MOLECULAR GENOTYPING OF ANTITHETICAL *KEL* POLYMORPHISMS BY EXON-TARGETING PCR-RFLP

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Background: Besides the Rh system, the *KEL* polymorphic epitopes constitute the second most immunogenic blood group system. The Kell protein is a 93 kDa type II membrane glycoprotein that is encoded by the *KEL* locus at chr 7q34 and exerts endopeptidase activity. It forms a heterodimer with the multipass membrane protein XK that is stabilised by a single SS bond (XKcys347-KellCys72) and which is altered in McLeod red cell phenotypes that lack XK protein. The *KEL* locus contains 19 exons which harbor various single nucleotide polymorphisms (SNP) establishing antigen diversity. By the ISBT, 31 distinct antigens of the Kell protein are currently recognized, among them 4 antithetic pairs and one antithetic triplet (K/k, Jsa/Jsb, K11/K17, K14/K24, Kpa/Kpb/Kpc). Clinically, the antithetic antigens constitute the most relevant polymorphic sites of Kell glycoprotein. The antithetic SNPs are located in exon 6, 8 and 17 of *KEL*. Due to lack of specific antisera, not all antithetic sites can be typed serologically. We therefore designed a PCR-RFLP procedure to genotype for all 5 antithetic SNPs by targeting exon 6, 8 and 17.

Methods:

Table 1: PCR-Primer for amplification of antithetic SNPs of exon 6, 8 and 17

Name	Sequence (5' - 3')	Exon	Product (bp)
Kex6-F	GAAAGTATCAGGACCTTGGG	6	450
Kex6-R	AGGTAGGGTTGTTTCCTATA		
Kex8-F	CCTCTGGCACCTGTGACTGA	8	320
Kex8-R	AGGTATTAAGGGCACTAGGA		
Kex17-F	TCACCTAGGCAGCACCAACC	17	115
Kex17-R	TGAGCTTCCTGGAGGGCATG		

Table 2: SNP specific restriction of amplicons of exon 6, 8 and 17

Exon	Enzyme	Locus	SNP	RFLP (bp)
6	BsmI	C698T	K	231 + 219
			k	450
	HaeIII	G659C	K14	255 + 104 + 91
			K24	187 + 104 + 91 + 68
8	NlaIII	T961C	Kpa	143 + 118 + 59
			Kpb	261 + 59
	PvuII	G962A	Kpb	251 + 69
			Kpc	176 + 75 + 69
	MscI	T1025C	K11	320
			K17	205 + 115
17	MnlI	T1910C	Jsa	110 + 5
			Jsb	90 + 20 + 5

Table 1 shows the customized primer for exon amplification flanking the targeted SNPs in the respective exons. Primers were designed to provide distinguishable product sizes for the resp. exons as well as to exclude non-informative restriction sites in the amplification products. The three exons were amplified in separate tubes applying identical amplification conditions. The product digestions were performed in separate tubes using the SNP specific endonucleases **(Table 2)**. 10 ul of each restriction product was separated on agarose gel 2.0%. On the gel, the products were aligned in rising order of the exons. The first line was used for simultaneous run of unrestricted products of all three exons, the last line contains the molecular marker at 100 bp.

Conclusions:

1. **A reliable molecular approach to genotype all antithetical poly-morphic sites of *KEL* locus is provided. Applying multi-dye labelling of primers, the protocol might be improved to preform single tube multiplex genotyping.**
2. **Molecular genotyping of antithetical *KEL* epitopes provides evidence for rare anti-Kell specificity of anti-public alloantibodies.**
3. **Molecular genotyping of *KEL* locus does not necessarily predict correct Kell phenotype. Especially rare K_{null} phenotypes need to be excluded by phenotype targeting approches such as serological typing or flowcytometry using generic anti-Kell glycoprotein antibodies.**

Results:

Figure 1: Molecular Genotyping of 3 Patient carrying various Kell phenotypes as by serology ( )

Figure 1 A: Normal Blood Donor (K-/k+)

