Loss of heterozygosity analysis for deciphering two cases with challenging RHCE serology

M. Gueuning[1], G.A. Thun[1], S. Sigurdardottir[2], N. Trost[2], K.Engehausen[2], L. Wagner[3], B. Mayer[3], S. Koller[1,2], S. Meyer[1,2]

1 Blood Transfusion Service Zurich (SRC), Research and Development, Schlieren, Switzerland
2 Blood Transfusion Service Zurich (SRC), Molecular Diagnostics and Cytometry, Schlieren, Switzerland
3 Institute for Transfusion Medicine, Charité-University Medicine, Berlin, Germany

Background: Patients with absent RH4 (c) antigen in combination with partial or absent antithetical RH2 (C) antigen are challenging to transfuse. Resolving the genetic underpinnings of such a phenotype may help to provide matching blood units more efficiently. Here, we present two cases in which genetic analyses identified exceedingly rare *RHCE*02* alleles which appeared completely homozygous across coding regions of the *RHD* and *RHCE* genes. Such results do not allow the unequivocal distinction between hemi- and homozygosity of the rare alleles.

Aims: The aim of this study was to determine zygosity of the identified rare alleles using a combination of diagnostic methods targeting *RHD*. Furthermore, we aimed to shed light on the occurrence of loss of heterozygosity (LOH) at this locus and on its extension by third-generation sequencing by Oxford Nanopore Technologies.

Methods: Conspicuous serological findings related to the RH2 and RH4 antigens were initially analysed by Sanger sequencing of all *RHCE* exons. To characterise the zygosity of *RHD*, we used MALDI-TOF mass spectrometry, PCR-SSP and digital PCR (dPCR), targeting specific SNVs within selected *RHD* exons, as well as the hybrid Rh box. Finally, adaptive sampling, an enrichment method for Nanopore sequencing, was used to construct both *RH* haplotypes and investigate potential LOH at this locus of chromosome 1.

Results: The first case was a pregnant woman that was serologically tested RH:1,2(C+ partial),-3,-4,5. By Sanger sequencing we identified RH2-associated variants along with c.143A>G in *RHCE* exon 1 (p.Tyr48Cys, rs758379880), all in homozygous state. Given the rarity of this variant, population frequency <10(-6), we either suspected a deletion over *RH* on one haplotype (i.e., hemizygosity) or copy-neutral LOH. While dPCR, MALDI-TOF mass spectrometry and PCR-SSP analyses pointed to two *RHD* genes, Nanopore sequencing revealed complete absence of heterozygous variation across the entire *RH* locus (~150 kb) and extending up to 400 kb beyond, consistent with copy-neutral LOH. The second case involved a patient with a serological -D- (RH:1,-2,-3,-4,-5). Sanger sequencing failed for exons 3 to 9, which suggested the homozygous presence of a rare *RHCE*02N.08* allele (i.e., a *RHCE*CE-D(3-9)-CE* hybrid). This was confirmed by gene dosage analysis of different *RHD* exons using dPCR and haplotyping via Nanopore sequencing. The latter allowed narrowing down the breakpoints of *RHCE*02N.08* to regions of 4.1 (intron 9) and 4.3 kb (intron 1 to 2). Further analysis of *RH* flanking regions revealed copy-neutral LOH extending over ~7 Mb.

Summary/Conclusions: Extensive genetic analyses of the *RH* locus revealed two very rare *RHCE* alleles, one of them novel, both presented in homozygous state. Copy-neutral LOH extended far beyond the *RH* locus in both cases. These allelic constellations may be explained either by mitotic interstitial events (gene conversion) occurring in early embryogenesis or by inheritance of a DNA segment identical by descent from both parents. Nanopore sequencing demonstrates its potential to disentangle complex cases where alternative methods only provide partial or ambiguous results.