35th Regional Congress of the ISBT, 2025, Milan, Italy

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

G. A. Thun[1], M. Gueuning[1], S. Sigurdardottir[2], N. Trost[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 of Translational Medicine, Triesen, Liechtenstein

Background

We genotyped more than 36,000 blood donors for the three *ABO* variants rs8176719, rs41302905 and rs8176747 using MALDI-TOF mass spectrometry. These variants characterise the two main alleles for blood group O (*ABO*O.01*, *O.02*) and that of blood group B (*ABO*B*) enabling us to verify or challenge ABO serology. Discrepant results were further analysed by Sanger sequencing, but an accurate allele determination was not always possible due to the inability to phase variants. By their ability to sequence long reads, third generation sequencing technologies can generate haplotypes across the entire gene and elucidate the allelic background in such samples.

Aims

Here**,** we used third-generation sequencing by Oxford Nanopore Technologies to resolve genotype-phenotype discrepant cases indicating unusual allele compositions, which we further assessed by aligning them to allele-specific *ABO* reference haplotypes.

Methods

We selected four *ABO* null alleles that could not be fully resolved by Sanger sequencing. These encompassed either silent *ABO*A*-alleles (n=2) or suspected hybrid *ABO*O.01* alleles carrying the variant 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. Full-length haplotypes were aligned to *ABO* reference sequences (OM283861-OM284014) and compared to the ISBT blood group allele table for ABO.

Results

We identified two new *A1.01* derived null alleles. The first showed a combination of indels in exon 7 causing a frameshift (NM_020469.3:c.758_766del; NM_020469.3:c.768_769insG). These indels have so far been observed only four times among more than 1.4 million sequenced alleles in the GnomAD exome study and have always occurred in combination. The second novel allele contained a nonsense c.542G>A variant (defining *ABO*O.06*) along with four downstream variants representing *ABO*O.09.01*. Aligning the allele to *ABO* reference sequences suggested a rearrangement between *ABO*A1.01* (up to end of exon 6) and *ABO*O.01.02*. Heterozygous variation in exons 3 and 4 was in both cases phased to the *ABO*O.01* allele (*ABO*O.01.68*). The alignment for the third case pointed to a hybrid allele between *ABO*O.01.02* and *ABO*B.01* (similar to *O.01.24*, but showing c.220C>T) with the break point at beginning of intron 6. Finally, the last sample harboured the *O.01.57* defining

variants. Variation downstream of exon 7 is suggesting a past recombination event between *O.01.01* and *O.02.01* within exon 7.

Summary/Conclusions

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 highly useful to categorise the high genetic diversity found in *ABO*. Modern sequencing methods may soon trigger a more adequate representation of allelic variation in blood group genes than currently depicted in blood group allele tables.