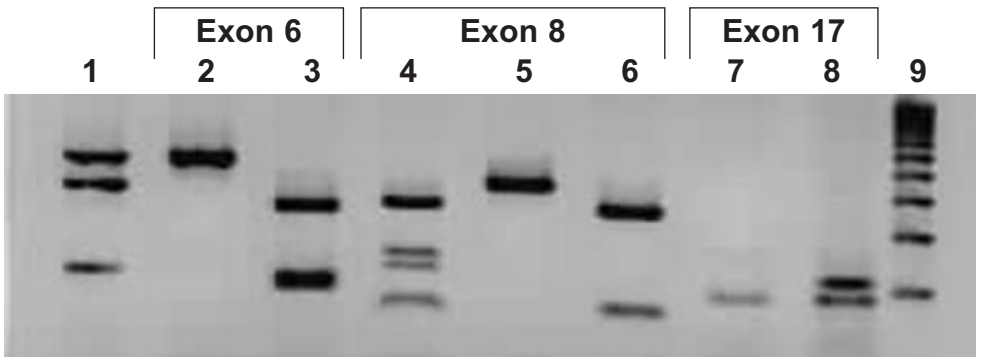

Figure 1 B: 2nd Gravida carrying Anti-K11

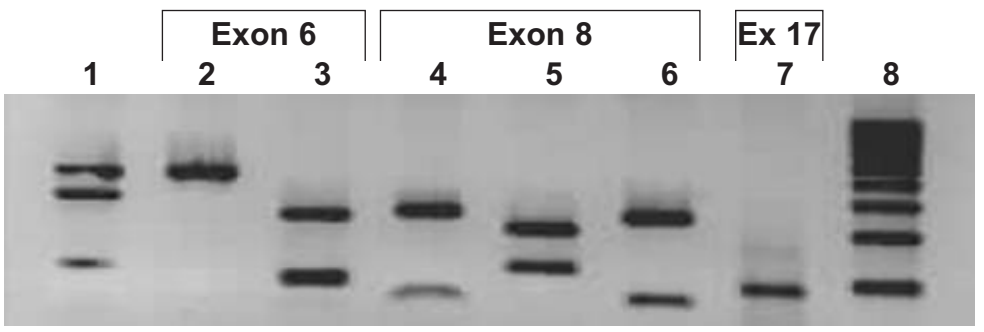

Figure 1 C: Patient with Anti-Ku, Anti-K, Anti-E (K_{null})

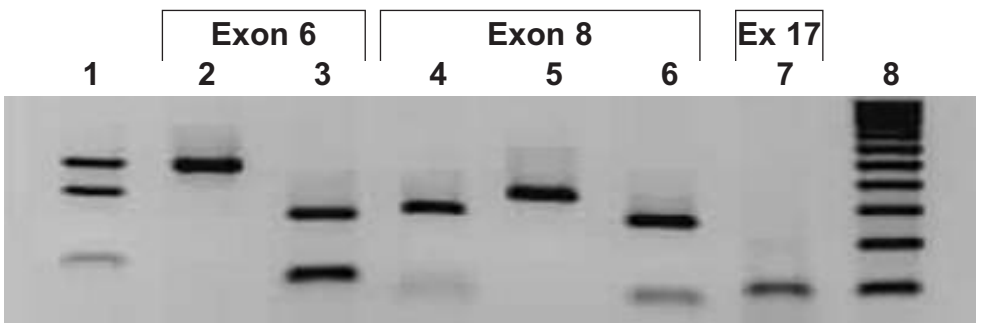

Figure 1 shows three individuals with differently affected *KEL* locus causing various phenotypes with significant clinical consequences: **1A**: normal blood donor with common Kell phenotype. In this case, the amplification product of exon 17 was run in lane 7 and 8 in duplicate. Lane 7: after digestion with MnlI. Lane 8: mixture of digested and undigested product. **1B**: 2nd gravida with anti-public anti-K11 alloantibody, indicating the very rare K17/K17 phenotype, which was confirmed by molecular genotyping (lane 5, exon 8). **1C**: Patient carrying anti-Ku, anti-K, anti-E. The suspected **(K_0)** phenotype was confirmed by flowcytometry. The molecular genotyping of *KEL* is unremarkable and excludes a deletional mutation as underlying molecular mechanism of the K_{null} phenotype. The mechanism of *KEL* silencing in this case is currently under investigation.

Table 3: Serological phenotype (red) and predicted phenotype (green) by molecular genotyping of samples 1A - 1C:

1A:	**K-1, K+2, K+3, K+4, K-6** **K-/k+, Kpa+/b+, Jsa-** **K-1, K+2, K+3, K+4, K-6, K+7, K+11, K+14, K-17, K-21, K-24**
1B:	**K-1, K+2, K-3, K+4, K-6** **K-/k+, Kpa-/b+, Jsa-** **K-1, K+2, K-3, K+4, K-6, K+7, K-11, K+14, K+17, K-21, K-24**
1C:	**K-1, K-2, K-3, K-4, K-6** **K-/k-, Kpa-/b-, Jsa- (K_0), Kx+** **K-1, K+2, K-3, K+4, K-6, K+7, K+11, K+14, K-17, K-21, K-24**