38th International Congress of the International Society of Blood Transfusion (ISBT), 23.-27. Juni 2024, Barcelona, Spain

The potential of adaptive sampling in blood group genomics: a new horizon?

M. Gueuning[1], G. A. Thun[1], L. Wagner[2], B. Mayer[2], S. Meyer[3], M. P. Mattle-Greminger[1]

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

[2] Institute for Transfusion Medicine, Charité–Universitätsmedizin Berlin, Berlin, Germany

[3] Blood Transfusion Service Zurich (SRC), Department of Molecular Diagnostics and Cytometry, Schlieren, Switzerland

Background: Adaptive sampling (AS), a computational enrichment method from Oxford Nanopore Technologies (ONT), could mark a significant advancement in blood group molecular diagnostics. Leveraging the power of long-read sequencing, it shows considerable promise in accurately identifying complex structural variants (SVs) in the RH and MNS blood group systems — areas historically challenging for conventional diagnostic techniques. Moreover, AS provides a novel avenue for comprehensive analysis of the entire blood group genome, highly relevant for patients requiring chronic transfusions, such as those with thalassemia or sickle cell disease. Complementing methods for high-throughput genotyping of donors, sequencing by AS aims at accurately matching patient and donor profiles, which could considerably reduce risks associated with alloimmunization and delayed hemolytic transfusion reactions.

Aims: This study aims to evaluate the effectiveness of AS in resolving complex SVs in the RH and MNS systems while in parallel assessing its ability to provide complete and precise sequence information for all blood group genes.

Methods: We assessed AS using four distinct samples: a sample with a suspected deletion in the *RHCE* gene, one with a suspected *RHD*01N.06* hybrid allele, one with an unresolved hybrid allele in the MNS system, and finally a sickle cell patient with an unresolvable SV in the RH system. Sequencing was performed on ONT's PromethION P2 solo platform using the latest V14 chemistry. The reference FASTA file, conveying the genomic regions to enrich, contained all known red cell blood group genes (n=51), 2 transcription factors, 7 platelet, and 4 neutrophil antigen genes. We included 50 kb flanking regions for each locus to increase chances of retrieving long on-target reads. In sum, we targeted ~8.6Mb; corresponding to ~0.27% of the human genome. Reads were mapped to the novel human reference genome (T2T-CHM13v2.0), and variants called using Clair3 and Sniffles2. To assess variant calling accuracy, ONT sequencing results were compared to pre-typed genetic data for up to 17 blood groups systems.

Results: Raw sequencing output ranged from 40.1 to 50.9 Gb per PromethION flowcell. Mean coverage for the targeted genomic regions was between 37.4x and 46.6x. The decision for on- or off-target during sequencing was taken on average after ~650 bp, corresponding to approximately 1.5 seconds. N50 values fluctuated between 18 and 22 kb for the 4 libraries, with the longest read being 684,247 bp long. In the first sequenced sample, a perfect concordance was observed on 73 pre-typed variants spread across 17 blood group systems. For this same sample, long reads across the *RHCE* gene (45.3x coverage; 29 reads > 25 kb; max length of 98 kb) facilitated the detection of a novel 8,636 bp deletion

spanning from intron 8 to intron 9 of *RHCE*, representing to our knowledge the largest deletion ever reported in this gene. Detailed analyses for the three remaining cases will be presented at the congress.

Summary/Conclusions: AS represents a significant advancement in the genomic characterization of blood group systems, offering a powerful tool for resolving complex SVs as well as providing access to the whole blood group genome. Its application has proven to be exceptionally versatile and accurate, providing a new avenue for the comprehensive genetic analysis of blood group genes. This method holds promise for improving patient outcomes, particularly for those requiring chronic transfusions.