Immunohematology Case Studies 2018 – 5

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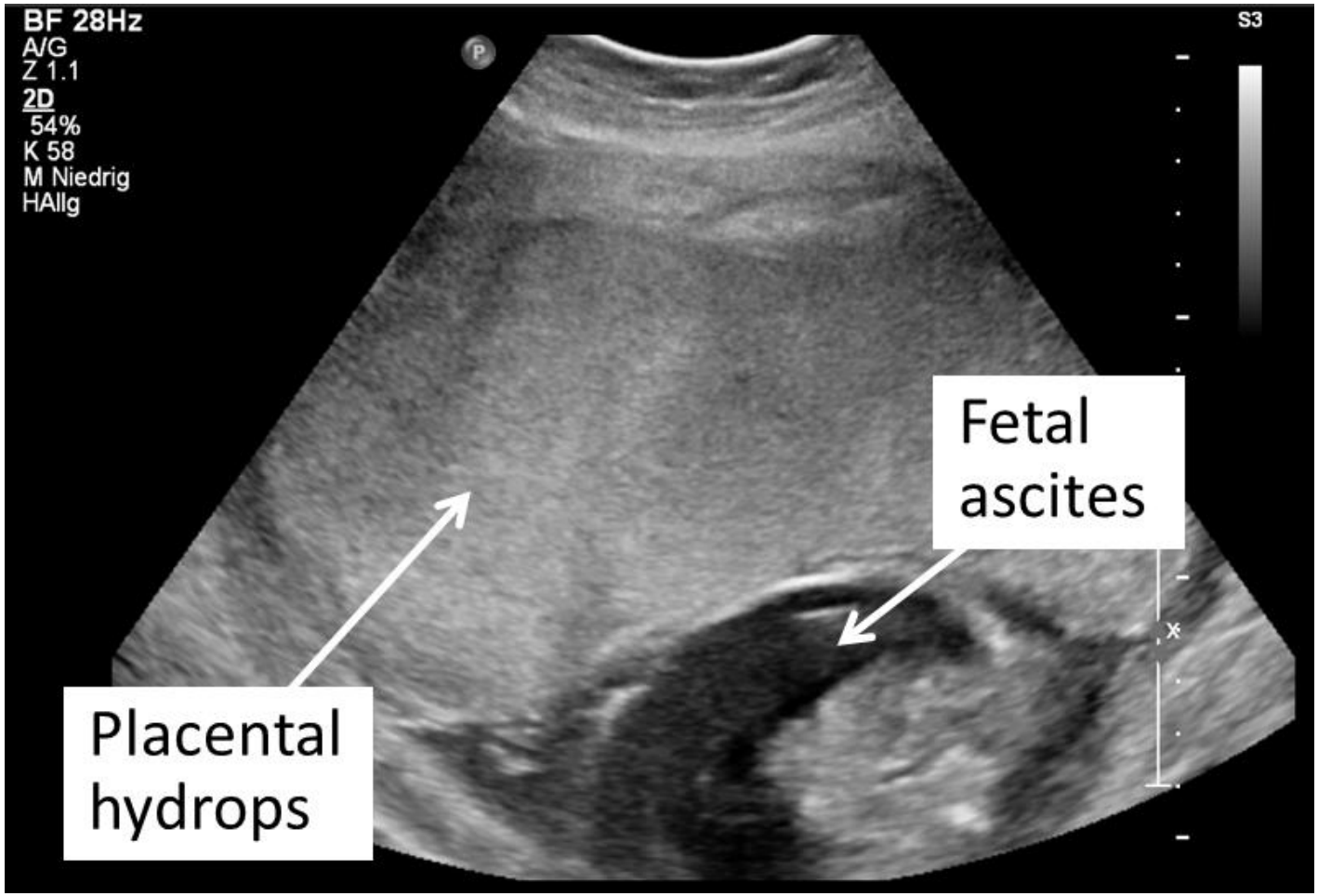
Clinical History 1

- A pregnant Caucasian woman (39 years old, gravida 2, para 1) with no transfusion history hospitalized because of the Mirror Syndrome with edema, ascites, massive fetal hydrops and polyhydramnios

