Nanopore sequencing to resolve Lutheran blood group discrepancies

Nadine Trost[1], Gian Andri Thun[2], Charlotte Engström[3], Morgan Gueuning[2], Sonja Sigurdardottir[1], Naemi Larbes[3], Beat M. Frey[1,2,3], Maja P. Mattle-Greminger[2], Stefan Meyer[1]

[1] Blood Transfusion Service Zurich (SRC), Molecular Diagnostics and Cytometry, Schlieren, Switzerland
[2] Blood Transfusion Service Zurich (SRC), Research and Development, Schlieren, Switzerland
[3] Blood Transfusion Service Zurich (SRC), Immunohematology, Schlieren, Switzerland

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. 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 mass spectrometry (MS).

Aims: We evaluated genotype-phenotype concordance of Lutheran-typing. Rare discrepancies were resolved by third-generation Oxford Nanopore sequencing as well as standard Sanger sequencing for inter-performance comparison.

Methods: Phenotyping of donors was performed using standard and extended serological techniques. MALDI-TOF MS based genotyping of *LU*01/02* relied on SNV-detection of c.230G>A. In case of genotype-phenotype discrepancies, once confirmed with commercial PCR-SSP kits (sequence-specific priming; inno-train GmbH, Germany), the entire coding region as well as intronic and flanking regions of *BCAM* and *KFL1* were amplified in specific long-range PCRs (˜13.5 and 11.0 kb, respectively). Both amplicons of all samples were sequenced using Nanopore sequencing, which allowed allele haplotype generation along the entire genes. Sanger sequencing of gene regions of interest were used to confirm the results.

Results: Among ~15,000 donors for whom both serology and MALDI-TOF MS data for the Lutheran system were available, we identified six discrepant cases. Our sequencing approach on one *LU*01/02* heterozygous and three *LU*02* homozygous samples revealed one new (c.874A>G; p.Lys292Glu) and three rare known *KLF1* alleles (*KLF1*BGM21*, **BGM62*, **BGM66*), all leading to the dominant In(Lu) phenotype. The other two discrepant cases, both genotyped as *LU*01/02*, were linked to a novel *LU*02* null allele (c.1427G>A; p.Arg476His) and a rare *LU*02.-12.1* (c.100_105del; p.Arg34_Leu35del) allele, respectively. The latter is phenotypically characterized by the loss of the high frequency antigen LU12 (LU:-12), accompanied by strong weakening of LU2 expression. Genetic findings were confirmed by Sanger sequencing and further serologically refined by adsorption/elution techniques.

Conclusions: Using third-generation Nanopore sequencing of the *BCAM* and *KLF1* genes, both critical for Lutheran phenotype expression on red blood cells, we resolved all LU genotype-phenotype discordances of the last seven years of donor screening at our center. Four cases were linked to *KLF1* alleles, one of which was novel, leading to In(Lu) phenotype. The remaining two discrepancies were based on a new *BCAM* allele and a very rare allele lacking LU12 expression. In summary, our long-read sequencing strategy appeared well-suited for elucidating genotype-phenotype discrepancies in relation to the respective allelic *LU* background.