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# Discovery and phasing of a novel null allele in a *FY*A/FY*B* individual with Nanopore sequencing

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**Background:** In the field of blood group genetics, direct determination of novel variants to their respective allelic background can enhance the accuracy of blood group phenotype prediction. In fact, haplotype-resolved sequences may be essential to interpret the functional consequences of identified variants, particularly when phenotypic data are unavailable. For example, it is important to know on which allelic background a novel silencing variant lies for deducing its effect on the blood group phenotype. Phasing variants, however, is not possible with the majority of standard techniques in molecular diagnostics, such as Sanger sequencing, PCR-SSP or other genotyping approaches.

**Aim:** We present here a case study illustrating the advantages of long-read Oxford Nanopore Technologies (ONT) sequencing for resolving a novel null allele in the Duffy blood group as complete gene haplotype.

**Methods:** The presented sample was initially identified as a genotype-phenotype discrepancy for the Duffy blood group in course of the routine donor typing at Blood Transfusion Service Zurich. Phenotyping was conducted via established serological methodologies. Genotyping was performed via MALDI-TOF mass spectrometry (MS) targeting the *FY*A*/*FY*B* defining single-nucleotide variant (c.125G>A), as well as the most common weak (c.265C>T) and null (c.-67T>C) defining variants. To elucidate the cause of the discrepancy, the entire length of the *ACKR1* gene, including flanking regions, was amplified (~2.1 kb) and sequenced using an ONT MinION flow cell. The results were further confirmed by Sanger sequencing of both *ACKR1* exons.

**Results:** The sample was identified as having a Fy(a-b+) phenotype. MALDI-TOF MS analysis, however, revealed heterozygosity for *FY*A*/*FY*B* and did not detect the presence of c.265C>T or c.-67T>C variants. This was the only genotype-phenotype discrepancy observed among ~15,000 donors for which both types of data was available. Subsequent Nanopore sequencing of the discrepant sample uncovered novel a 1-bp deletion (c.655delG) and an adjacent c.657C>G SNV (rs748896745) in the second *ACKR1* exon of the *FY*A* allele. Both variants were verified by Sanger sequencing.

**Conclusions:** Nanopore long-read sequencing was found to be effective and accurate for resolving incongruities between Duffy blood group genotype and phenotype. It proved to be clinically beneficial by facilitating direct phasing of a newly discovered frameshift mutation to the corresponding *FY*A* allelic background. This study serves as an illustrative example of the potential of functionally characterizing blood group alleles by sequencing them as complete gene haplotypes, which could become the emerging standard.

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