Clinical History 2



Clinical History 2

- Progressive HELLP syndrome of the mother
- Delivery of the child by a Cesarean section in the 25 week of gestation
- Child´s weight: 1,070 g
- Critical condition with the APGAR score of 1/3/5
- Severe anemia (3.1 g/dl)
- Immediate transfusion and intensive care unit
- Child´s death 9 days after delivery

Serologic History

- Child´s red blood cells (RBCs):
  Blood group A, rr
  Direct antiglobulin test positive (IgG 3+)
- Mother´s blood group: A, rr
- Mother´s plasma:
  Antibody screening test negative
  Several antibody identification panels negative

Standard Panel

Untreated and papain treated RBCs in indirect antiglobulin test; gel technique

	System			Rh						Kell				Duffy		Kidd		Lewis		P	MNS				Xg	Lutheran		Dombrock		Auberger				Results	
	Rh		Component Lot	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Xg^a	Lu^a	Lu^b	Do^a *	Do^b *	Au^a *	Au^b *	Spezial-Antigene Special types		IAT	Enzyme	
1	$C^wCD.ee$	$R1^wR1$	70118427019	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	0	+	Co(b+)	1	O	O	
2	CCD.ee	R1R1	70218347834	+	+	0	0	+	0	+	+	0	+	+	0	+	+	0	0	$+^s$	+	0	+	0	+	0	+	+	+	+	+	0	Bg+	2	O	O	
3	ccD.EE	R2R2	70518489874	+	0	+	+	0	0	0	+	+	+	+	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	+	Bg+ $Rg(a+^w)^\$$	3	O	O	
4	ccD.ee	Ror	70218922414	+	0	+	0	+	0	0	+	0	+	0	0	+	0	0	+	$+^{vs}$	0	+	0	+	+	0	+	+	0	+	$Di(a+)^{*\$}$, KCAM–* $Yt(b+)^*$, WH+*	4	O	O	
5	Ccee	r´r	70118205755	0	+	+	0	+	0	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	$Yk(a+^w)^\$$	5	O	O	
6	ccEe	r´´r	70418371629	0	0	+	+	+	0	0	+	0	+	+	0	+	0	+	0	$+^s$	0	+	0	+	+	0	+	+	+	+	0		6	O	O	
7	ccee	rr	70418107317	0	0	+	0	+	0	+	0	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	$Cs(a+^{vw})^\$$ $Rg(a+^{vw})^\$$, $Yt(a-b+)^*$	7	O	O	
8	ccee	rr	70218347809	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	$+^s$	+	0	0	+	0	+	+	+	+	+	+		8	O	O	
9	ccee	rr	70518552548	0	0	+	0	+	0	0	+	0	+	+	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	$Cs(a+^{vw})^\$$ $Rg(a+^w)^\$$, $Yk(a-)^{*\$}$	9	O	O	
10	ccee	rr	70418243501	0	0	+	0	+	0	0	+	0	+	+	+	+	0	0	+	+	0	+	+	0	0	0	+	+	0	+	+	$Ch(a+^w)^\$$	10	O	O	
11	ccee	rr	70218126814	0	0	+	0	+	0	0	+	0	+	0	0	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	$Kn(a+^{vw})^\$$ $Yk(a+^{vw})^\$$	11	O	O	
	Patientenzellen Patient´s cells																														Patientenzellen Patient´s cells		O	nt	

