Nanopore sequencing to resolve Kidd blood group discrepancies

Résoudre les cas de divergences phenotype/genotype dans le groupe sanguin Kidd avec Nanopore

M Gueuning[1], L Schneider[2], GA Thun[1], N Trost[2], S Sigurdardottir[2], C Engström[3], G Rizzi[3], Y Merki[2], K Neuenschwander[2], C Gassner[4, 5], B Frey[1, 2, 3], M Mattle-Greminger[1*], S Meyer[2*]

[1]Department of Research and Development, Blood Transfusion Service Zurich, Schlieren, Switzerland

[2]Department of Molecular Diagnostics and Cytometry, Blood Transfusion Service Zurich, Schlieren, Switzerland

[3]Department of Immunohematology, Blood Transfusion Service Zurich, Schlieren, Switzerland

[4] Institute for Translational Medicine, Private University in the Principality of Liechtenstein, Triesen, Liechtenstein

[5] Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany

*Authors should be considered as senior co-authors

Background: Read accuracy from long-read sequencing technologies has recently experienced a surge, placing third-generation sequencing on the verge of entering clinical diagnostics. Its use in Transfusion Medicine is particularly promising in cases where well-established genotyping or sequencing approaches, typically targeting only exons, cannot explain serologic phenotypes (e.g. due to involvement of regulatory regions or presence of structural variation). Since 2015, Blood Transfusion Service Zurich routinely genotypes blood donors for 46 blood group antigens including the clinically relevant antigens of the Kidd system (Jk, *SLC14A1*) using MALDI-TOF mass spectrometry (MS). The goal of this study was to assess genotype/phenotype concordance of Kidd-typing and resolve rare discrepancies by third-generation Nanopore sequencing.

Methods: Donors were serotyped using standard techniques. Genetic discrimination between *JK*A*/*B* was performed by MALDI-TOF MS using the c.838G>A SNV. Two relevant null allele causing SNVs, c.582C>G (*JK*01N.03*) and c.342- 1G>A/C (*JK*02N.01/.02*), were also routinely assessed in all donors. Discrepant results were sequenced using Nanopore as well as Sanger sequencing for performance comparison. For the former, the entire coding region of the *SLC14A1* gene (~24 kb, exons 3 to 10) was amplified in two overlapping long-range PCRs (~13 kb each) and sequenced using a MinION (R9.4.1) flow cell.

Results: In ~12,000 donors, for whom both serology and MALDI-TOF MS data for the Kidd system were available, we identified 10 discordant cases. Both sequencing approaches revealed concordantly two known weak (*JK*01W.05*, *JK*02W.04*) and three known null alleles (*JK*02N.06*/*08*/*09*). Additionally, two donors were found to harbour new null alleles linked to *JK*A* (Gly40Asp) and *JK*B* (Gly242Glu), respectively. Remarkably, in the last three cases we identified an identical and yet unknown *JK*A* linked ~5 kb deletion spanning over exon 9 to 10, which could only be resolved by Nanopore sequencing. To our knowledge, it is the largest deletion ever described for the *JK* system.

Conclusions: Using long-range PCRs in combination with Nanopore sequencing, we resolved all *JK* phenotype/genotype discrepant cases gathered over six years of donor screening. Overall, Nanopore sequencing proved reliable for SNV as well as for structural variant calling. These results confirm the potential of Nanopore sequencing to become a robust tool in the molecular diagnostic portfolio, in particular for challenging cases.