57. Jahrestagung der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämatologie e.V. (DGTI), 11.-13. September 2024, Düsseldorf, Deutschland

Long-read sequencing to characterize conspicuous *ABO* null alleles

G. A. Thun[1], M. Gueuning[1], S. Sigurdardottir[2], N. Trost[2], S. Koller[2], C. Engström[3], C. Gassner[4], M. P. Mattle-Greminger[1], S. Meyer[2]

1 Blood Transfusion Service Zurich (SRC), Research and Development, Schlieren, Schweiz
2 Blood Transfusion Service Zurich (SRC), Molecular Diagnostics and Cytometry, Schlieren, Schweiz
3 Blood Transfusion Service Zurich (SRC), Immunohematology, Schlieren, Schweiz
4 Private University in the Principality of Liechtenstein, Institute for Translational Medicine, Triesen, Liechtenstein

Background: We genotyped more than 36,000 blood donors for three *ABO* variants characterising *ABO***O.01*, *O.02*, and *B*-alleles using MALDI-TOF mass spectrometry with high-throughput capability. Discrepant results with serological values were further analysed by Sanger sequencing, but an exact allele determination was not always possible due to inability of phasing variants. To address this, we used long-read sequencing to elucidate the allelic background of unresolved samples by producing full haplotypes.

Methods: We analysed four cases with third-generation sequencing by Oxford Nanopore Technologies (ONT) that previously showed genotype-phenotype discrepancies. Those either pointed to silent *ABO***A*-alleles (n=2) or to suspected hybrid *ABO***O.01*-alleles harbouring variants defining *ABO***O.02*-alleles (n=1) or *ABO***B*-alleles (n=1). The entire *ABO* gene was amplified in two long-range PCRs of ~13 kb each with an overlap of almost 5 kb. Samples were barcoded and sequenced on a MinION flow cell according to standard protocols by ONT. Full-length haplotypes were aligned to *ABO* reference sequences and compared to the ISBT blood group allele table for ABO.

Results: Our findings identified two new *A1.01*-derived null alleles: one with a combination of indels in exon 7 causing a frameshift (NM_020469.3:c.758_766del; NM_020469.3:c.768_769insG) and one containing the nonsense c.542G>A variant (defining *O.06*) along with four downstream variants representing *O.09.01*. Aligning the latter to *ABO* reference sequences suggested a rearrangement between *A1.01* (up to end of exon 6) and *O.01.02*. The alignment for the third case pointed to a hybrid allele between *O.01.02* and *B* (similar to *O.01.24*, but showing c.220C>T) with break point at beginning of intron 6. Finally, the last sample showed the *O.01.57* allele whose variation downstream of exon 7 suggested a former recombination between *O.01.01* and *O.02.01* in exon 7.

Conclusion: Long-range PCR in combination with long-read Nanopore sequencing allowed straight-forward allele characterisation over the complete

ABO gene, a cumbersome task with alternative technologies. We found three novel null alleles and argue that the increasing revelation of intronic information owing to long-read sequencing proves useful to categorise the high diversity found in *ABO*. Modern sequencing methods may soon trigger a more adequate representation of allelic variation in blood group genes.