Special Panel with rare RBCs 1

Untreated RBCs in indirect antiglobulin test; gel technique

	System				Rh - Hr						Kell				Duffy		Kidd		Lewis		P	MNS				Xg	Lutheran		Dombrock		Auberger			Results	
	Rh - Hr		Component Lot	Spezial-Antigene Special types	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Xg^a	Lu^a	Lu^b	Do^a *	Do^b *	Au^a *	Au^b *		IAT	
1	ccddee	rr	70116502273S	Vw+, Yk(a-)*	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	+	+	1	O	
2	ccddee	rr	70116343351S	Mi(a+)$, Yk(a-)*	0	0	+	0	+	0	0	+	0	+	+	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	+	2	O	
3	CcD.ee	R1r	70216363164S	BARC+*, D^{VI+} Co(b+)	$+^w$	+	+	0	+	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	3	O	
4	CCD.ee	R1R1	80216615674S	PARG+*$	+	+	0	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	+	0	+	nt	nt	nt	nt	4	O	
5	CCD.ee	R1R1	70116200802S	Wu+*$	+	+	0	0	+	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	+	0	+	+	+	+	0	5	O	
6	CCD.ee	R1R1	70216286891S	Js(a+)*	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	+	+	6	O	
7	ccddee	rr	70216303772S	Lu(b-), Co(b+)	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	7	O	
8	ccddee	rr	70216202383S	KCAM–*	0	0	+	0	+	0	0	+	0	+	+	+	+	+	0	+	+	+	0	+	+	+	0	+	+	+	+	0	8	O	
9	Ccddee	r´r	70216344250S	Ch(a-)$	0	+	+	0	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	9	O	
10	ccddee	rr	70116563586S	Cs(a-)$, Yk(a-)*	0	0	+	0	+	0	0	+	+	+	0	+	0	+	+	0	+	+	0	0	+	+	0	+	+	+	+	+	10	O	
11	CCD.ee	R1R1	70216323293S	Vel–*$	+	+	0	0	+	0	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	+	+	11	O	
12	--D.--		646316010984S	Rh:–17	+	0	0	0	0	0	0	+	0	+	+	0	+	+	0	+	+	+	+	0	+	+	0	+	nt	nt	nt	nt	12	O	
	Patientenzellen Patient´s cells																																		

Special Panel with rare RBCs 2

Untreated RBCs in indirect antiglobulin test; gel technique

	System				Rh - Hr						K	k	Kp^a	Kp^b	Duffy		Kidd		Lewis		P	MNS				Xg	Lutheran		Dombrock		Auberger			Results	
	Rh - Hr		Component Lot	Spezial-Antigene Special types	D	C	c	E	e	C^w					Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Xg^a	Lu^a	Lu^b	Do^a *	Do^b *	Au^a *	Au^b *		IAT	
1	ccD.EE	R2R2	70216405046S	Sc2+$^{\$*}$	+	0	+	+	0	0	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	+	1	O	
2	CCD.ee	R1R1	70118243066S	Rb(a+)$^{\$*}$ Co(b+) , Bg+	+	+	0	0	+	0	0	+	0	+	+	+	+	+	+	0	0	0	+	0	+	0	0	+	+	0	+	+	2	O	
3	CcD.ee	R1r	70318101722S	Wr(a+)	+^{w}	+	+	0	+	0	0	+	0	+	0	+	+	0	0	+	+	+	+	+	+	+	0	+	nt	nt	nt	nt	3	O	
4	ccD.Ee	R2r	70217361109S	Ew+*	+	0	+	+^{w}	+	0	0	+	0	+	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	0	+	0	4	O	
5	ccddee	rr	70117284883S	LW(b+)$^{\$*}$	0	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	5	O	
6	ccD.EE	R2R2	70117581581S	Yt(b+)$^{\$*}$ Kn(b+)* , Kn(b+^{w})$^{\$}$	+	0	+	+	0	0	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	6	O	
7	ccddee	rr	70216163589S	Rg–$^{\$}$	0	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	0	+	+	+	7	O	
8	ccddee	rr	70317110508S	AnWj–$^{\$\#}$	0	0	+	0	+	0	0	+	0	+	+	+	0	+	0	+	+	0	+	0	+	0	0	0	0	+	+	0	8	O	
9	ccddee	rr	70216322548S	Vw+ Yk(a-)$^{\$*}$	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	+	+	9	O	
10	CcD.ee	R1r	70216360431S	Co(a-b+)	+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	+	0	10	O	
11	ccD.ee	Ror	70217912735S	KCAM–* Sl(a-)* , Vil+*	+	0	+	0	+	0	+	+	0	+	0	0	0	+	0	+	+	+	0	0	+	0	0	+	+	+	+	+	11	O	
12	ccddee	rr	70117527370S	Jr(a-)$^{\$}$ Bg+	0	0	+	0	+	0	0	+	0	+	+	+	+	+	0	0	0	0	+	+	+	0	0	+	0	+	+	0	12	O	
	Patientenzellen Patient´s cells																																		

