58. Jahrestagung der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämagologie e.V. (DGTI), 17.-19. September 2025, Mannheim, Deutschland

In-depth genetic analyses for resolving two cases with challenging RhCE serology

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Background: Patients with absent RH4 antigen in combination with partial or absent antithetical RH2 antigen are challenging to transfuse. Resolving the genetic underpinnings of such a phenotype may help to provide matching blood units more efficiently. We present two cases in which a comprehensive set of genetic approaches — including Nanopore sequencing — was used to identify causal variants and determine the zygosity of the rare alleles involved.

Methods: Conspicuous serological findings related to the RH2/RH4 antigens were initially analysed by Sanger sequencing of all *RHCE* exons. To characterise the zygosity of the *RH* locus, we applied a range of genetic methods, including MALDI-TOF mass spectrometry, PCR-SSP and digital PCR (dPCR). These approaches targeted specific SNVs within selected *RHCE* and *RHD* exons, as well as the hybrid Rh box. Finally, long-read Nanopore sequencing was used to construct both *RH* haplotypes and investigate potential instances of loss of heterozygosity (LOH) that could explain the homozygous presence of exceedingly rare alleles.

Results: First case involved a pregnant woman, serologically 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 or copy-neutral LOH. While dPCR analysis did not support an *RH* deletion, 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. 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 CE-D(3-9)-CE hybrid). This was confirmed by gene dosage analysis 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.

Conclusions: Extensive genetic analyses of the *RH* locus revealed two very rare *RHCE* alleles, one of which novel, both at homozygous state. By detecting copy-neutral LOH stretching well beyond the *RH* locus, Nanopore sequencing fosters its potential to disentangle complex cases where results of alternative methods remain partial or ambiguous.