24, 1996	33.8	0.8
5	Serum (before infusion)	March 26, 1996	1.5*	0.66†
	Serum (after infusion)	April 3, 1996	7.1	0.42
	Serum (before infusion)	April 10, 1996	2.1*	0.65†
	Serum (after infusion)	April 17, 1996	17.1	0.37
		Mean ± SD	3.28 ± 1.93* (n = 9)	1.43 ± 0.89† (n = 9)
Control 1 (n = 29)	Normal serum	Mean ± SD		0.79 ± 0.35
Control 2 (n = 12)	ITP serum	Mean ± SD		2.04 ± 0.88
Control 3 (n = 7)	Aplastic serum	Mean ± SD		18.53 ± 12.37

* Platelet count before plasma infusion.

† TPO level of serum before plasma infusion.