Special Panel with rare RBCs 3

Untreated RBCs in indirect antiglobulin test; gel technique

	System				Rh - Hr						Kell				Duffy		Kidd		Lewis		P	MNS					Xg	Lutheran		Dombrock		Auberger			Results	
	Rh - Hr		Component Lot	Spezial-Antigene Special types	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Xg^a	Lu^a	Lu^b	Do^a *	Do^b *	Au^a *	Au^b *		IAT	
1	ccD.ee	Ror	70418133713S	Mi(a+)§	+	0	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	+	0	+	+	0	+	+	1	O	
2	CcD.ee	R1r	70119440243S	Di(a+)*§ Yt(b+)*§	+	+	+	0	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	0	+	0	+	+	0	2	O	
3	CCD.ee	R1R1	70118525656S	Lu14+*§ Yt(a-b+)*§, HLA+§	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	3	O	
4	ccD.EE	R2R2	70216145721S	Sc4+* HLA+§	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	+	0	+	4	O	
5	ccee	rr	70518529378S	Mt(a+)§	0	0	+	0	+	0	0	+	0	+	0	+	+	0	0	0	+	+	+	0	+	+	+	+	+	+	+	+	5	O	
6	CcD.ee	R1r	70216445439S	Tar+*, D^{VII}*	+w	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	+	0	+	+	+	+	0	+	+	+	+	+	6	O	
7	ccee	rr	70316169967S	Kp(b-) LW(b+)*	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	0	+	+	+	7	O	
8	ccee	rr	70116168909S	Lu8–*§, Lu14+*§ HLA+§	0	0	+	0	+	0	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	8	O	
9	CCD.ee	R1R1	70219180014S	Sd(a-)§	+	+	0	0	+	0	0	+	0	+	+	+	+	+	0	+	+	0	+	0	+	+	0	+	nt	nt	nt	nt	9	O	
10	ccD.Ee	R2r	70216123656S	Vel–*§	+	0	+	+	+	0	0	+	0	+	+	+	+	+	0	+	0	+	0	0	+	+	0	+	+	+	+	0	10	O	
11	$C^wCD.ee$	$R1^wR1$	70116427087S	Co(a-b+)	+	+	0	0	+	+	0	+	0	+	+	+	+	+	0	+	0	+	0	+	+	+	0	+	nt	nt	nt	nt	11	O	
12	$C^wC^wD.ee$	$R1^wR1^w$	70318210066S	MAR–*§# (MAR-like negative)	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	0	+	0	+	+	+	12	O	
	Patientenzellen Patient´s cells																																		

Serological Results 1

→ Probably antibody to a low prevalence antigen

Antibodies to following low prevalence antigens ruled out:

Wr^{a}, Di^{a}, Wu, Rb^{a}, Lu14, Kp^{a}, Js^{a}, K17, K25, V, VS, Crawford, JAL, JAHK, PARG, E^{w}, C^{x}, C^{w}, DAK, FPTT, BARC, Tar, Go^{a}, Kn^{b}, Vil, Co^{b}, Yt^{b}, LW^{b}, Ls^{a}, Ul^{a}, Tc^{b}, Sc2, Sc4, Vw, M^{g}, Mi^{a}, Hut, Mur, Hil, Miny, He, Dantu, Mt^{a}, St^{a}, Mit, Vr

