Two complementary long-read sequencing approaches of the *RH* locus reveal a novel *RHCE* hybrid allele

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Introduction

Long-read sequencing has proven reliable to resolve blood group alleles as full haplotypes, but successful determination of hybrid alleles between *RHD* and *RHCE*, in particular novel ones, remains challenging. Here, we present the case of a pregnant RH1-positive patient with weak RH4 (3+) and inconsistent serological reactions with anti-RH2 (0-2+) and anti-RH5 (0-3+). While genetic data of routine methods only revealed common exonic variants in both genes, further clarification of the allelic composition provided ambiguous results and remained unresolvable due to loss to follow-up. Three years later, phenotypic results of a pregnant sister were very similar and routine genotyping showed the same genetic variants. Third generation sequencing approaches by Oxford Nanopore Technologies (ONT) were applied to clarify the cryptic findings of routine methods in both sisters.

Methods

Standard serological techniques and commercially available PCR-SSP kits were applied to pheno- and genotype RH antigens. Multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing data were additionally generated. Long-range PCR was used to co-amplify the homologous *RHD* and *RHCE* genes with five generic primer pairs allowing an overlap of at least 1.1 kb between adjacent amplicons to facilitate variant phasing. Finally, we used an in-house *RH* long-read hybridization capture assay to mitigate the chance of mismappings and enable phasing between the two genes.

Results

Genotyping and Sanger sequencing pointed to *RHD*04.01*/*RHD*10.00* and homozygous *RHCE*01.07.01*, which could not explain the serological results. MLPA pointed to a *RHCE* hybrid, but the interpretation of height ratios of several nucleotide positions were ambiguous. ONT data revealed skewed allelic proportions of ~2-1 in certain *RHD* variants. Moreover, loss of heterozygosity across several kb in *RHCE* strengthened and refined the hybrid hypothesis. Finally, split read analysis of the capture-based ONT sequencing revealed a novel hybrid allele *RHCE*01-D(2,3)-CE(4)-D(5-9)-CE(10)* with the 4 *RHD*04.01* defining SNVs that was in accordance with the sister's MLPA data. The hybrid allele, which appeared causative for the weak RH2 expression (pseudo RhC), was phased on the same haplotype with *RHD*04.01*.

Conclusion

The combination of two effective enrichment methods of the *RH* locus for Nanopore sequencing — an *RHD-RHCE*-unspecific amplicon-based approach and a capture-based method — allowed disentangling a novel complex heterozygous *RHCE* hybrid allele.