PA19-L06 | Nanopore sequencing to resolve Kidd blood group discrepancies

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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).
Aims: We assessed genotype/phenotype concordance of Kidd-typing and resolved rare discrepancies by third-generation Nanopore sequencing as well as standard Sanger sequencing for performance comparison.
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*01 N.03*) and c.342-1G > A/C (*JK*02 N.01/.02*), were also routinely assessed in all donors. Discrepant results between phenotype and genotypes, once confirmed with commercial PCR-SSP kits (sequence-specific priming; inno-train GmbH, Germany), were further investigated by Sanger sequencing. In parallel, we applied long-read sequencing by Oxford Nanopore Technologies. For this, we amplified the entire coding region of the *SLC14A1* gene (~24 kb, exons 3–10) in two overlapping long-range PCRs (~13 kb each). Amplicons were barcoded and sequenced on one MinION Mk1B (R9.4.1) flow cell. We used *cuteSV* to detect structural variants. The presence of such variants like larger deletions was validated using bridge-PCRs.
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*01 W.05*, *JK*02 W.04*) and three known null alleles (*JK*02 N.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.
Summary/Conclusions: Using long-range PCRs in combination with Nanopore sequencing, we resolved all *JK* phenotype/genotype discrepant cases gathered over 6 years of donor screening. Five out of the 10 cases were linked to rare weak and null alleles not included in the routine MALDI-TOF MS assay. The other five cases were linked to novel null alleles either caused by SNVs ($n = 2$) or by a large *JK*A* linked ~5 kb deletion ($n = 3$). Overall, Nanopore sequencing proved reliable for SNV as well as for structural variant calling. It has the potential to become a robust tool in the molecular diagnostic portfolio, in particular to overcome challenges with respect to hybrid genes, deletions, and duplications.

Parallel Session 20 - Immunobiology - Antibodies that like platelets & granulocytes

PA20-L01 | Platelet antibody testing

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Detection and identification of anti-platelet antibodies is essential for the diagnosis of clinical conditions of immune-mediated thrombocytopenia, including fetal-neonatal alloimmune thrombocytopenia (FNAIT), platelet transfusion refractoriness (PTR) and post-transfusion purpura (PTP).
Various antigens are expressed on the surface of human platelets, including blood group ABO antigens, human leukocyte antigens (HLA) and human platelet antigens (HPA).
Presently, various methods, including commercial ones, are available for the platelet antibody testing. However, the availability and the preference of the methods, including the commercial ones, differs from region to region or from lab to lab. As an example, the mixed-passive hemagglutination (MPHA) assay, a highly sensitive and easy-to-handle method with similar principle of solid-phase red cell adherence (SPRCA), is commercially available only in Japan. Since the sensitivity of detection and the range of antibodies specificities that may be reliably identified varies among the various methods, it is recommended to combine the various methodologies for the reliable detection and identification of anti-platelet antibodies. Recent advances in antibody identification methods, combined with accurate molecular technologies for platelet antigen typing, have provided a framework for the development of clinical algorithms to support patients with FNAIT and PTR. In suspected FNAIT cases, following an initial screening of maternal serum for the presence of anti-platelet alloantibodies, the crossmatch between maternal serum and paternal/newborn's platelets is recommended, followed by the identification of an incompatible SNP in the father/newborn, in case positive. The same applies for suspected PTR. In case a platelet antibody is identified in patient's serum, there is need to find a compatible donor from the HLA-typed donor registry pool (cohort), and the crossmatch between patient's serum and donor's platelets is recommended. Interestingly, anti-HLA antibodies are the most involved in PTR, but their role in FNAIT has been doubted or even denied. Antibody functional assays, such as cytotoxic methods and phagocytosis assays, have been shown to be superior to microarray- or beads-based assays in predicting clinical PTR, and a deeper investigation