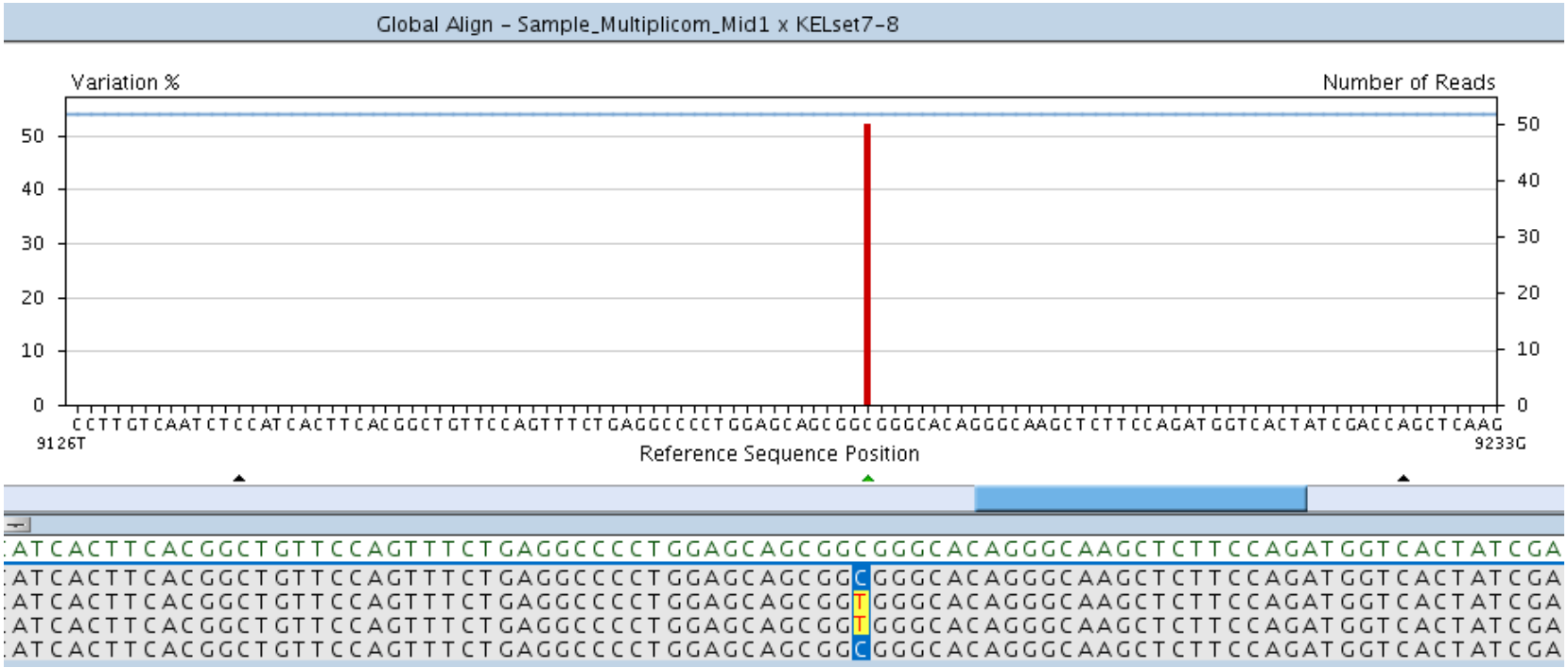
Serological Results 2
Serological Family Studies

- Mother´s plasma reactive (2+ in indirect antiglobilin test/gel) with the RBCs (all ABO compatible) of:
  the child´s father
  the father's mother
  the child's 3 years old brother
- Titer 16 in indirect antiglobulin test in gel
- Reaction slightly enhanced (titer 32) when the RBCs papain treated
- Reaction negative when the RBCs DTT (200 mmol) treated

Interim Antibody Identification
Possible Answers and Next Steps

- The severity of the hemolytic disease of the newborn and the serological results indicated it might be an antibody to low prevalence antigen of the Kell blood group system
- Next step:
  Sequencing of all exons of the *KEL* gene of the child's father using 454-sequencing technology

Genotyping Results



Exon 8 revealed C>T heterozygosity at position 877
The 877C>T mutation is predicted to cause a single amino acid change (Arg293Trp) in the Kell protein and defined a new low prevalence Kell antigen named KEAL (KEL39) (*KEL*02.39*) recognized by the ISBT 2016

Genotyping Results

- Next step:
  Establishing a PCR-SSP method for genotyping of the 877C>T mutation

