Swisstransfusion Jahreskongress, 05.-06. September 2024, Neuchâtel

Rapid full-length haplotyping method of *RHD*/*RHCE* by nonspecific long-range PCR-based sequencing

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

1 Department of Research and Development, Blood Transfusion Service Zurich SRC, Schlieren, Schweiz

2 Department of Molecular Diagnostics and Cytometry, Blood Transfusion Service Zurich SRC, Schlieren, Schweiz

3 Department of Immunohematology, Blood Transfusion Service Zurich SRC, Schlieren, Schweiz

Resolving allele-specific sequences (haplotypes) for the *RHD* and *RHCE* gene can be highly challenging. Molecular techniques like cloning or cDNA sequencing have been utilized for this purpose, but they are not suitable in time-sensitive situations. Here, we introduce a rapid long-read sequencing method for haplotype reconstruction at the *RH* locus, exemplified by three distinct cases for which both serological and genotypic tests failed to offer unambiguous *RHCE* allele assignment.

Standard serological methods showed RH:1,2,3,4,5 phenotype in two donor cases. Additionally, PCR-SSP kits revealed heterozygous c.733C>G resulting in V+VS+ antigen expression. The third case was a patient urgently requiring transfusion; serotyped RH:1,2,-3,4,5 and molecular analyses showed presence of c.667G>T. To fully cover exons 1 to 10 from *RHD* and *RHCE*, we designed five generic primer pairs co-amplifying both homologous genes. PCR amplicon lengths ranged from 12.3 to 15.2 kb for *RHD* and 13.3 to 15.2 kb for *RHCE*. Overlaps of PCR amplicons comprised >1 kb and were chosen to lie within polymorphic regions to facilitate variant phasing. Pooled PCR products were barcoded allowing multi-sample Nanopore sequencing on MinION flow cells.

Long-range PCRs and library preparation were completed within half a day. Sequencing produced >20,000 reads in ~4 hours. Variant calls enabled full-length haplotype reconstruction in all three samples by identifying variants in overlapping regions. The c.733C>G variant was phased to different *RHCE* backgrounds: one donor exhibited a *RHCE*ce* allele (*RHCE*01.20.01* | *RHCE*04*), while the other displayed the V+VS+ causing variant on a *RHCE*Ce* background (*RHCE*02.30* | *RHCE*03*). Contrary to frequency-based expectations, the c.667G>T variant found in the patient was linked to *RHCE*Ce* (*RHCE*02.22*) associated with weak C rather than lying on *RHCE*ceMO* (*RHCE*01.07*, partial c), resulting in transfusion recommendation from a RH:1,-2,-3,4,5 donor.

Here, we present an amplicon-based Nanopore sequencing approach capable of rapidly and reliably obtaining complete *RHD* and *RHCE* haplotypes, at least in the absence of complex structural variation. This method is particularly well-suited for urgent scenarios where precise genetic allele data is critical for ensuring optimal blood provision. To the best of our knowledge, no other technique offers the ability to accurately infer *RHD*/*CE* haplotype sequences within a time frame of one working day.