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

NEW

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

RESULTS

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*** NEW
Lu(a+b+)	Lu(a+b-) °°	c.100_105delCGCTTG; p.Arg34_L35del	***LU*02.-12.1***
KLF1			
Lu(a-b+)	Lu(a-b-)	c.874A>G; p.Lys292Glu	***KLF1*BGMXX*** NEW
Lu(a+b+)	Lu(a-b-)	c.977T>G; p.Leu326Arg	***KLF1*BGM21***
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***

° Adsorption/Elution: Lu(b-) → *LU*02N.XX*

°° Adsorption/Elution: Lu(b+) → Phenotypically characterized by the loss of the high frequency antigen LU:-12, accompanied by strong weakening of LU2 expression.

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.

33rd Regional Congress of the ISBT, 2023, Gothenburg, Sweden

Abstract