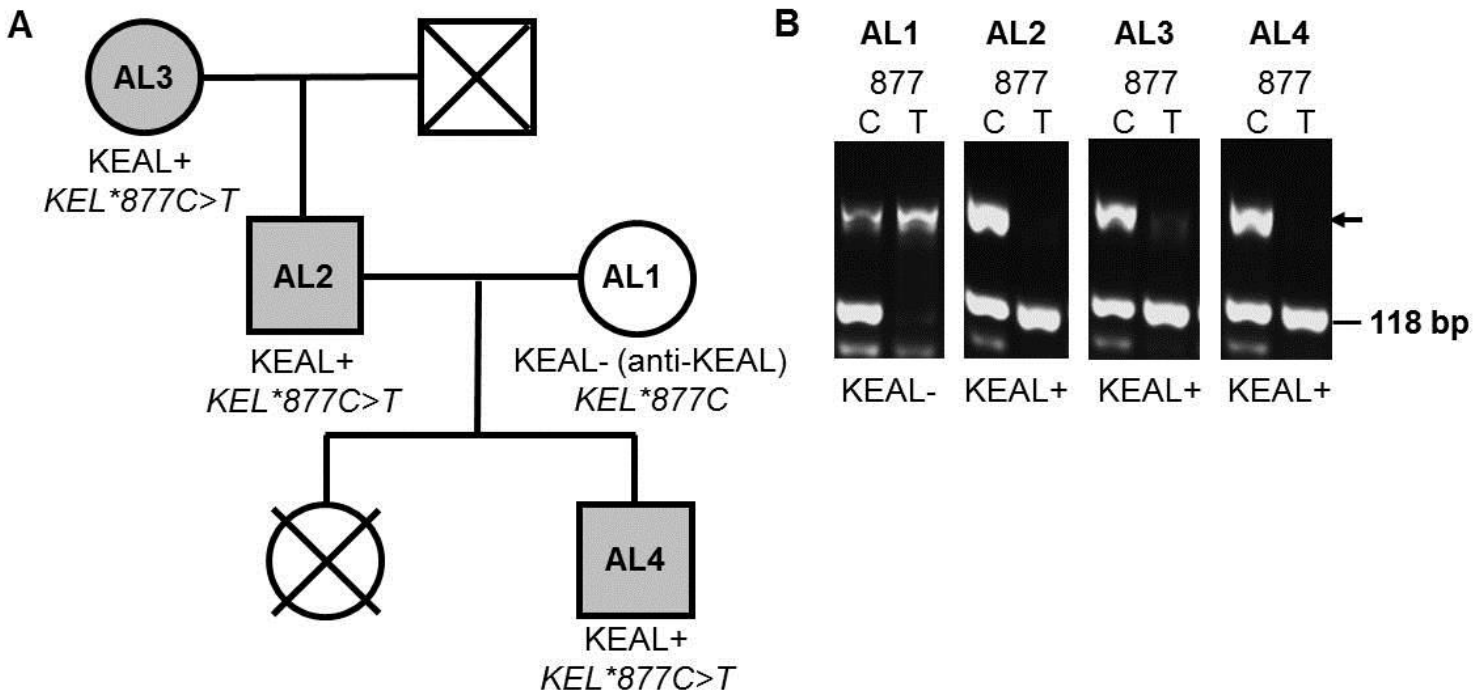
Table 1. PCR-SSP primers used for *KEL* genotyping.

Name	Location	Direction	Sequence (5'-3')	Amplicon size (bp)
KELex8-F1	*KEL* exon 8	sense	CCTCCACACCTCCGAGT	
KEL877C-R1g	*KEL* exon 8	antisense	GAGCTTGCCCTGTGCCC**G**	118
KEL877T-R1a	*KEL* exon 8	antisense	GAGCTTGCCCTGTGCCC**A**	118
betaglob-F	*HBB*	sense	GGTTGGCCAATCTACTCCCAGG	536
betaglob-R	*HBB*	antisense	GCTCACTCAGTGTGGCAAAG	

Genotyping Results

- Next step:
  Genotyping of the available family members for KEAL (KEL39)

