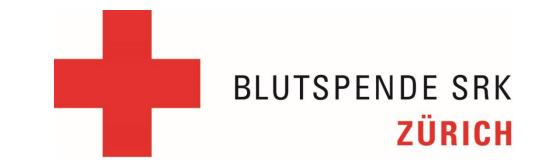
NANOPORE SEQUENCING TO RESOLVE LUTHERAN BLOOD GROUP DISCREPANCIES

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BACKGROUND



The Lutheran (LU) blood group system comprises 25 antigens encoded by the *BCAM* gene. There are four pairs of antithetical antigens, including LU1/LU2 (c.230G>A; p.Arg77His). The others represent independently expressed high frequency antigens.



The Lutheran null phenotype commonly arises either from recessive inactivating mutations in the **BCAM** gene (LU_{null}) or from dominantly inherited loss-of-function mutations in the transcriptional activator gene *KLF1* (In(Lu) phenotype).



Since 2015, Blood Transfusion Service Zurich has routinely been genotyping blood donors for 46 blood group antigens including LU1 and LU2 using MALDI-TOF MS. Among ~15,000 donors with both serotype and genotype available, we identified six discrepancies.

METHODS

Genotyping MALDI-TOF MS BCAM, LU*01/*02 Standard Phenotyping Lu(a/b) LU Genotype-Phenotype Discrepancies confirmed by PCR-SSP kits

Long-Read Sequencing by **Oxford Nanopore** *BCAM* (13.5 kb) *KLF1* (11.0 kb) as one amplicon each; confirmed by Sanger



RESULTS

Two new BCAM and KLF1 blood group alleles resolved as haplotypes by Oxford Nanopore sequencing

Genotyping MALDI-TOF MS (~45,000 donors)	Standard Phenotyping (~15,000 donors)	Causal variants detected by Nanopore Sequencing (6 discrepancies)	ISBT Allele name
BCAM			
Lu(a+b+)	Lu(a+b-) °	c.1427G>A; p.Arg476His	LU*02N.XX
Lu(a+b+)	Lu(a+b-) °°	c.100_105delCGCTTG; p.Arg34_L35del	LU*0212.1
° Adsorption/Elutio	n: Lu(b-) 🝎 LU*02	2N.XX	
° Adsorption/Elution		otypically characterized by the loss of the hig 2, accompanied by strong weakening of LU2	
KLF1			
Lu(a-b+)	Lu(a-b-)	c.874A>G; p.Lys292Glu	KLF1*BGMXX NE
Lu(a+b+)	Lu(a-b-)	c.977T>G; p.Leu326Arg	<i>KLF1*BGM21</i>
Lu(a-b+)	Lu(a-b-)	c.954G>C; p.Trp318Cys	KLF1*BGM62
Lu(a-b+)	Lu(a-b-)	c.858C>A; p.Cys286Ter	KLF1*BGM66

CONCLUSIONS

Resolution of all six LU genotype-phenotype discrepancies over the last seven years.

- Nanopore long-read sequencing enabled the identification and phasing of variants on their respective *LU* background.



Nanopore sequencing appeared well-suited for resolving genotype-phenotype discrepancies and is a reliable, emerging tool for routine diagnostics.



Abstract