Genotyping Results

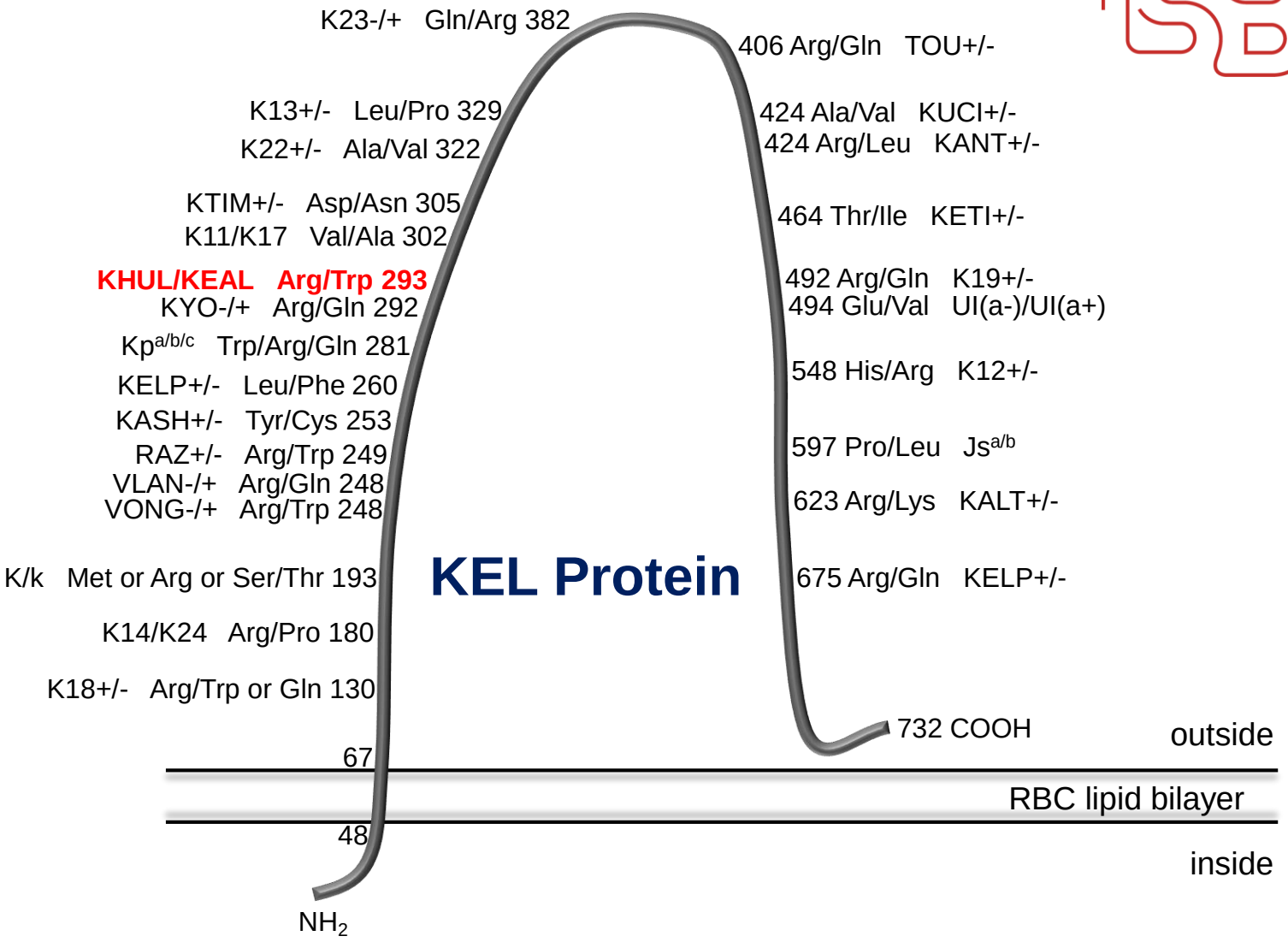


A. Pedigree of the family with the KEAL antigen.
The child's mother (AL1) represented the anti-KEAL index individual.

B. Results of PCR-SSP analysis of the *KEL* 877C>T mutation.
In accordance with serology AL2, AL3 and AL4 were positive (heterozygous) for the 877T allele (amplicon size: 118 bp), whereas, AL1 was negative.

Further Investigations

- Homozygosity for *KEL* 877C>T mutation is the genetic background for the rare KHUL (KEL37) negative phenotype
- Further heterozygous KHUL positive RBCs were positive with plasma of the mother
- **KEAL (KEL39) is antithetical to KHUL (KEL37)**



(according to: Reid ME, Lomas-Francis C, Olsson ML. The Blood Group Antigen Factsbook. 3rd edition, 2012)

Lessons Learned by the Case

- KEAL a low prevalence Kell antigen, which is characterized by a 877C>T mutation in exon 8 of the *KEL* gene and a predicted Arg293Trp substitution in the Kell protein caused a severe hemolytic disease (HDN) of the newborn
- Antibodies to Kell antigens are known to be highly clinically significant in pregnancy
- KEAL (KEL39) is antithetical to the high prevalence KHUL antigen (KEL37)

References

- Scharberg EA, et al. Fatal hemolytic disease of the newborn caused by an antibody to KEAL, a new low-prevalence Kell blood group antigen. Transfusion 2017;57;217–218.
- Lomas-Francis C, Vege S, Velliquette RW, et al. Expansion of the Kell blood group system: two new high-prevalence antigens and two novel K0 (Kellnull) phenotypes. Transfusion 2013;53:2887-91
- Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. London (UK): Academic Press; 2012